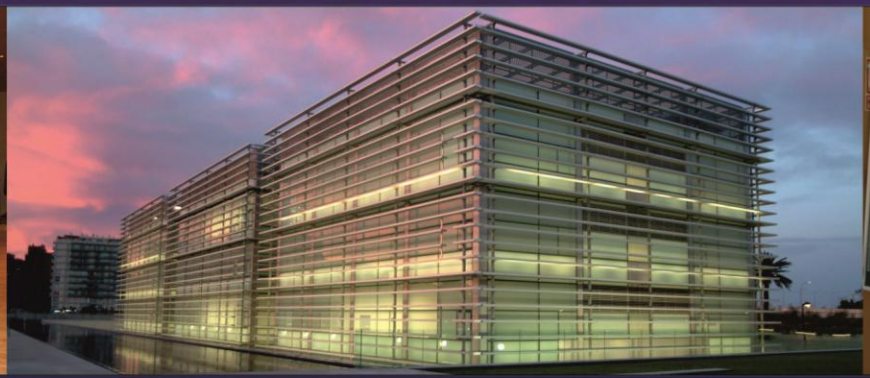
PRINCIPE FELIPE
CENTRO DE INVESTIGACION

CRS
Spanish-Portuguese
Local Chapter

Xth Spanish-Portuguese Conference on Controlled Drug Delivery

10th-12th November 2013
Centro de Investigación Príncipe Felipe,
Valencia, Spain


Xth Spanish-Portuguese Conference on Controlled Drug Delivery

Drug Delivery Systems from Lab to Clinic. New trends and Opportunities.

Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain

Sunday 10th November – Tuesday 12th November 2013

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SPONSORS

SCOPE

- A growing number of drug delivery technologies have been approved by Regulatory Authorities for routine clinical use and others are progressing through clinical trials as single agents or as components of combination therapy regimes.

- Novel carriers and synthetic technologies are being developed for different applications ranging from macro- to nano-sized controlled drug delivery technologies. Ever more sophisticated synthetic chemistry is leading to complex three dimensional polymeric architectures, hybrid constructs and self-assembling micro and nano-sized particles. Many carriers and hybrid systems are being developed as imaging agents and theranostics.

- As clinical applications broaden to include treatments for infectious and inflammatory diseases, tissue repair and regeneration (including cell therapy), and diseases of the ageing population. Importance will be given to combination therapy and the key issues of the manufacture/development process to achieve transfer from lab. to clinics.

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Programme

Sunday 10th November

11.00 - 13.00 ***Registration and Poster Mounting***

13.00 ***Welcome*: María J. Vicent (CIPF),**
Isabel Muñoz (CIPF Director), Paula Llobet (Inndea Valencia Foundation)

Plenary 1

Chairman: María J. Vicent

13.15 P1. **Lawrence Mayer** (Celator Pharmaceuticals Inc., Canada) • CombiPlex Anticancer Drug Combinations: Bridging Bench to Bedside

Session 1. New Technologies and Novel Therapeutic Targets

Chairmen: Julio San Román and Vicent Nebot

14.00 IL1. **Pablo Botella** (UPV-CSIC, Spain) •Stimuli-responsive hybrid materials for intracellular drug delivery

14.30 IL2. **Patricia Horcajada** (Univ. Versailles, France) • MOFS as novel Drug Delivery Platforms

15.00 IL3. **Ramón Martínez-Máñez** (UPV, Spain) • Gated Materials for Controlled Drug Delivery

15.30 O.1. **Ana Armiñán** (CIPF, Spain) • Polymer-based Combination Conjugates as Breast Cancer Therapy

15.40 O.2. **Joao Nuno Moreira** (Univ. Coimbra, Portugal) • Nucleolin-specific targeting of the Tumor Microenvironment in Bevacizumab-Resistant Lung cancer

15.50 O.3. **Sara Zalba** (Univ. Navarra, Spain) • Cell uptake mechanism involved in EGFr-targeted formulations

16.00-16.30 **Coffee/Tea • Posters**

Session 2. Novel preclinical technologies/disease targets

Chairmen: Dolores Torres and Emilia Barcia

16.30 IL4. **Rocío Herrero-Vanrell** (Univ. Complutense Madrid, Spain) • Novel Protein encapsulation Protocols for Long Term Delivery in the Treatment of Intraocular Diseases

17.00 IL5. **María J. Alonso** (Univ. Santiago Compostela, Spain) • Polymer Nanocarriers for Transmucosal Peptide Delivery

17.30 O.4. **Lucía Martín-Banderas** (Univ. Sevilla) • Preparation and Evaluation of a Caspase Inhibitor-Loaded Chitosan Nanoparticles

17.40 O.5. **Philipp Seib** (Univ. Stratclyde, UK) • Multifunctional Silk-Heparin Biomaterials for Vascular Tissue Engineering Applications

17.50 O.6. **Carmen Evora** (Univ. La Laguna, Spain) • Osteogenic Effect of Bioactivated Electrospinning System on a Bone Critical Deffect

18.00 O.7. **Petra Kocbek** (Univ. Ljubljana, Slovenia) • Formulation of Nanofibers with Incorporated Growth Factors and Their Effect on Cell Response In Vitro

Session 3. PhD Thesis Award (Co-sponsored: Evonik and SPLC-CRS)

Chairmen: **Javier Palacín and María J. Vicent**

18.10 **PhD Award Ceremony**

1st Prize. Susana A. Marques Martins (Univ. Porto)
Drug delivery across blood-brain barrier by means of intravenous administration of lipid nanoparticles

2nd Prize. Inmaculada Conejos Sánchez (CIPF, Valencia)
Polymer conjugates for the treatment of neurodegenerative disorders

2nd Prize. Ligia C. Gomes da Silva (Univ. Coimbra)
A novel multifunctional lipid- based nanoparticle for the delivery of siRNA to cancer cells and the tumor microenvironment

3rd Prize. Edurne Imbuluzqueta (Univ. Navarra)
Nanostructured biomaterials as a platform for the controlled delivery of antibiotics

18.30 O.8. **Susana A. M. Martins** (Univ. Porto, Portugal) • Drug delivery across blood-brain barrier by means of intravenous administration of lipid nanoparticles

19.00 - 20.30 **Poster Viewing • Refreshments will be provided**

Monday 11th November

Session 4. Novel Drug Delivery Approaches in clinical development
Chairmen: María José Alonso and Lawrence Mayer

9.00 IL6. **Jeff Hrkach** (BIND Biosciences, USA) • BIND-014: Preclinical Development and Clinical Translation of a PSMA-Targeted Docetaxel Nanoparticle with a Differentiated Pharmacological Profile.

9.30 IL7. **Andrés Cervantes** (INCLIVA Health Research Institute, Spain) • Open-label extension study of the RNAi therapeutic ALN-VSP02 in cancer patients responding to therapy?

Session 5. YOUNG SECTION SPLC-CRS
Chairmen: Amaya Niño and Lucía Martín-Banderas

10.00-11.00 **Short Talks selected from abstracts**

O.9. **Paola S. Apaolaza** (UPV/EHU, Vitoria) • Novel vector based on hyaluronic acid and solid lipid nanoparticles for gene therapy. **10 min**

O.10. **José Crecente** (CIMUS/USC, Santiago Compostela) • Enhancing the stability of grow factors: PLGA vehicles encapsulating heparin based nanocomplex for sustained released of BMP-7. **10 min**

O.11. **Susana P. Egusquiaguirre** (UPV/EHU, Vitoria) • Efficient intracellular delivery of PLGA nanoparticles conjugated to G-Proline derived cell-penetrating peptides for the treatment of Dyskeratosis Congenita. **10 min**

O.12. **Garazi Gainza**. (UPV/EHU, Vitoria) • A novel strategy for the treatment of chronic wounds based on the topical administration of RHEGF loaded lipid nanoparticles. **10 min**

O.13. **Juan Aparicio** (UCM, Madrid) • Elaboration and evaluation of CBD-Loaded Lipid nanocapsules as a new approach to bypass the Blood-Brain Barrier. **5 min**

O.14. **Ana Cadete** (CIMUS/USC, Santiago de Compostela) • Preparation of hyaluronic acid nanocapsules by a self-emulsifying method for cancer therapy. **5 min**

O.15. **Aroa Duro** (CIPF, Valencia) • Well-defined synthetic polypeptide based architectures as nanocarriers for drug delivery or imaging probes. **5 min**

O.16. **Sonia Reimondez** (IDIS/CIMUS/USC, Santiago de Compostela) • Protamine: Dextran nanoparticles for cancer gene therapy. **5 min**

11.00-11.30 **Coffee/Tea • Posters**

Chairmen: Aroa Duro

11.30 IL8. **Marcelo Calderon** (Freie Univ. Berlin, Germany) • Dendritic thermoresponsive nanogels for externally triggered drug delivery.

Chairmen: Sonia Reimondez and Ana Cadete

12.00-13.30 **Short Talks selected from abstracts**

O.17. **Judit Huarte** (UNAV, Pamplona) • Pegylated nanoparticles for oral delivery of Camptothecin. **10 min**

O.18. **Beatriz Lasa** (UNAV, Pamplona) • Lipid nanoparticles of an antitumor Alkyl-lysophopholipid edelfosine as a novel antileukemia treatment. **10 min**

O.19. **Adriana Martínez-Ledo** (CIMUS/USC, Santiago de Compostela) • Design and characterization of novel nanosystems for the co-encapsulation of peptides and nucleic acids. **10 min**

O.20. **Alexander Parra** (UB, Barcelona) • Formulation of alginate coated Poloxamer 407 particles for the

treatment of vulvovaginal candidiasis. **10 min**

O.21. Teresa Simon (UNAV, Pamplona) • PLGA microparticles carrying VEGF: Preparation and efficacy studies in combination with COQ10 PLGA nanoparticles in an animal model of myocardial ischemia. **10 min**

O.22. María J. Martin (UGR, Granada) • Viability of lactobacillus fermentum cect5716 encapsulated in gelatine and gastroresistant capsules **10 min**

O.23. Ana D. Bonillo (UGR, Granada) • Influence of Compritol on the controlled release of Dexketoprofen Trometamol from matrices containing polyesteramide PADAS. **5 min**

O.24. Patricia Marcianes (UCM, Madrid) • Multiparticulate controlled delivery systems of Tolcapone for Parkinson's disease. **5 min**

O.25. Rebeca Peñalva (UNAV, Pamplona) • Encapsulation of resveratrol in polymeric nanoparticles to improve its oral bioavailability. **5 min**

O.26. Juan P. Sánchez (UV, Valencia) • Evaluation of mesoporus silicon particles: studies in vivo with insulin. **5 min**

O.27. Edorta Santos (UPV/EHU, Vitoria) • Inactivation of encapsulated cells and their therapeutic effects by means of TK-GFP-Luciferase plasmid. **5 min**

O.28. Martha L. Vázquez (UB, Barcelona) • Enhance of hyaluronic acid release: development of the transformation of liposomes into planar lipid bilayers. **5 min**

13.30-14.45 **Lunch • Posters**

Plenary 2

Chairman: Ruth Duncan

14.45 P2. Collen Masimirembwa (African Institute of Biomedical Science & Technology, Zimbabwe) • The challenge of diseases of poverty in Africa and what Drug Delivery Technologies can achieve

Session 6. Tools to optimise the safety and efficacy of novel DDS

Chairmen: Antonio Pineda-Lucena and Marianne Ashford

15.30 IL9. Iola Duarte (CICECO, Univ. Aveiro Portugal) • Metabolomics as a new tool to monitor safety and efficacy of nanomedicines

16.00 IL10. Vicent Nebot (Polypeptide Therapeutic Solutions SL, Spain) •Well-Defined Synthetic Drug Delivery Carriers

16.15 O.29. Martina Palomino (CIPF, Spain) • A metabolomics perspective of controlled drug delivery

16.25-16.50 **Coffee/Tea • Posters**

16.50 O.30. Jennifer Hare (AstraZeneca, UK) • Improving the Pre-clinical to Clinical Translatability of Nanomedicines: Re-Investigating the EPR Effect Across Solid Tumours

17.20-18.30 **SPLC-CRS GENERAL ASSEMBLY**

19.30 **Turistic Tour Valencia City Center**
Meeting Point: Torres de Serrano
(to finalise at 'La Embajada' for dinner)

21.00 **Conference Dinner • La Embajada**
Pl. Alfonso el Magnánimo 7, 1º

Tuesday 12th November

Plenary 3

Chairman: María Blanco

9.15 **P3. Felipe Prosper** (Univ. Navarra, Spain) • Cell Therapy and Controlled Release Materials for Tissue Regeneration

Session 7: Novel preclinical technologies/disease targets

Chairmen: **Julie Movellan and Rogerio Gaspar**

10.00 **IL11. Damiá Tormo** (BiOncotech Pharmaceuticals SL, Spain) • BO-110 an RNA based therapeutic from Lab to Clinic

10.30 **IL12. Africa González** (Univ. Vigo, Spain) • Drug Delivery Technologies interplay with the immune systems

11.00-11.30 **Coffee/Tea • Poster**

Session 8: Challenges for development of 'DDS from Lab to Clinic'

Chairmen: **Ruth Duncan**

11.30 **IL13. Rogerio Gaspar** (Univ. Lisbon, Portugal) • Biosimilars and Generics. Issues for development and approval.

12.00 **IL14. Dolores Hernan** (EMA) • How does the EU facilitate the development of nanomedicines? Recent guidance.

12.30 **ROUND TABLE DISCUSSION.** Coordinator: Ruth Duncan (Cardiff, UK)

13.15 **Reflections on SPLC-CRS Local Chapter.**
The 10th Conference Anniversary.
Where are we now and where are we going?

13.30 **Oral and Poster Communication Prizes**
Concluding Remarks

ABSTRACTS OF PLENARY LECTURES

PL_1

CombiPlex Anticancer Drug Combinations: Bridging Bench to Bedside

Lawrence D. Mayer, Ph.D.
President and Chief Scientific Officer
Celator Pharmaceuticals, Ewing, NJ and Vancouver, BC

Abstract

Anticancer drug combinations can act synergistically or antagonistically against tumor cells *in vitro* depending on the ratios of the individual agents comprising the combination. The ability to take advantage of this relationship *in vivo* requires that drug ratios be controlled after administration. Nano-scale drug delivery vehicles are well suited for this application since they can be designed to coordinate the release of drug combinations after injection so that synergistic drug ratios can be delivered to tumors. This fixed-ratio dosing approach, referred to as CombiPlex, leads to dramatic increases in preclinical efficacy and clinical trials with CPX-351, a co-encapsulated liposome formulation of cytarabine:daunorubicin at a synergistic 5:1 molar ratio, have revealed marked efficacy improvements in certain AML populations. In addition, such formulations can be reproducibly manufactured at commercial scale and exhibit robust pharmaceutical stability. Nano-scale drug delivery vehicles allow *in vitro* drug ratio-dependent synergy informatics to be translated *in vivo* and these relationships appear to further translate from preclinical models to the clinic. Ratiometric dosing using CombiPlex technology allows drug combination synergy to be fully exploited *in vivo*, thereby increasing the likelihood of favorable therapeutic outcomes in patients.

PL_2

THE CHALLENGE OF DISEASES OF POVERTY AND WHAT DRUG DISCOVERY TECHNOLOGIES CAN ACHIEVE

Collen Masimirembwa

African Institute of Biomedical Science and Technology, Zimbabwe

INTRODUCTION

Poor economic development in African countries has meant that diseases peculiar to this continent do not receive attention from the pharmaceutical industry with respect to the discovery of new drugs hence their being referred to as poverty related diseases (PRD) among which are tuberculosis, malaria, trypanosomiasis, and schistosomiasis. Most of the drugs in use for the treatment or prevention of these diseases were discovered more than 50 years ago and are either unsafe, have variable efficacy, or are bring lost to drug resistance. The major cause of these liabilities has been shown to be inadequate pharmacokinetics. Advances in drug delivery systems are showing promise in addressing the DMPK parameters important for the safe and effective use of these drugs. In this presentation, cases were such exploratory work has or is being done will be highlighted.

CASES & OPPORTUNITIES

Malaria: In the treatment of malaria, traditional successful drugs such as chloroquine and fansider have been lost to drug resistance. We are now left with the artemisinin based combination therapy (ACTs) for which there are reports of emerging drug resistance and/or poor effectiveness in some parts of Africa. The highly potent artemisinins (artemisinin, artemether, artesunate or dihydroartemisinin) have the limitation of short half lives associated with incomplete cure rates. Studies to improve their half lives through use of liposomes (1) and their absorption using pheroids have been reported (2).

Tuberculosis: Treatment of tuberculosis is with a cocktail of drugs which include key components such as rifampicin and isoniazid. Three to six months of continuous daily drug intake is required for complete treatment. This long time and the tablet burden are associated with poor patient compliance. Rifampicin has additionally been associated with many undesirable drug-drug interactions due to its inductory effects on drug metabolizing enzymes. Nano-formulations of anti-TB drugs are showing promising results to reduce the dosing regimens and the length of treatment (3,4).

Schistosomiasis: The only drug effective against all forms of schistosomiasis is praziquantel. The drug has however been shown to exhibit variable cure rates. This has been attributed to its variable bioavailability which in turn is due to its poor solubility and high metabolic clearance. Nano-formulation is being explored to address these limitations (5,6).

Conclusion: Whilst none of the above formulations and delivery systems for drugs against PRDs are in clinical use, the efforts of some dedicated research groups in Africa and their international collaborators promise to yield tangible results in the near future.

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PL_3

Cell Therapy and Controlled Release Materials for Cardiac Regeneration

FR Formiga[1], JJ Gavira[2], G Abizanda[2], E Garbayo[1], B Pelacho[2], MJ Blanco-Prieto[1] and F Prosper[2]

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[2]Hematology, Cardiology and Cell Therapy, Clínica Universidad de Navarra and Foundation for Applied Medical Research, University of Navarra, Pamplona, Spain

INTRODUCTION

Cardiovascular diseases remain the first cause of morbidity and mortality in the developed countries accounting for almost 30% of all deaths (1). Despite recent evidence indicating that the heart is endowed with a regerative potential based on the presence of cardiac progenitors/stem cells, this is insufficient overall to prevent the development of cardiac failure after MI in the majority of patients (2). While heart transplant remains the only curative option for patients with end-stage heart failure, new approaches such as gene and stem cell therapy (3) or even the direct administration of pro-angiogenic growth factors have been explored in recent years (4). In the case of cell therapy, the current view suggests that stem cells contribute to cardiac repair through a paracrine effect associated with the release of growth factors rather than by directly contributing to tissue regeneration (5).

RESULTS AND DISCUSSION

We have assessed the potential of administration of different growth factors using DDS based of PLGA microparticles in models of myocardial infarction, including small (rat) as well as large preclinical models (pig). As a proof of concept we employed (PLGA) microparticles loaded with VEGF165 and compared with free VEGF or control non-loaded microparticles in a rat model of ischemia-reperfusion. VEGF165 loaded microparticles could be detected in the myocardium of the infarcted animals for more than a month after transplant and provided sustained delivery of active protein in vitro and in vivo. One month after treatment, an increase in angiogenesis, arteriogenesis along with a positive remodeling of the heart was detected in the VEGF-microparticle group. This was associated with an improvement in cardiac function (6). These results led us to initiate further studies using growth factors involved not only in angiogenesis but also in cardyogenesis and cardiomyocyte survival such as Neurogeulin 1 (NRG1) and FGF1 in a rat and a pig model of MI.

In order to characterize the mechanisms by which the cytokine loaded MP exerted its action, tissue cardiomyocyte proliferation was examined by double immunostaining for cTnT and Ki-67+. A significant increase in the number of adult cardiomiocytes Ki-67^{+} was detected in the hearts treated with NRG-1, 3 months post-treatment. Furthermore, c-Kit+CD45- progenitor cells were also detected in the myocardium, although tissue quantification did not show significant differences among groups, 3 months post-injection. However, a significant increase was detected 1 week post-implantation in the NRG-1-MP group in comparison with the NL-MP control group. Finally, growth factor loaded MP used in rats proved to contribute to an improvement in cardiac function. The strategy was successfully scaled-up to be tested in an ischemia-reperfusion porcine model. NRG1-MP, FGF1-MP and non-loaded MP were next injected locally into the infarcted myocardium using NOGA® XP Cardiac Navigation System, a highly accurate electro-guided methodology which creates precise 3-D heart images, 1 week after the infarct. NRG-1-MP, FGF-1-MP and non-loaded MP were injected into the infarcted myocardium using NOGA® XP Cardiac Navigation System 1 week after the infarct. The long-term functional efficacy of growth factor-loaded MP treatment is currently being evaluated. The validation of this therapeutic approach could make significant progress for patients with ischemic heart disease.

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ABSTRACTS OF INVITED LECTURES

IL_1

Stimuli-responsive hybrid materials for intracellular drug delivery

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INTRODUCTION

In cancer therapy, to achieve complete eradication of tumors, cytotoxic drugs must be administrated systematically in high dose. However, this may lead to severe side-effects due to non-specific uptake of antitumor drugs by healthy tissues/organs.[1] In this sense, an ideal drug carrier should deliver a significant quantity of the pharmaceutical payload with no premature release of the drug prior to reaching the targeted cells.[2] Therefore, it is compulsory to develop drug delivery systems (DDS) able to discharge their cargo under specific stimuli that may trigger drug release under the internalization process takes place within cancer cells. Here, organic/silica hybrid materials have been considered to be excellent candidates for the preparation of DDS, as their textural properties favor the loading of important amounts of therapeutic molecules and their silanol-containing surface can be easily functionalized, introducing additional features that allow imposing a stimuli-responsive controlled drug release.[3,4] Herein, we present some novel organic/silica nanohybrids for the intracellular delivery of anticancer drugs that can release the therapeutic load under specific stimuli.

RESULTS AND DISCUSSION

We have developed DDS responsive to physical (vis/NIR light), chemical (glutathione) or biological (esterases) stimuli. In the first case, the therapeutic nanoplatform associates the optical activity of gold nanoclusters with the cytotoxicity of camptothecin (CPT).[5] Gold nanoparticles were assembled into stable clusters with a tailored absorption cross-section in the vis/NIR spectrum. These clusters were further encapsulated in a mesoporous silica coating containing CPT. After internalization in 42-MG-BA human glioma cells, these protected gold nanoclusters produced photothermolysis under fs pulse laser irradiation of 790 nm. Moreover, incorporated CPT was released during the process, provoking significant cell death increase. In the second example, a (pyridin-2-yldisulfanyl)alkyl carbonate CPT derivative was directly coupled with thiol groups of silica hybrid nanoparticles containing a non-porous core and a mesoporous shell. Upon internalization in HeLa cells, the reducing activity of cytosolic gluthathione provoked disulfide bridge cleavage, releasing the naked drug after an intramolecular cyclization mechanism.[7] Finally, amorphous non-porous silica nanoparticles were surface-modified to covalently link CPT through an ester bond.[8] Direct coupling of 20-O-trifluoroglycinylcamptothecin with carboxylate groups of silica nanoparticles gave a highly stable in plasma nanodrug. After uptake in HeLa and HT29 cells, CPT release took place due to the activity of cytosolic esterases.

Overall, we foresee a great future for these multifunctional hybrid materials as novel DDS, as their tailored physical and chemical properties allow imposing controlled release over delivered compounds under specific stimuli.

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IL_2

Metal Organic frameworks as novel drug delivery platforms

Patricia Horcajada

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Porous metal-organic frameworks (MOF) belong to a fascinating class of porous crystalline materials and currently receive much attention in regard to their potential applications in strategic fields such as gas storage, separation, sensing or heterogeneous catalysis.[1] Among these, bioapplications have emerged in 2006 as a very promising field,[2,3] due to their high and regular porosity and their highly versatile chemical composition. In particular, biocompatible and non toxic nanoparticles of porous iron(III) carboxylates have shown exceptional loadings of different challenging drugs (antitumoral, antiretroviral, cosmetics) with controlled releases of the active form of the therapeutic molecule.[4]

This talk discloses an overview of the most interesting MOF structures developed at the Institut Lavoisier of Versailles as well as some of their competitive performances and/or limitations in the biomedical field.

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IL_3

GATED MATERIALS FOR CONTROLLED DRUG DELIVERY

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[3] CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN)

INTRODUCTION

Gated nanochemistry, although highly topical and rapidly developing, is still in its infancy. Recently it has been demonstrated the possible incorporation of "gates" into mesoporous supports. In this field, molecular or supramolecular gates can be defined as nanoscopic-based devices in which mass transport can be triggered by target external stimuli that can control the state of the gate; i.e., closed or open. In fact in the last few years, nano-containers bearing gated scaffoldings have proved to be excellent candidates for the design of controlled-release "nano-machines" at different levels. In this area, the use of gated ensembles built up using silica mesoporous materials containing on-off triggered gated systems have proved their suitability. These systems show an ideal "zero release" until opened via suitable stimuli. Mesoporous supports show stable structures (pores of ca. 2-3 nm), large surface areas (up to 1200 m^2/g), tunable pore sizes and volumes, and well-defined surface properties for hosting molecules and for site-specific delivery. The mesoporous support can additionally be obtained in a nanometric size, resulting in suitable materials for the design of "nanodevices" for the controlled delivery of drugs and other species. Moreover, a second novel application involves the use of gated material in sensing protocols.

RESULTS AND DISCUSSION

Selected examples of triggered silica mesoporous gated materials able to deliver their cargo by changes in temperature,[1] irradiation with light,[2] and by the presence of small molecules or biomolecules [3,4,5] will be shown.

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IL_4

NOVEL PROTEIN ENCAPSULATION PROTOCOLS FOR LONG TERM DELIVERY IN THE TREATMENT OF INTRAOCULAR DISEASES

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INTRODUCTION

Chronic and multifactorial pathologies affecting the back of the eye represent visual impairment and blindness. Biotechnological products are often employed for the treatment of these posterior segment disorders. Treatments are performed by intravitreal injections due to the difficulty in delivering effective doses of these active molecules to intraocular target tissues. This therapeutic approach allow to reaching effective tissue drug levels. However, successive intraocular injections are associated to side effects (retinal detachment, hemorrhage, endophthalmitis, and cataract) and the risk increases with the frequency of administrations. Intraocular drug delivery systems (IODDS) are under evaluation to avoid successive injections. Depending on their size, the devices can be implanted through a relatively large surgical incision or through a smaller tissue perforation. Among the IODDS, microparticles (1-1000µm size) are emerging therapeutic tools for the treatment of posterior segment diseases as they are able to release the active substance during weeks or months. Biodegradable microspheres can be injected through small gauge needles avoiding surgical procedures and disappear from the site of injection after delivering the drug. Furthermore, they can be used in personalized medicine as different amounts of microspheres can be injected for individualized therapy. Since several years ago, the encapsulation of proteins has been one of the most interesting challenges in the field of pharmaceutical technology. The encapsulation technique should guarantee the maintenance of the biological activity of the product throughout manufacturing, storage and use.

RESULTS AND DISCUSSION

Preservation of protein biological activity can be achieved by the use of a novel protein encapsulation procedure based on a solid-in-oil-water (S/O/W) emulsion technique. To this, the bioactive substance is encapsulated without any preliminary manipulation. With this technique, the use of additives promotes additional protection of the biological product before and after sterilization. Protein is released from microspheres in its active form for long periods of time. This technological strategy has been applied to the encapsulation of a neurotrophic factor (Glial-cell derived neurotrophic factor) in PLGA microspheres. Efficacy studies in an animal model of glaucoma have confirmed the activity of the GDNF released from the microspheres after 12 weeks of their intravitreal injection.

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IL_5

POLYMER NANOCARRIERS FOR TRANSMUCOSAL PEPTIDE DELIVERY

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Our group, being committed with the translation of ideas from the university through novel pharmaceutical technology, has designed novel polymer nanocarriers intended to transport peptides and proteins across biological barriers and to deliver them to the target tissue. In particular, we pioneered the development of nanoparticles and nanocapsules made of chitosan in combination with other biomaterials, such as glucomanan, hyaluronic acid, cyclodextrins, phospholipids and oils. During my presentation I would like to focus on the analysis of the potential of a variety of nanocarriers for the oral and nasal administration of therapeutic peptides, i.e. insulin and salmon calcitonin. Within this frame, I will also describe the activities being implemented within TRANS-INT European Consortium and those planned for the coming years (for more information visit www.trans-int.eu). Finally, I will present the potential of different types of nanoparticles for intranasal immunization using deferent protein and peptide antigens. As a critical example, I will describe our recent advances towards the development of a peptide-based nasal HIV vaccine, in collaboration with the University of Manitoba. Overall, the results have shown that packaging 12 peptide antigens within nanoparticles is a powerful strategy for achieving significant immune responses following nasal administration to macaques.

More information about these applications and the literature associated to them can be found at:

http://webspersoais.usc.es/mariaj.alonso

IL_6

BIND-014: Preclinical Development and Clinical Translation of a PSMA-Targeted Docetaxel Nanoparticle with a Differentiated Pharmacological Profile

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INTRODUCTION

The challenge for all drugs is to maximize the net clinical benefit by increasing the desired therapeutic effect and reducing adverse effects. This is especially difficult in cancer, where the goal is to destroy or inhibit growth of cancer cells without damaging similar healthy cells. Accurins™ are polymeric nanoparticles that incorporate a therapeutic payload and are designed to have prolonged circulation within the bloodstream, enable targeting of the diseased tissue or cells, and provide for the controlled and timely release of the therapeutic payload. Accurins are designed with specified physical and chemical characteristics to target specific cells or tissues and concentrate a therapeutic payload at the site of disease to enhance efficacy while minimizing adverse effects on healthy tissues.

RESULTS AND DISCUSSION

BIND-014 is a prostate-specific membrane antigen, or PSMA, targeted Accurin that contains docetaxel. PSMA is a clinically-validated tumor marker expressed on prostate cancer cells and the blood vessels of many types of non-prostate solid tumors, including non-small cell lung cancer.

We compared the pharmacokinetics of BIND-014 and Taxotere in cynomolgus monkeys, with equal doses of BIND-014 or Taxotere administered and blood samples collected at various times over a 70-hour period to measure the total docetaxel concentration. The docetaxel concentration was approximately 10 to 100 times higher with BIND-014 than Taxotere for the entire duration of the experiment. We also studied the levels of accumulation of docetaxel in a mouse model of human prostate cancer when treated with BIND-014 or Taxotere. After 12 hours, the docetaxel concentration was more than seven times higher in the animals treated with BIND-014. We also compared the efficacy of BIND-014, a version of BIND-014 without targeting ligand, which we refer to as PTNP, and docetaxel in a mouse model using the LNCaP human prostate cancer cell line. BIND-014 treatment resulted in significantly increased shrinkage of the tumors when compared to docetaxel and was also significantly more effective than PTNP.

To date, we have clinically tested BIND-014 in over 45 patients with advanced or metastatic cancer who failed prior therapies. In our Phase 1 clinical trial, of the 28 patients who received BIND-014 once every three weeks, to date there have been one complete response in a patient with cervical cancer and three partial responses in patients with NSCLC, mCRPC and ampullary cancer. Five additional patients had stable disease lasting longer than 12 weeks. BIND-014 is in Phase 2 clinical trials for non-small cell lung cancer and metastatic castrate-resistant prostate cancer.

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IL_7

OPEN-LABEL EXTENSION STUDY OF THE RNAi THERAPEUTIC ALN-VSP02 IN CANCER PATIENTS RESPONDING TO THERAPY

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IL_8

DENDRITIC THERMORESPONSIVE NANOGELS FOR EXTERNALLY TRIGGERED DRUG DELIVERY

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INTRODUCTION

The effort to design and develop materials on the nanometer scale has been accelerating at a fast pace in recent decades. In the field of biomedicine, stimuli-responsive and biocompatible materials have emerged as the new generation of smart molecules/materials. In general, the operating principle behind responsive architectures lies in the fact that different environmental triggers can lead to structural/chemical changes within the scaffold of such materials. This unique feature enables their use in diversified biomedical applications.[1]

In an attempt to create very well-defined, monodisperse, stable nanostructures at the molecular level, highly branched dendritic polymers have been used for last couple of decades. Special interest has been devoted to the fabrication of nanogels which are high molecular weight cross-linked polymers that combine the characteristics of dendritic polymers with that of cross-linked macroscopic gels, to yield soluble particles within the useful size range between 20 and 200 nm.

Stimuli-sensitive nanogels can shrink or swell rapidly by expelling or absorbing water in response to external stimuli such as temperature, pH, electrical, and magnetic fields.[2] The combination of nanogel properties and thermo-responsiveness generates a promising candidate for the development of smart nanocarrier systems. Their potential properties can be influenced by temperature changes with high responsiveness, reveal high loading capacity, can improve drug stability, and thus can be used for stimuli-controlled drug release.

RESULTS AND DISCUSSION

Our hypothesis treats the preparation of thermoresponsive glycerol-based nanogels and the investigation of their phase behavior with respect to their potential biomedical applications. Initial focus was given to fabricate nanogels with size control over the range of 50 and 200 nm and narrow size distributions.[3] Preliminary results about their low cytotoxicity, their capability to penetrate cell membranes, and their potential to delivery and release bioactives upon external triggers like temperature and light will be presented.

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IL_9

Metabolomics as a new tool in nanotoxicology and nanomedicine: Validation in vitro using human keratinocytes exposed to silver nanoparticles

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INTRODUCTION

There is growing concern regarding the potential toxicity of nanomaterials in respect of accidental human exposure and also during their development as nanomedicines. Silver nanoparticles (Ag-NPs) are among the nanomaterials with highest propensity for human exposure, arising from their established use in wound dressings and increasing incorporation into consumer products (e.g. clothing, food packaging), mainly due to their remarkable antimicrobial properties. However, there is a narrow window between bactericidal activity of Ag-NPs and their toxicity to human cells[1], making the further understanding of their biological effects a relevant up-to-date subject. Development of metabolic profiling (metabolomics) strategies for assessing the cellular effects of these nanoparticles may provide a unique and important tool that can be broadly applied in the areas of nanotoxicology and nanomedicine[2].

RESULTS AND DISCUSSION

Human epidermis keratinocytes (HaCaT cell line) grown in DMEM medium, have been exposed for 24 and 48h to well-characterised Ag-NPs of different average diameters (10, 30 and 60 nm) at (sub)toxic doses, and their endo- and exo-metabolomes characterised by ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy, in tandem with multivariate analysis. Cell proliferation and cytotoxicity evaluations were conducted by microscopic evaluation and MTT cell viability assay. A number of metabolites involved in different biochemical processes (e.g. antioxidative response, aminoacid and lipid metabolisms) were found to be altered upon Ag-NPs exposure, in a dose- and time-dependent manner. Ag-NPs size and aggregation pattern were also seen to influence the metabolic responses. The results show the potential of NMR metabolic profiling for highlighting new endpoint markers of Ag-NPs effects, thus demonstrating the value of metabolomics as a novel tool in the area of *in vitro* nanotoxicology.

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IL_10

WELL-DEFINED POLYMERS FOR THERAPEUTIC APPLICATIONS: VERSATILE POLYGLUTAMATES VIA NCA POLYMERISATION

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INTRODUCTION

Polymer therapeutics have demonstrated excellent potential as drug delivery vehicles for localised delivery of cytotoxic drugs, with several carriers now in the marketplace or clinical trials as treatments for cancer and other medical conditions.[1] Biopersistent carriers (PEG, HPMA) present disadvantages if chronic parenteral administration and/or high doses are required as there is the potential to generate 'lysosomal storage disease' syndrome. Therefore, there is a need for the development of new synthetic strategies to access biodegradable carriers with well-defined architectures. On this context, the use of biodegradable polypeptides is gaining interest within the field due to their molecular diversity and multifunctionality.[2]

RESULTS AND DISCUSION

Here we present a wide and versatile family of polyglutamates with well-defined architectures obtained via ring opening polymerisation of N-Carboxyanhydrides. To this end, we have developed new synthetic strategies allowing the access to several polyglutamate architectures including linear, branched and star homopolymers but also block copolymers with narrow polydispersities and batch to batch reproducibility. The use of amphiphilic block copolymers opens the possibility to design polymeric micelles for different applications. An exhaustive study on solution conformation of the final carriers is of key importance to value their possible therapeutic output. Interestingly, the post-polymerisation modification of the polymers has proven to be an effective and straightforward strategy towards the preparation of multifunctional polyglutamates with potential applications as carriers in drug delivery approaches.[3,4]

Finally, it is worth mentioning that PTS has recently developed a hybrid drug delivery system combining a PGA-derived hydrogel including polymeric micelles that opens the possibility for local treatment, with particular interest on tissue regeneration.

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IL_11

BO-110, A NEW CONCEPT OF ANTICANCER THERAPY

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BO-110 is an antitumoral drug product for parenteral administration with high efficacy and a broad spectrum of action that eludes mechanisms of cell survival in malignant cells. BO-110 is formed by combination of a RNA and a polycationic polymer and exhibits a multiple mechanism of action in tumor cells with a solid proof of concept. Bioncotech Therapeutics supposes a platform for chemistry, manufacturing and control of BO-110 drug product. In addition, Bioncotech is now involved in nonclinical and clinical evaluation of the drug.

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IL_12

DRUG DELIVERY TECHNOLOGIES INTERPLAY WITH THE IMMUNE SYSTEM

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INTRODUCTION

The potential toxicity of nanomaterials (NMs) has raised many concerns in the nanotechnology research field and in the regulatory/advisory committees. Nanostructures can interact with biological systems, such as the immune system, which includes a large variety of cells and soluble elements, being its main role to detect and to eliminate foreign elements. The understanding of the interaction between NMs with biological fluids (such as serum) and the immune system is essential (1). The interaction of serum proteins with the NMs can have negative consequences, such as the induction of conformational changes, leading to functional loss or important modifications in some proteins. NMs can induce immune responses such as complement activation, phagocytosis, induction of oxidative stress, and the activation or inhibition of the immune cells. We have tested (*in vitro* and *in vivo)* the effect induced by non-biodegradable and biodegradable NMs on the immune system and biological fluids.

RESULTS AND DISCUSSION

The interaction of four different metal oxide Nps (ZnO, TiO_2, CeO_2 and Al_2O_3) with human albumin, fibrinogen and globulins showed that for ZnO Nps, a strong interaction was observed, which induced a decrease in the thermal stability of both fibrinogen and albumin at a low temperature, interfering with the clotting activity of fibrinogen. TiO_2 and CeO_2 Nps showed lower effects, while for Al_2O_3 Nps only a slight interaction was observed (2). Thus, some metal oxide nanoparticles induce conformational changes in the secondary structure of human serum proteins. None of the four Np tested induced complement activation, oxidative stress or *in vivo* immunotoxicity, although ZnO Nps showed high cytotoxicity *in vitro* (3).

We also tested several prototypes of biodegradable nanostructures containing chitosan (in the presence or not of a toll like receptor 7 ligand) for improving immunization against hepatitis B infection (using recombinant hepatitis B surface antigen). Comparison with the conventional alum-rHBsAg vaccine was performed. The results indicate that a polymer/oil based nanovaccine can be used as a single-dose immunization approach eliciting long lasting and protective immune responses (4), and that the co-delivery of viral proteins and a TLR7 agonist from polysaccharide nanocapsules, can be useful in a needle free vaccination strategy (5).

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IL_13

Biosimilars and Generics. Issues for development and approval.

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INTRODUCTION

The increased complexity of factors involved in drug discovery, design, development and usage (3DU) makes way for new approaches that can integrate Science & Technology serving the needs of patients[1]. Among those approaches the need to establish more efficient strategies to transform Science in better Healthcare faces challenges from Industrial organization (and business model), from Science gaps (in certain areas evident lack of adequate models for translation to first in man or clinical trials) and from non-harmonized views in certain areas between regulators and scientists.

Systems approaches will become more common through the introduction of complex analytical and predictive tools, by opening new doors for systems toxicology (allowing room for the introduction of modern toxicology methods), systems pharmacology (a whole new paradigm currently addressed by a number of NIH initiatives), systems therapeutics (integrating also pharmacoepidemiology and pharmacogenetics, as well as efficacy and effective evaluation tools, paving the way for health technologies assessment with a better scientific base), systems technologies (Quality by Design or QbD approaches, through the use of PAT), and complex systems, through the development and use of new hybrid and increasingly complex structures that will allow to combine different therapeutic targets with a combination of diagnostics, therapeutics and monitoring. These five systems approaches are the Science base for modern Regulatory Science[2].

Among different innovative approaches for development of better medicinal products based in existing technologies a number of issues have been arising regarding the changes in biosimilar medicinal products introduced in Europe in 2004 and the current discussions on follow-on non-biological complex drugs.
An overview of major problems arising in this particular area will be established according to current scientific and regulatory discussions[3].

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IL_14

HOW DOES THE EU FACILITATE THE DEVELOPMENT OF NANOMEDICINES? RECENT GUIDANCE.

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INTRODUCTION

Nanomedicine is an emerging interdisciplinary scientific research field with a wide applicability in the context of the development of medicinal products. It can offer many advantages over conventional medicines, including target-specific delivery, improved solubility and bioavailability, and reduced adverse effects.

Over the last three decades several first-generation nanomedicines have successfully entered routine clinical use. As this science evolves, several "follow-on" nanomedicines ("nanosimilars") and second generation nanomedicines are being developed. To ensure a timely introduction of high quality, safe and efficacious medicinal products it is important to identify and address gaps in scientific knowledge and to prepare for their evaluation.

DISCUSSION

Over the last decade the European Medicines Agency (EMA) has established different initiatives to support innovation of nanomedicines and protect public health.

The EMA, in collaboration with its network of experts, has published several guidance documents to provide appropriate regulatory guidance and assist researchers and companies interested in the development of nanomedicines for clinical applications. The presentation will focus on the key aspects of the recently published reflection papers for liposomes; block copolymer micelles and iron nanoparticles.

The accumulation of experience is allowing, on an on-going basis, to assess the need for further guidance specific to nanomedicines or for the update of existing ones.

Applicants developing nanomedicinal products are encouraged to establish a dialogue with the EMA from the early stages of development through the EMA Innovation Task Force and/or the Scientific Advice/Protocol Assistance procedure.

By establishing expert groups, international collaborations and convening stakeholders in public workshops, the EMA continues to adapt and prepare the regulatory system for the development, evaluation and successful market entry of nanomedicines for the benefit of patients.

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ABSTRACTS OF ORAL INTRODUCTION TO POSTERS

O_1

Polymer-based combination conjugates as breast cancer therapy

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INTRODUCTION

Due to the molecular complexity of cancer, the use of polymer-drug conjugates in combination therapy represents an important opportunity to enhance tumour response rates.[1] The polymeric carrier provides an ideal platform for the simultaneous delivery of drug cocktails.[2] In the treatment of hormone-dependent cancer, it has been demonstrated that the combination of endocrine therapy with a chemotherapeutic agent could bring significant advantages.[3,4] This novel approach includes drug synergism and patience compliance. We have previously reported the first endocrine-chemotherapy combination HPMA copolymer-AGM-Dox conjugate.[3,4] The conjugate containing both drugs showed markedly enhanced cytotoxicity compared with HPMA copolymer-Dox which has already showed clinical activity in breast cancer patients[4]. Currently our efforts are directed towards the understanding of the molecular mechanisms of action for the combination *vs.* single Dox conjugates and the achievement of the *in vivo* proof for synergism in breast cancer mice models.

RESULTS AND DISCUSSION

To evaluate the HPMA combination conjugates, an orthopic breast cancer mice model was optimised.[5] In 6 weeks balb/c mice female, 5 million of 4T1 murine breast cancer cells were injected in the third breast. Tumours were analysed in order to control the aromatase enzyme levels together with the tumour vascularisation. EPR effect can significantly vary between tumour type and stage, therefore is highly important to properly characterise the *in vivo* models used in order to evaluate the therapeutic value of a conjugate based on passive targeting. The vascular permeability was studied using BSA-Evans blue.[5] We were able to determine 0.1 cm^3 as the maximum accumulation of Evans Blue in tumours. After 8 days (0.1 cm^3 tumour size) HPMA-AGM-Dox combination conjugate, HPMA-Dox conjugate, HPMA-Dox+ HPMA-AGM and free Dox were injected i.v. at 5mg/mL Dox-equiv. 3 times every 3 days. Tumor growth and animal weigh were measured daily until the end point. HPMA-Dox and HPMA-AGM-Dox diminished tumor growth without any animal weigh loss in comparison to the Dox and control groups. Moreover, after the second dose, the antitumour activity of HPMA copolymer-AGM-Dox conjugate compared to HPMA copolymer-Dox conjugate was significantly greater. As expected, the combination of both single conjugates (HPMA copolymer-Dox + HPMA copolymer-AGM) showed a different effect in comparison with HPMA copolymer-AGM-Dox confirming the importance of conjugating both drugs in the same polymer backbone.

The molecular mechanisms responsible for the observed antitumor synergism were also studied using tumour tissues. The differences observed in cell death mechanisms (autophagy *vs*. apoptosis) together with a different VEGF modulation seem to be the key factors responsible for a greater antitumour effect with the combination conjugate.

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O_2

NUCLEOLIN-SPECIFIC TARGETING OF THE TUMOR MICROENVIRONMENT IN BEVACIZUMAB-RESISTANT LUNG CANCER

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INTRODUCTION

In recent decades, the treatment options for lung cancer have expanded beyond traditional chemotherapy to include targeted therapies that act specifically against key components involved in tumorigenesis. Considering the limitations associated with chemotherapies and the possibility of interrupting a tumor vascular network, there has been great interest in targeting the tumor vasculature and much effort has been directed towards the development of agents that disrupt angiogenesis. In addition to the target accessibility, the endothelial cells of the tumor-associated vasculature have greater genetic stability than cancer cells. Anti-angiogenic drugs, such as *bevacizumab*, have become a standard treatment option in lung cancer. In spite some clinical successes with these inhibitors, disappointing results have been reported, with a substantial number of lung cancer patients who have become resistant to angiogenic inhibitors[1-3]. Recently, a novel nanotechnology-based strategy, using lipid-based nanoparticles containing doxorubicin (DXR) and targeted to nucleolin (overexpressed on the surface of both cancer and endothelial cells from tumor blood vessels) has achieved a significant antitumor effect in a murine model of human breast cancer[4]. Such promising results led us to investigate the impact of this therapeutic approach against human lung cancer models resistant to *bevacizumab*.

RESULTS AND DISCUSSION

In vitro studies demonstrated an improved association and intracellular delivery of encapsulated DXR in liposomes targeted to nucleolin, leading to a significant impact on cell death. Along with this, immunohistochemical analysis revealed that nucleolin was highly expressed in different cells in the tumor microenvironment of patient-derived lung tumors, in a tumor-specific manner. The generated results render an important indication of the therapeutic potential of the nucleolin-targeted strategy against lung cancer.

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O_3

Cell uptake mechanism involved in EGFr-targeted formulations

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Introduction

Targeted therapy is the current strategy used in the treatment of several types of tumors that over-express certain receptors, such as the endothelial growth factor receptor (EGFr). This strategy consists in the use of ligands able to block those specific receptors involved in the growth and progression of cancer cells. One example is the application of monoclonal antibodies (mAbs), like Cetuximbab. This mAbs has higher affinity by EGFr than the endogenous factors, providing the inhibition of the receptor-activity [1].

Nanomedicine applied in the cancer treatment, is focused in the development of targeted-nanosystems to increase their selectivity by the tumor [2]. Therefore, the coupling of mAbs to the surface of liposomes shows certain advantages at the therapeutic levels compared to stealth liposomes or non-targeted liposomes [3]. However, the presence of Fc' fragments of the mAb induce a rapid withdrawal from circulation and produce several undesirable immunoreactions. Thus, the current targeted therapies are based on the coupling of small molecules, like the Fab' fragment of an antibody or others even smaller than Fab'[4]. On the other hand, the endogenous ligand of EGFr, EGF, has been also conjugated to liposomes to obtain a more efficient gene delivery in tumor cells that over-express this receptor [5].

Therefore, the aim of this work was to develop nanoliposomes coupled to EGF or Fab' for EGFr targeting and explore the mechanism involved in the cell uptake using four human colorectal cancer cell lines with different expression level of the receptor.

Results and discussion

The Fab' fragment, obtained from Cetuximab by enzymatic digestion with pepsin, was purified for the coupling to the surface of liposomes previously formulated with a fluorescent probe. EGF was also covalent linked to liposomes by a previous modification of the protein. These ligands did not influence parameters like particle size and polydispersity index, as was expected. *In-vitro* studies showed that the time profile of the liposomes internalization for both targeted formulations, Fab' and EGF, was significantly higher in comparison with non targeted liposomes. In order to explore the role of the receptor in this process, a pretreatment with Cetuximab induced a reduction in the uptake of the targeted formulation. Therefore, although the EGFr targeted strategy seems to be a promising approach to increase the accumulation of these formulation in tumor cells, the mechanism observed deserves to be investigated, especially in *in-vivo* studies.

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O_4

PREPARATION AND EVALUATION OF A CASPASE INHIBITOR-LOADED CHITOSAN NANOPARTICLES

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INTRODUCTION

Activation of microglia and inflammation-mediated neurotoxicity are suggested to play a decisive role in the pathogenesis of several neurodegenerative disorders. Recently, it has been demonstrated that caspase-8 and caspase-3/7 are involved in regulating microglia activation. Therefore, the inhibition of these caspases in microglial cells could be neuroprotective [1]. Chitosan is a linear polysaccharide characterized by a high biodegradability with applications in the biomedical and pharmaceutical fields. Chitosan nanoparticles play an important role as drug delivery to the brain specially neuroptotection [2]. To further enhance the stability and biocompatibility of chitosan nanostructures in vivo, several authors have synthesized graft copolymers of chitosan with the hydrophilic polymer PEG. The resulting PEG-chitosan graft copolymers have shown indeed improved biocompatibility[3]. Here, we propose CS-PEG nanoparticles (NPs) as possible carriers for the caspase-8 inhibitor Z-Ile-Glu(O-ME)-Thr-Asp(O-Me) fluoromethyl ketone (Z-IETD-FMK).

RESULTS AND DISCUSSION

Chitosan (CS) NPs (Protasan® UP CL113, Novamatrix) were produced by ionic gelation with sodium tripolyphosphate (TPP, Sigma-Aldrich) [2]. Z-IETD-FMK loaded-nanoparticles were produced by dissolving the inhibitor in DMSO at 571 ng/mg NPs. Particles were collected by centrifugation (10,000 rpm, 4ºC, 30 min).

Cationic *(zeta potential = +30 mV),* monodisperse *p*articles around 345 nm in diameter (Pdi ≈ 0.2) were obtained. Drug content, was determined by measuring the amount of free drug in the supernatant. For this purpose we have developed an LC-MS method for the quantification of the peptide. A QTRAP**:** Hybrid triple quadrupole-linear ion trap mass spectrometry (QqQLIT) technique was used. The loaded amount was 485.7 ± 86 ng/mg NPs with high encapsulation efficiency (85%). Loaded NPs were *in vitro* evaluated and added to BV2 microglial cell cultures and caspase-8 activity was measured (Caspase-Glo 8 Assay, Promega). Next, to study microglial activation we analyzed iNOS, IL6 and TNFα levels by qPCR. In this case we used modified NPs expressing PEG and biotin on their surface.

NPs loaded with Z-IETD-FMK inhibited caspase-8 activity and microglial activation induced by LPS. This work is the first step on the application of Z-IETD-FMK loaded-CS NPs for their targeted delivery into the CNS.

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O_5

MULTIFUNCTIONAL SILK-HEPARIN BIOMATERIALS FOR VASCULAR TISSUE ENGINEERING APPLICATIONS

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INTRODUCTION

Scaffold materials with suitable biological and mechanical properties are necessary for vascular tissue engineering[1]. One potential scaffold material of interest is silk[1,2]. Many in vitro studies have proposed the use of silk for vascular applications; for example, as a stent coating for sustained drug release[3], in blood vessel engineering, and as a material for small vascular grafts[4]. Silk sutures are well tolerated in humans[5], but the use of silk for vascular engineering applications still requires extensive biocompatibility testing[2]. The aim of this study was to develop multifunctional silk-heparin biomaterials and determine their hemocompatibility in a humanised *in vitro* test system.

RESULTS AND DISCUSSION

Over the past 30 years, silk has been proposed for numerous biomedical applications that go beyond its traditional use as a suture material[5]. Some studies have indicated a need to modify silk to yield a hemocompatible surface[2,5]. This study examined the potential of low molecular weight heparin as a material for refining silk properties by acting as a carrier for vascular endothelial growth factor (VEGF) and improving silk hemocompatibility. Heparinized silk showed a controlled VEGF release over 6 days; the released VEGF was bioactive and supported the growth of human endothelial cells. Silk samples were then assessed using a novel humanized hemocompatibility system that employs whole blood and endothelial cells. The overall thrombogenic response for silk was very low and similar to the clinical reference material polytetrafluoroethylene. Despite an initial inflammatory response to silk, apparent as complement and leukocyte activation, the endothelium was maintained in a resting, anticoagulant state. The low thrombogenic response and the ability to control VEGF release support the further development of silk for vascular applications.

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O_6

OSTEOGENIC EFFECT OF BIOACTIVED ELECTROSPINNING SYSTEM ON A BONE CRITICAL DEFECT

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INTRODUCTION

Constructs combining porous 3D scaffold, GFs and cells have been proposed to bone healing. Electrospinning is a relatively simple and cost-effective technique that allows fabricating synthetic as well as natural micro- and nanoscale fiber scaffolds for bone tissue engineering to mimic the native extracellular matrix (ECM) [1]. Electrospun structures typically present a high specific surface area for cell attachment, and porosity for improved cell infiltration and nutrient diffusion [2]. Technical parameters (polymer concentration and molecular weight, flow rate, voltage, distance from the collector) are easily modified for different geometries, structural properties, and application targets [3. The first part of the present study was dedicated to the in vitro characterization of electrospun membranes of PLGA 75:25, its degradation over time, biocompatibility, and promotion of rat mesenchymal stem cells (rMSC) proliferation and differentiation. The objective of the in vivo part was to evaluate the osteogenic effect of a sandwich-like system for implantation in a 8 mm rat calvarial critical-size defect. The system was bioactivated with BMP-2 in microspheres or rMSCs pre-seeded in the electrospun microfiber membranes at varying densities or combinations of both.

RESULTS AND DISCUSSION

Electrospun membranes of 120±10 μm thickness, uniform distribution of fibers (diameter 1.65±0.39μm) and an interconnected porous 3D structure (70±3.9%) were obtained. The contact angle was 117º and dropped to 100º in 30 min. PLGA 75:25 membranes favored environmental conditions for osteogenic differentiation. The *in vivo* analysis showed that the bone formation started on the defect borders and progressed into the center, the void zone being frequently localized in the middle of the defect. The systems with the higher cell density induced a more pronounced progress in growth than the one implanted with fewer cells. However, the 40% of repair achieved after 12 weeks was still less than the 60% obtained in the BMP-2 group. Combinations of rMSCs with BMP-2 did not further enhance the regeneration response obtained with solely BMP-2. In addition, as the system disrupted the microspheres spread and interacted with the tissue, the rest of membranes were progressively degraded and tissue was progressively integrated into the remaining fibers. In conclusion, this study presents good bone defect repair outcomes within 8–12 weeks using an electrospun system combined with BMP-2.

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O_7

FORMULATION OF NANOFIBERS WITH INCORPORATED GROWTH FACTORS AND THEIR EFFECT ON CELL RESPONSE *IN VITRO*

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INTRODUCTION

The ongoing demographic changes bring, beside other health related problems, increased incidence of patients suffering from chronic wounds. Novel materials and therapeutic approaches are therefore needed to achieve effective healing. Polymer nanofibers as a new class of nanostructured materials show high potential as tissue scaffolds, modern wound dressings and new drug delivery systems[1]. They mimic the fibrillar elements of natural extracellular matrix; therefore, their application may induce better and faster tissue regeneration[2]. On the other hand, it is well known from clinical practice that application of various growth factors enhances tissue regeneration[3]. The combination of nanofibers with simultaneous delivery of growth factors is, therefore, expected to exert synergistic effects in wound healing. Fabrication of nanofibers can be performed using electrospinning. It is a modern, versatile, one-step and widely used method, which enables the formation of nanofibers from numerous biocompatible polymers.

The objective of this research was formulation of chitosan-based nanofibers with incorporated blood-derived growth factors and evaluation of *in vitro* cell response upon contact with nanofibers.

RESULTS AND DISCUSSION

In the current study chitosan was selected as a biocompatible, and biodegradable material for nanofiber formation, since it has been widely investigated for various biomedical applications, due to its haemostatic activity, promotion of normal tissue regeneration, bacteriostatic and fungistatic effects[4]. To improve its spinnability chitosan solution was supplemented with polyethylene oxide as a spinnable carrier. The optimization of process and solution parameters enabled preparation of electrospun bead-less chitosan-based nanofibers with incorporated blood-derived growth factors with diameters ranging from ~70 nm to ~90 nm as determined by SEM analysis. Keratinocyte and dermal fibroblasts were seeded on nanofibrillar supports. Evaluation of cell proliferation indirectly revealed that bioactivity of growth factors was preserved in electrospinning process. However, the cell mobility was hindered and cell morphology was changed, depending on concentration of growth factors in the cell culture. To sum up, the results show that chitosan-based nanofibers with incorporated growth factors support cell growth *in vitro*, therefore, showing a good potential for *in vivo* application in management of chronic wounds.

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O_8

DRUG DELIVERY ACROSS BLOOD-BRAIN BARRIER BY MEANS OF INTRAVENOUS ADMINISTRATION OF LIPID NANOPARTICLES

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INTRODUCTION

The blood-brain-barrier (BBB) is a selective barrier that generally restricts the passage of drugs setting serious limitations for CNS pharmacotherapy. Looking at high mortality of malignant gliomas and the lack of efficient treatment, this field is a primary area for new intravenous formulations with BBB crossing ability. The focus of this work was to use nanotechnology in cancer therapy, based on the use of solid lipid nanoparticles (SLN) to assess the suitability of these carriers for anticancer delivery to the brain. Bearing that in mind, novel formulations of SLN loaded with camptothecin (CPT), a potent anticancer candidate, were designed. To assess the suitability of the CPT-loaded SLN for brain drug delivery small and wide angle X-ray scattering (SAXS/WAXS) analyses, cellular internalization studies (flow cell cytometry) and cytotoxicity studies (MTT) in four human glioma cell lines and one human macrophage cell line were performed. Additionally, *in vivo* fluorescence and biodistribution studies of injected rhodamine 123-loaded SLN and CPT-loaded SLN were performed in rats.

RESULTS AND DISCUSSION

Particles with a mean particle size below 200 nm, a homogenous size distribution, high encapsulation efficiency (> 90%) and high stability were developed[1,2,3]. SAXS/WAXS studies suggested favorable interactions of SLN and the cells lipid bilayers[4]. A higher affinity of the SLN to the porcine brain capillary endothelial cells (BCEC) and gliomas was shown in comparison to macrophages[3,5]. Cytotoxicity studies revealed that CPT-loaded SLN induced glioma cell death with the lowest maximal inhibitory concentration (IC50). The mechanism of internalization was found to be mainly through a clathrin-dependent endocytic pathway. In this work it was demonstrated not only the brain targeting ability of SLN coated with polysorbate 60 and 80, but also the superiority of the antitumor activity of CPT-loaded SLN compared with CPT in solution/suspension or in physical mixture with SLN[4,6]. These studies indicate that the CPT-loaded SLN are a promising drug brain delivery system worth to be exploited as a novel formulation for brain tumor therapy.

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O_9

NOVEL VECTOR BASED ON HYALURONIC ACID AND SOLID LIPID NANOPARTICLES FOR GENE THERAPY

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INTRODUCTION

Non-viral gene therapy based on solid lipid nanoparticles (SLNs) is a promising alternative for the treatment of several diseases[1,2]. As non-viral vectors, their main advantage is the safety profile, but the low efficiency of transfection of SLNs makes them to be far from an ideal vector. The incorporation of different components to overpass the main limiting steps for transfection is one of the strategies used for the improvement of their efficency.

In this work, we have designed and evaluated a new vector composed by SLNs, protamine (P) and hyaluronic acid (HA) of different molecular weights. P is a potent DNA condenser that enhances the active transport of DNA to the nucleus and also improves the intranuclear transcription. HA is a biocompatible anionic biopolymer that provides the particles with desirable properties, rendering them non-immunogenic, biocompatible, and biodegradable.

The SLNs were prepared by a solvent emulsification-evaporation technique previously described by del Pozo-Rodríguez et al.[3] .Firstly, a P-DNA complex was prepared. Then, an aqueous solution of HA was added to form the HA-P-DNA complex, that was put in contact with a suspension of previously prepared SLNs.

Once the vectors were characterized, the nanoparticle transfection efficacy of the reporter plasmid pCMS-EGFP and cell viability in HEK-293 cells were studied. Moreover, in order to insight into the mechanisms by through the HA influences the transfection, we studied the cellular uptake and the endocytosis mechanism of the vectors labelling the SLNs with Nile Red, and the intracellular disposition of DNA into the cytoplasm by using EMA-labeled-DNA vectors.

RESULTS AND DISCUSSION

The particle size of the vectors ranged from 240 nm to 340 nm and the surface charge varied from +30 mV to +40 mV. All formulations were able to protect the DNA from the enzymatic degradation and to release it in presence of SDS. The vectors were able to efficiently transfect HEK-293 cells (60% EGFP positive cells at 72 h), without compromising the cell viability. The cellular uptake was about 60% at 8h. The vectors were internalized by both clathrin- and caveoale/lipid raft-mediated endocytosis, regardless of the presence of HA and protamine on the surface of the nanoparticle. Since caveoale/lipid raft-mediated endocytosis is quantitatively much more active than clathrin-mediated endocytosis in HEK-293 cells, to efficiently transfect this cell line, vectors should be designed to once internalized, the transfection does not depend on the lysosomal activity, being this the case of the HA-P-DNA-HA vectors. We have previously shown that when the vector was prepared with protamine and SLNs but without HA, it was unable to transfect this cell line due to high condensation of DNA by the protamine[4]. The intracellular disposition of EMA-labelled DNA indicated that the HA is able to modulate the high degree of condensation of the DNA due to the protamine; this is especially important if the vector is uptaken mainly by caveoale/lipid raft, since optimizing the formulation we can influence on the decondensation of the DNA inside the cell, and therefore, on the transfection rate.

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O_10

ENHANCING THE STABILITY OF GROWTH FACTORS: PLGA VEHICLES ENCAPSULATING HEPARIN BASED NANOCOMPLEX FOR SUSTAINED RELEASE OF BMP-7

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INTRODUCTION

Morphogens are naturally occurring compounds responsible for cellular differentiation. Their function is to convert normal stem cells in differentiated cells of a specific tissue or organ. Bone morphogenetic proteins (BMPs), a group of morphogens, are essential for the normal formation of bone and cartilage. Moreover, BMPs has recently been pointed out as key components in some pathological processes such as cancer[1,2], osteoporosis or osteoarthritis. However, the therapeutic use of these proteins is limited by their high instability and low half life[3]. To overcome this problem, several strategies have been proposed. Some of them make use of heparin, a natural polymer that binds to BMPs protecting them from the environment[4]. In this work, we design a new system that entraps nanocomplexes of BMP-7-heparin-Tetronic® in a PLGA matrix. In this way, a sustained release of the BMP-7 is achieved in its antigenically-active form. This system could have a potential impact in tissue engineering.

RESULTS AND DISCUSSION

Two different vehicles for growth factors were synthesised. Firstly, microparticles of PLGA were prepared following a modified S/O/O emulsification method where the morphogen is introduced as a complex with heparin-Tetronic®. With this strategy sustained release rates for, at least, one month are achieved while preserving the antigenically-active form of the protein. The size and morphology of microparticles were evaluated using scanning electron microscopy. Secondly, nanocomplexes of growth factor-heparin-Tetronic® were also encapsulated in scaffolds of PLGA. These scaffolds were synthesised using a solvent-casting salt-leaching method. Both platforms, microparticles and scaffolds, were used to achieve the differentiation of human mesenchymal stem cells to chondrocytes. The formation of cartilage cells was confirmed by histological analysis and RT-PCR of cartilage specific genes.

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O_11

EFFICIENT INTRACELLULAR DELIVERY OF PLGA NANOPARTICLES CONJUGATED TO γ-PROLINE DERIVED CELL-PENETRATING PEPTIDES FOR THE TREATMENT OF DYSKERATOSIS CONGENITA

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INTRODUCTION

Dyskeratosis Congenita (DC) is a rare, inherited, multi-systemic disorder exhibiting premature ageing, bone marrow failure and increased cancer predisposition; initially described by the mucocutaneous triad (nail dystrophy, oral leukoplakia and abnormal skin pigmentation). There is an association of DC with defective telomerase, leading to the accumulation of short telomeres. Mutations in seven genes encoding components for telomere maintenance have been identified, and are responsible to cause the three different modes of inheritance of the disease[1]. It has been described that the peptide GSE 24-2, corresponding to an internal domain of DCK1 gene (encoding a component of telomerase complex) is able to reactivate the telomerase[2]. Unfortunately, peptides lose their therapeutic activity due to their high susceptibility to degradation[3]. Furthermore, since GSE 24-2 target is localized within the nucleus, it must be adequately internalized into cells and reach the nucleus. Therefore, in this study we propose a method to reduce the mentioned inefficiencies, encapsulating GSE 24-2 into biodegradable poly-lactic-co-glycolic acid nanoparticles (PLGA NPs) conjugated to different γ-proline derived cell-penetrating peptides (CPPs) which display the capacity of cellular and nuclear internalization[4].

RESULTS AND DISCUSSION

The average size of the NP formulations was around 220 nm and, according to the polydispersity index, (PDI < 0.15), all formulations displayed a narrow size distribution. ζ potential values were also measured; showing that all CPPs conjugated NPs resulted in negative surface charge. Besides, the encapsulation efficiencies and surface adsorbed peptide of the NPs were quite variable, depending on the CPP attached. According to the cytotoxic assay, MEF cells maintained a moderate viability level, especially at low concentrations of NPs. The intracellular uptake and the time course of internalization of PLGA NPs bound to the different CPPs were evaluated in HeLa cells, showing that, depending on the CPP, the uptake of the NPs into the nucleus and their internalization time varied. Finally, the bioactivity of GSE 24-2 encapsulated into PLGA NPs was analyzed *in vitro* by the incubation of the peptide with F9 A353V cells. As a result, it was observed that, GSE 24-2 incorporated into PLGA NPs preserves the *in vitro* biological activity, rescuing DNA damage and therefore reactivating the telomerase.

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O_12

A NOVEL STRATEGY FOR THE TREATMENT OF CHRONIC WOUNDS BASED ON THE TOPICAL ADMINISTRATION OF rhEGF LOADED LIPID NANOPARTICLES

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INTRODUCTION

The increasing incidence of suffering chronic wounds represents a major clinical challenge for health care systems worldwide. Chronic wounds are characterised by the persistence of inflammation, the disordered extracellular matrix and a lack of re-epithelialisation[1]. The epidermal growth factor (EGF) plays an important role in tissue remodelation by stimulating dermal and epidermal regeneration and granulation tissue formation. However, the short half-life of rhEGF *in vivo* requires multiple administrations and this restricts its clinical application[2]. To overcome these limitations, in the current work rhEGF loaded Solid lipid Nanoparticles (rhEGF-SLN) and Nanostrucutres Lipid Carries (rhEGF-NLC) were prepared and their efficacy was tested *in vitro* in BALB/c fibrosblast and after their topical administration in a full-thickness excisional wound model in db/db mice.

RESULTS AND DISCUSSION

rhEGF-SLN and rhEGF-NLC were prepared by a simple emulsification-ultrasonication method. Interestingly, the *in vitro* proliferation studies, evidenced that rhEGF-SLN and rhEGF-NLC bioactivity was even higher than free rhEGF, suggesting that nanoencapsulation of rhEGF may promote its affinity to EGF receptors, and/or may enhance nanoformulations cell internalization. Finally, the *in vivo* studies revealed that 4 topical administrations of 20 µg rhEGF-NLC and rhEGF-NLC reduced wound area by days 8 and 11 more than the control groups (untreated control and blank nanoparticles) . Moreover, the re-epithelisation and the resolution of the inflammatory state reached earlier for rhEGF-SLN and rhEGF-NLC groups in comparison with controls. Besides, it should be pointed out that both 20 µg rhEGF-SLN and rhEGF-NLC improved the re-epithelisation even more than 4 doses of 75 µg free rhEGF administrated intralesionally. Overall, we demonstrate the promising effect of rhEGF-SLN and rhEGF-NLC to promote faster and more effective wound healing, and suggest its possible application in chronic wounds treatment.

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O_13

ELABORATION AND EVALUATION OF CBD-LOADED LIPID NANOCAPSULES AS A NEW APPROACH TO BYPASS THE BLOOD-BRAIN BARRIER

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INTRODUCTION

The poor access of the majority of therapeutic agents through the blood-brain barrier (BBB) often limits the effective non-invasive treatment of diseases of the central nervous system (CNS). For this reason, research has been focusing on ways to bypass such a barrier. In this context, the lipid nanocapsules arise as a promising approach to achieve such an ambitious aim, since their small size (ranged from 18 to 70 nm) and their lipophilic nature enable them to carry drugs into the CNS, otherwise unable to go through. The above mentioned nanocarriers, obtained according to an expanded phase inversion-based method, are loaded with cannabidiol (CBD). Phase inversion temperature was determined by conductimetry and drug stability during heating cycles was evaluated in order to establish nanoparticle elaboration conditions. Nanoparticles obtained were then characterized in terms of size and amount of drug entrapped. Afterwards, the colorimetric MTT metabolic activity assay was used to measure cell viability/cytotoxicity of cannabidiol and unloaded lipid nanocapsules on the rat brain RBE4 endothelial cells for 30 min, 3 h and 24 h.

RESULTS AND DISCUSSION

CBD inclusion to the mixture of lipids forming nanocapsules resulted in a slight decrease in the phase inversion temperature (the change in conductivity was detected in the range of 67- 72ºC). After three temperatures cycles from 40 to 90ºC, loss of drug lower than 20% was observed. Three batches of CBD-loaded lipid nanocapsules were obtained, with average size of 22nm, 41nm and 75nm. These diameter values were slightly higher when compared to unloaded nanoparticles, what is in agreement with the expected behavior of CBD, soluble in the oily core (as a proof of it, such an increase in size was higher for those formulations containing higher proportions of the drug). High encapsulation efficiency was obtained in all cases. According to viability *in vitro* results, cannabidiol did not markedly affect RBE4 cell viability in the concentration range of 10-40 µM at the different incubation periods assayed. On the other hand, the treatment of RBE4 cells with unloaded lipid nanocapsules (from 0.1 mg/mL to 10 mg/mL) revealed that cell viability was reduced in a concentration and time-dependent manner. The optimal concentration range for unloaded lipid nanocapsules was established.

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O_14

PREPARATION OF HYALURONIC ACID NANOCAPSULES BY A SELF-EMULSIFYING METHOD FOR CANCER THERAPY

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INTRODUCTION

Nanocapsules (NC) are flexible drug delivery systems with potential applications in several medical conditions such as oncology.[1] Coating of NC with hyaluronic acid (HA) is an attractive approach to achieve active targeting. In addition to its biocompatibility, non toxicity and biodegradability, HA is effectively recognized by CD44 receptor, which is over-expressed in many tumor cell types; consequently, HA-modified nanocarriers can increase the concentration of anticancer drugs at the target site. [2] NC can be produced using different technologies, but nowadays, special attention has been given to self-emulsifying methods that generate NC without requiring organic solvent and heat. Self-emulsification is interesting for the pharmaceutical industry since it does not require management of organic solvents, and it can be scaled-up with ease. Furthermore, the production of NC by this method allows the incorporation of thermolabile drugs. [3, 4] The aim of this study was to formulate HA NC using a self-emulsifying method and to characterize the physicochemical properties of the resulting nanocarrier.

RESULTS AND DISCUSSION

Hyaluronic-acid nanocapsules (HA NC) were prepared by a self-emulsifying method, without the use of solvents, at room temperature and gentle stirring. In a first step an O/W nanoemulsion (NE) was designed and its surfactant concentration and oil/water ratio were optimized. The best NE formulation was obtained by using a medium chain triglyceride and Tween80® as the oily phase and an aqueous phase composed of Solutol HS 15®. HA NC were obtained by the addition of a cationic surfactant to the oil phase and a 50KDa molecular weight HA to the aqueous phase. The optimized formulation presented a particle size below 150 nm and negative zeta potential that confirmed the effective HA coating. HA NC were stable at room temperature for 48h and in PBS 0,02M for 2h. This versatile system offers several sites amenable for drug encapsulation: a core for hydrophobic anticancer agents and a surface for loading hydrophilic drugs such as antibodies. Drug loading studies are underway to investigate the full potential of this system.

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O_15

WELL-DEFINED SYNTHETIC POLYPEPTIDE BASED ARCHITECTURES AS NANOCARRIERS FOR DRUG DELIVERY OR IMAGING PROBES

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INTRODUCTION

A large variety of natural and synthetic polymers have been described as carriers for drug delivery or imaging probes. Nevertheless, only a few have been successfully transferred into patients (mostly poly(ethyleneglycol) (PEG), N-2-Hydroxy-propilmethacrylamide (HPMA) copolymers and poly-L-glutamic acid (PGA) conjugates)[1]. Polyglutamates are highly biocompatible, biodegradable and multifunctional polymers, which have been effectively used as building blocks in polymer drug conjugates and polymeric micelles for various medical applications ranging from cancer[2-4] to ischemic processes.[5,6] Moreover, it is expected its FDA approval after approval of PGA-paclitaxel conjugate, Opaxio™ for the treatment of various cancers alone or in combination (Opaxio™ has been recently designated as orphan drug in combination with radiotherapy for glioblastoma treatment).[1,7,8]

RESULTS AND DISCUSSION

We report the development of synthetic pathways to a plethora functional polyglutamates with well defined structure, adjustable molecular weight and low dispersity (D = Mw/Mn <1.2) applying the ring opening polymerization (ROP) of N-carboxyanhydrides (NCA) with novel initiators.[9]

Furthermore, a number of architectures based on PGA, including stars, grafts, and hybrid diblock copolymers have been designed. In addition, a variety of functionalities such as alkyne, azides, reactive disulphides, protected amines… can be easily introduced by "post-polymerization modification" reactions yielding a set of orthogonal reactive attachment sides[9] suitable for further bioconjugations. Those different structures, after an adequate labelling with fluorescence/NIR probes or/and complexing agents for MRI and/or PET techniques allowed us to study polymer *in vivo* fate (pK and biodistribution) in animal models.

Summarizing, new polymeric libraries with a range of attractive and sometimes unexpected properties result of the contribution of the different architectures can be built, characterized and investigated for different applications in the nanomedicine field. The knowledge of the structure/*in vivo* fate relationships will allow us to regulate the polymer properties to each desired application.

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O_16

PROTAMINE:DEXTRAN NANOPARTICLES FOR CANCER GENE THERAPY

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INTRODUCTION

Gene therapy is considered to be a highly potent tool including the regulation of certain genes implicated in cancer processes. Developing an innovative and successful cancer treatment based on gene therapy requires effective therapeutic genes and efficient, safe and cheap gene delivery systems. Nanoparticles based on the combination of biodegradable polypeptides and polysaccharides may be an interesting approach for the design of new nanomedicines [1]. Nanoparticles can efficiently associate different nucleic acids (pDNA and siRNA) and successfully deliver them to cancer cells. The aim of this work was to develop and characterize protamine:dextran-based nanoparticles, as delivery systems in gene therapy, using plasmid DNA and interference RNA as model molecules.

RESULTS AND DISCUSSION

Protamine:dextran (Pr:Dx) nanoparticles were prepared in very mild conditions by ionic gelation technique [2], which avoids the use of organic solvents and high energy sources. Therefore, this technique is highly recommended for the encapsulation of labile molecules such as nucleic acids. In a first step, different Pr:Dx ratios (8:1, 4:1, 2:1, 1:2, 1:4, 1:8; w/w) were studied in terms of (i) physicochemical properties (ii) yield of reaction and (iii) nucleic acid association efficiency in order to establish the optimal ratio between the components. The selected formulation was Pr:Dx 4:1 due to its high yield of reaction (75%±12), and good association of pDNA and siRNA confirmed by agarose gel electrophoresis and UV spectroscopy (≥90%). The mean particle size of the system was below 200 nm and it presented positive zeta potential. TEM images showed homogenous populations of spherical nanostructures. The nanoparticles presented adequate stability in cell culture medium and simulated physiological conditions.

In vitro studies were carried out in U87MG cancer cell line. Cellular uptake studies were performed by confocal microscopy and showed that these nanoparticles have the capacity to interact and be efficiently internalized by cells. Cell proliferation was evaluated by the colorimetric MTT assay, which confirmed low/non-toxicity of the nanoparticles. The hemolytic capacity of the system was also studied and no hemolytic effect was detected after the incubation of nanoparticles with red blood cells. Additionally, transfected cells with pDNA encoding GFP-F were directly examined under a fluorescence microscope.

Results evidenced the promise of these nanocarriers in gene therapy. In conclusion, the developed nanosystem shows potential for cancer gene therapy and therefore, subsequent studies will be carried out in the near future to explore their activity in tumour-bearing animal models.

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O_17

PEGYLATED NANOPARTICLES FOR ORAL DELIVERY OF CAMPTOTHECIN

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INTRODUCTION

Camptothecin (CPT) is a Topoisomerase I inhibitor that was first isolated from the bark of *Camptotheca acuminata* in 1966. Although it shows powerful anticancer activity, it has great limitations such as poor solubility and stability [1]. When administered orally, camptothecin and its analogues display a high variability and a low bioavailability due to their physicochemical characteristics and their presystemic metabolism (P-glycoprotein and Cytochrome P450) [2].

Gantrez® AN is a copolymer of methyl vinyl ether and maleic anhydride (PVM/MA) that shows low toxicity and excellent biocompatibility. Polyethyleneglicols (PEGs) are hydrophilic polymers. Pegylation can provide nanoparticle's surface with slippery properties and, thus, the resulting carriers would be capable to cross the mucus layer [3]. Interestingly, PEGs appears to inhibit the intestinal P-gp and the CYP isoenzyme 3A4 [4]. Taking all this into account, it is reasonable to think that pegylated polymeric nanoparticles can be suitable carriers to enhance the oral bioavailability of camptothecin. These pegylated nanoparticles would conduct the loaded drug to the surface of the enterocytes in which their cargo would be released. Then, the presence of the PEG would inhibit the activity of both P-gp and cythocrome P450 complex allowing the absorption of the anticancer drug.

RESULTS AND DISCUSSION

Nanoparticles were prepared by a solvent displacement method and subsequent lyophilization. The resulting carriers displayed spherical shape, negative zeta potential, and a mean size of 195 nm. The mean amount of CPT loaded in the nanoparticles was close to 10 μg/mg and the encapsulation efficiency was around 25 %. The *in vitro* release profile of the formulation exhibited a biphasic pattern, characterized by an initial non release period when the nanoparticles were incubated in the SGF followed by a rapid release of about 90% of the loaded drug when the nanoparticles were dispersed in the SIF. The release then is complete after 14 hours of incubation.

Pharmacokinetic studies were carried out in male Wistar rats. All the animals received a single oral dose of 1mg/kg of CPT, either encapsulated in nanoparticles or as a suspension. In addition, an intravenous CPT suspension was administrated at the same dose. The amount of CPT in plasma was quantified by HPLC. Results showed that the bioavailability of the encapsulated CPT was more than 6 fold higher than the bioavailability of the oral suspension of the drug.

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O_18

LIPID NANOPARTICLES OF AN ANTITUMOR ALKYL-LYSOPHOSPHOLIPID EDELFOSINE AS A NOVEL ANTILEUKEMIA TREATMENT

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INTRODUCTION

Cancer is one of the leading causes of death worldwide and leukemia represents 3% of all cancer cases[1]. Although there have been new advances in the treatment of these disease, current antitumor therapy is not always effective and relapses are frequent. Edelfosine (ET) is a selective antitumor against cancer cells[2]. Moreover, edelfosine has shown potent anticancer *in vitro* activity against several cancer cell lines. However, despite its high *in vitro* efficacy, edelfosine present *in vivo* side effects such as haemolysis when it is administered intravenously and gastrointestinal toxicity when it is administered by the oral route. These disadvantages led us to the vehiculization of the drug using nanotechnology. Edelfosine was encapsulated into lipid nanoparticles (LN) that are constituted by biodegradable lipids that are solid at room and body temperature[3]. The manufacturing process consists on a hot homogenization method that avoids the use of organic solvents[4]. This work evaluates the efficacy of lipid nanoparticles containing edelfosine (ET-LN) in treating leukemia[5]; gives further insight into lipid nanoparticles oral absorption and evaluates the *in vivo* toxicity profile of the vehicle and the treatments (free and encapsulated ET).

RESULTS AND DISCUSSION

Lipid nanoparticles produced by the hot homogenization method consisting on high shear homogenization and ultrasonication provided homogenous spherical nanoparticles with an average size around 100 nm suitable for intraperitoneal, intravenous and oral administration. PDI index was less than 0.3 in all cases and LN charge was negative and enough to maintain nanoparticle stability. Nanoencapsulated edelfosine preserved the potent apoptotic effect that free ET has in sensitive leukemia cells; moreover, only ET-LN were able to induce apoptosis in resistant leukemia cells. Additionally, *in vivo* studies showed that ET-LN were able to protect animals from gastrointestinal toxicity. Presently, efficacy of ET-LN is being evaluated in an *in vivo* xenogeneic model of human ALL in immune-deficient mice.

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O_19

DESIGN AND CHARACTERIZATION OF NOVEL NANOSYSTEMS FOR THE CO-ENCAPSULATION OF PEPTIDES AND NUCLEIC ACIDS

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INTRODUCTION

Despite the immune system is traditionally considered to act as a barrier against the formation and progression of tumors [1], recent experimental work shows that tumors are able to recruit certain immune cells from immunocompetent individuals to generate an immunosuppressive environment which allows their spread. Among these immune cells, one kind of partly differentiated myeloid progenitors known as "myeloid-derived suppressor cells" (MDSCs), has been identified as an important population [2].

In this work, we propose the use of gene medicines capable of promoting a differentiation of these MDSCs towards non-immunosuppressive phenotypes, with the aim of rescuing the immune response against the tumor. With that purpose, we have designed a nanocapsular system capable of co-encapsulating two different drugs: the CCL2 chemokine in the core, used as chemoattractant of immune cells [3], and a miR sequence capable of differentiating the MDSCs, complexed onto its surface. It can be hypothesised that the combination of these two biomolecules within the same platform could improve the targeting of the genetic therapy towards the myeloid cells, due to the chemotactic effect of the chemokine. Besides, the nanostructured nature of the platform may favor the accumulation of the treatment in the spleen, where most part of the MDSCs population remains.

RESULTS AND DISCUSSION

Polyarginine nanocapsules with a specific composition were developed for the appropriate encapsulation of CCL2. On the other hand, the therapeutic miR was successfully adsorbed onto their surface as demonstrated by agarose gel electrophoresis and UV absorption. Imaging and microstructural analysis of the nanocapsules has been performed. In addition, a transwell migration assay with RAW 264.7 macrophages [4] has been adapted in order to evaluate the functionality of the encapsulated CCL2.

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O_20

FORMULATION OF ALGINATE COATED POLOXAMER 407 PARTICLES FOR THE TREATMENT OF VULVOVAGINAL CANDIDIASIS

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INTRODUCTION

Vulvovaginal candidosis is estimated to be the second most common cause of vaginitis after bacterial vaginosis. Up to 75% of all women suffer at least one episode of this infection during their lifetime. Most patients with *Candida* vaginitis respond to topical treatment with nystatin (NYS) or imidazoles. However excessive use of azole antifungal drugs has increased the fungi resistances, and non-*C. albicans* related disease is less likely to respond to azole therapy. Moreover, azoles have a fungistatic effect, whereas Nystatine has both an antifungal and fungistatic activity1. NYS has been found to possess broader spectrum of activity than the former drugs towards fungi in *Candida* vaginitis too. For these reasons a special attention has been focused on the development of controlled release drug delivery systems to provide a long term therapeutic concentration of NYS following the application of a single dose in vagina2. In this sense, the use of microparticles have some advantages3. Thus based on these considerations, the aims of this study were: (*i*) to develop a NYS loaded microcapsules by complex coacervation coated with poloxamer 407 for the vaginal administration of nystatin, (*ii*) to characterize and to determine the antifungal activity of the Nys loaded microcapsules against a strain of *C. albicans*.

RESULT AND DISCUSSION

Uncoated alginate and poloxamer 407 coated microparticles (PCM) were successfully prepared by an emulsification/internal gelation method. Size of the systems increased with the incorporation of the drug, but not after storage periods except for NYS loaded PCM. These microparticulate systems showed spherical shape and slightly rough surface.PCM displayed some shape irregularities due to the sample preparation method for SEM study. Optimal values of percentage yield, loading capacity and encapsulation efficiency were obtained. Equally, release studies gave a good fit to first order kinetic model indicating that NYS release from microcapsules followed a concentration gradient pattern, based on the first Fick's law where the released amounts are directly proportional to the amounts remaining into the dosage form offering sustained release of drug. The ability of these systems, to adhere to the vaginal mucosa has great appeal for the treatment of localized infection. Moreover, NYS loaded microparticles exhibited a clear inhibition effect on the *C. albicans* growth, suggesting their clinical potential use, once assured the security of the treatment by the permeation studies performed.

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O_21

PLGA MICROPARTICLES CARRYING VEGF: PREPARATION AND EFFICACY STUDIES IN COMBINATION WITH CoQ_{10} PLGA NANOPARTICLES IN AN ANIMAL MODEL OF MYOCARDIAL ISCHEMIA

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INTRODUCTION

Myocardial ischemia is one of the leading causes of death worldwide[1]. VEGF therapy, with a proved angiogenic activity, has been investigated as a promising strategy to overcome ischemia and its consequences. Nevertheless, clinical trials involving VEGF suggested that incorporation of this cytokine into delivery systems could be an optimal way to face limitations of protein therapy[2,] In a previous study, our group demonstrated the efficacy of VEGF polymeric microparticles to promote neovascularization in an animal model of myocardial ischemia[3].
On the other hand, antioxidant agents are gaining relevance in pathologies that are mediated by oxidative stress, including heart ischemia[4]. CoQ_{10}, also named ubiquinone, poses a unique role in the electron transport chain and thus seems to be a good candidate. However new delivery strategies to increase its bioavailability are need, since it shows a high lipophilic nature[5]. Also it is necessary to establish the role of this antioxidant in the heart damage arena, since very few studies have been done to the date.

RESULTS AND DISCUSSION

VEGF stealth microparticles (VEGF-MP) and CoQ_{10} nanoparticles (CoQ_{10}-NP) have been prepared and tested in an animal model of myocardial ischemia. VEGF-MP (intramyocardial) and CoQ_{10}-NP (oral) were administered to female Sprague Dawley rats. 40 animals were divided into 8 different groups and the efficacy of the treatments was studied both separately and in combination, including also appropriate controls. Cardiac function was followed up by measuring ejection fraction before and after three months of therapy. Also animal hearts were analyzed by immunofluorescence technique.
Results of this study show that both the treatment with VEGF-MP and with CoQ_{10}-NP is effective when given separately. They are able to statistically improve cardiac function and to promote neovascularization after three months. On the other hand, the co-administration of the treatments did show neither synergistic effects nor benefits when compared to control groups. Immunohistology demonstrated a reduction in the formation of new vessels in this group.
In conclusion, VEGF-MP and CoQ10-NP can be considered as promising strategies to help tissue regeneration after cardiac ischemia. Combination of both agents needs to be studied in depth to establish a possible counteraction.

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O_22

VIABILITY OF *Lactobacillus F*ermentum CECT 5716 ENCAPSULATED IN GELATIN AND GATRO-RESISTANT CAPSULES

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INTRODUCTION

The intestinal microbiota plays an important role in human health as they contribute to inhibiting pathogen colonization, boosting the immune system, and metabolizing nutrients[1]. On the one hand, different factors like diet, antibiotics, and stress are reported to negatively influence bacterial population in human gastrointestinal tract. On the other hand, several conditions influence the bacterial survival during technological processes and the gastrointestinal transit.

The currently accepted definition of probiotics requires that the bacteria maintain its viability from production to consumption[2]. Consequently, the industry demands technologies ensuring probiotic stability for both economical and health reasons.

In this sense, the encapsulation of probiotics in capsules may provide an approach for protecting probiotics. Thus based on these considerations, the goal of the present study was to evaluate the survival of *lactobacillus fermentum* CECT 5716 stored into gelatin and gastro-resistant capsules during a period of 3 months at room temperature (RT).

RESULT AND DISCUSSION

The gastro-resistant and gelatin capsules were dosed aseptically by means of Capsunorm®, with a cell density of about 10^9 colony forming units (CFU). $2.7x10^9$ $5.7x10^8$ CFU/conventional capsule and $1x10^9 \pm 3{,}5x10^8$ CFU/gastro-resistant capsule, respectively. The capsules were placed in flasks containing silica-gel desiccants and stored at RT under darkness. Technological properties (mass uniformity, content uniformity and disintegration) were determined at time cero. The results show that gelatin capsules fulfill all the experiments, meanwhile gastro-resistant capsules release their content before 1 hour at gastric pH due to the opening of the capsules. The experiment was repeated using discs and without the discs. The tightness of capsule was tested prior to performing the assays. In both cases the capsule is opened before 1 hour.

The viability was also evaluated throughout a period of 3 months. The number of encapsulated cells remained relatively constant after this period in all of the cases. No significant differences ($p>0.05$) were found between samples at time cero and after 3 months. It means that both capsules are able to maintain a therapeutical level of bacteria (10^9 CFU) during the storage. However, future works are aimed at studying the functionality of the encapsulated probiotic and whether gastro-resistant capsules improve their survival through gastrointestinal tract. To this end, and to test the stability of the capsules at gastric pH, dissolution test will be carried out. Longer stability studies will be also made to elucidate if viability remains in the therapeutical level at least during 1 year.

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O_23

INFLUENCE OF COMPRITOL ON THE CONTROLLED RELEASE OF DEXKETOPROFEN TROMETAMOL FROM MATRICES CONTAINING POLYESTERAMIDE PADAS.

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INTRODUCTION

Polyesteramide PADAS poly (L-alanine-dodecanediol-L-alanine-sebacic) has been technologically characterized in previous studies (1) and applied as an inert matrix forming agent in tablets containing AINEs, demonstrating its ability to retain and control the release of ketoprofen and diclofenac sodium in different formulations (2, 3). However, for similar formulations containing dexketoprofen trometamol (DK-T), the polymeric matrix produced conventional release profiles (4). In order to prevent this drawback, this work has been designed to study the addition of a lipidic component (glyceryl behenate), Compritol 888 ato (C), to the matrix tablets (PADAS+ DX-T), representing respectively 3, 15, and 33% of the tablet weight. The influence of the amount of C on the release profile and the thermal treatment of 80ºC/30 min that the tablets were submitted to were assessed as a device to influence the porous matrix structure and, therefore, the release behavior of DK-T. The interaction between all tablet components was studied using differential scanning calorimetry (DSC), and environmental scanning electron microscopy (ESEM) was used to show pictures of the tablets before and after the dissolution assay.

RESULTS AND DISCUSSION

The study of the release profiles of DK-T showed the amount of drug released from matrix system. It has been observed that the presence of lipidic C improved drug retention, increasing the t_{80} value from 6 h (tablets without any lipids) to 12 and 16 h in tablets with C. Those tablets which were thermally treated experienced a higher retention of DK-T that the same formulations without this treatment. The obtained results showed that the models that best fit the formulations without thermal treatment followed the Higuchi and Korsmeyer-Peppas equations. On the other hand, the thermal treatment produced significant differences in the release rate between those formulations contained 3% of C (75,75% of the drug at 24 h) and those with a higher concentration (15 and 33 %) which release 56,24 % of the drug following Higuchi kinetics with a *n* value of 0.4 (Korsmeyer-Peppas equation). The two latter have an identic dissolution profile, suggesting that from a certain amount of C, this factor does not modify the release rate. Tablet components do not experience any interactions produced by the thermal treatment, as can be seen with the analysis by DSC.

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O_24

MULTIPARTICULATE CONTROLLED DELIVERY SYSTEMS OF TOLCAPONE FOR PARKINSON´S DISEASE

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease which affects 1% of the population older than 60 years (1). Tolcapone (TC) is a selective and reversible catechol-O-methyltransferase inhibitor that is used as adjunctive therapy to levodopa and a peripheral dopa-decarboxylase inhibitor in the treatment of PD (2). TC improves the pharmacokinetics of levodopa and increases the concentration of dopamine (3). Oral bioavailability of TC is 65% with a short elimination half-life (2 hrs) (4). In this study we develop two types of controlled release systems: microparticles (MPs) and nanoparticles (NPs). MPs were designed to achieve controlled release of TC for as long as possible. On the other hand, as the blood-brain barrier (BBB) plays an important role limiting strategies of therapy, NPs were developed to cross the BBB. MPs and NPs were prepared using poly D, L-lactic-co-gycolic acid resomers (PLGA 502®) and prepared by the solvent evaporation technique from an O/W emulsion for MPs and nanoprecipitation for NPs. The ratios of TC:PLGA used were 1:10 and 2:10 for MPs, and 0.5:10 and 1:10 for NPs. Particles were characterized by SEM, encapsulation efficacy and in vitro release at pH 7,4. Quantification of TC was performed by HPLC and spectrophotometry at 286 nm.

RESULTS AND DISCUSSION

SEM revealed that MP and NP were spherical and with smooth surfaces. Mean particle sizes were around 50 µm for MPs and 275 nm for the NPs prepared with a 0.5:10 TP:PLGA ratio. A 1:10 TC:PLGA ratio was not possible for the preparation of NPs. Encapsulation efficacy was higher than 70% for both systems. In vitro release profiles obtained from TC-MPs showed initial burst releases (1h) of 7.5% and 18% for MPs prepared with 1:10 and 2:10 ratios, respectively. A better controlled release of TC was obtained with the MPs prepared with a 2:10 ratio. In vitro release profiles obtained from TC-NPs showed an initial burst release higher than 20%. Thereafter, two release kinetics were observed. The first slower kinetics took place over 23 days, releasing 70% of drug. The second faster kinetics occurred until 96% of the drug was released from the system.

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O_25

ENCAPSULATION OF RESVERATROL IN POLYMERIC NANOPARTICLES TO IMPROVE ITS ORAL BIOAVAILABILITY

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INTRODUCTION

Resveratrol (3,5,4'-trihydroxystilbene), a naturally occurring polyphenol, has attracted considerable interest for its potential benefits for human health, which include anti-oxidant, anti-inflammatory, cardioprotective and anti-tumor activities [1, 2]. However, the in vivo biological effects of resveratrol appear strongly limited by its short biological half-life, labile properties and very low bioavailability, which hamper the development of therapeutic applications. This low oral bioavailability of resveratrol would be mainly due to its low aqueous solubility and is chemical unstable, being rapidly and extensively metabolized and excreted [2].

In order to overcome this drawback, one possible strategy may be the use of nanoparticles capable to carry the loaded drug till the surface of the mucosa and, once in it, control its release.

The aim of this work was to explore the potential of food protein nanoparticles as carriers for the oral delivery of resveratrol. For this purpose two types of nanoparticles were used: casein (CS-NP) and zein nanoparticles (Z-NP).

RESULTS AND DISCUSSION

Nanoparticles were prepared by coacervation and subsequent desiccation by Spray-drying technique. The resulting carriers displayed, in all cases, a mean size of about 300 nm, spherical shape and negative zeta potential. The amount of resveratrol incorporated in these nanoparticles was dependent on the food protein used and ranged from 24 µg/mg CS-NP to 80 µg/mg Z-NP. However, in both cases, the encapsulation efficiency was closed to 80 %. The in vitro release profiles of resveratrol from CS-NP and Z-NP exhibited a gastroresistant behavior and when nanoparticles were incubated in an intestinal simulated fluid, resveratrol was rapidly released.

Pharmacokinetic studies were carried out in male Wistar rats. All the animals received a single oral dose of 10 mg/kg resveratrol, either encapsulated in nanoparticles or as PEG 400: water solution. In addition, an intravenous resveratrol solution was administrated at the same dose. The amount of resveratrol in plasma was performed by High-resolution liquid chromatography. Results showed an increase in the plasma levels of resveratrol when encapsulated in polymeric nanoparticles, compared to the free antioxidant. Additionally, the relative oral bioavailability of resveratrol delivered in nanoparticles was calculated to be around 9% and 35% for CS-NP and Z-NP, respectively. In all cases, these values were 13-40 fold higher (on average) than the bioavailability estimated for the oral solution of the antioxidant (Fr=1%).

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O_26

EVALUATION OF MESOPORUS SILICON PARTICLES: STUDIES IN VIVO WITH INSULIN

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INTRODUCTION

Mesoporous silicon particles have proven to be an interesting delivery system for drugs (1). They present important advantages as long term stability at low pH and large specific surface area (>300 m^2/g), that make them a suitable carrier for proteins as they can load them by adsorption while protecting their structure into its small pores (2-50 nm). Some types of mesoporous silicon particles have already demonstrated biocompatibility and biodegradability (2). Nevertheless, these properties are not common to all types, as their properties rely on the particle structure (3). Actually, there are many sources of this kind of material. In our study, we focused on the material produced by EM-Silicon (MPS). Previous work on it showed the ability of easily loading high percentages of proteins (4). The objective of this part of the project was to test the performance of the particles loaded with insulin to ascertain if the protein maintains the activity in vivo and if the particles were able to enhance the oral absorption.

RESULTS AND DISCUSSION

Diabetic make rats were administered the particles, after an ip dose of glucose (1g/kg). Blood sampling was maintained for 8h, and glycaemia measured in plasma by BCA™ Protein Assay 23225, Thermo Scientific®. Four groups of animal were used: a) control; b) oral administration; c) ip administration; as blank MPS. Animals of b) and c) group received MPS loaded with insulin. The area under the curve (AUC) was estimated by the trapezoidal rule. The means of the two control groups (a and d) were compared by a t-Student test and no differences were demonstrated (α=0.05), so that they were considered as a unique control group. Considering this results, it can be concluded that the particles did not affect the glycaemia by themselves. Thus, the three groups were compared by one way ANOVA and the post-hoc Dunett D3 test, by means of SSPS v. 19®. Important features were noticed. The ip administration produces a significant effect, demonstrating that the insulin remains active into the MPS. Nevertheless, further studies on stability should be developed. Considering the oral group, even though it has a tendency to recover basal glycaemia faster than the control one, it did not show statistical differences. This result leads to the conclusion that no effect is attained by this route. Taking into account that the MSP show a good acidic resistance, it can be concluded that nor the particles nor the insulin are able to be absorbed into the enterocytes. The distinction between both possibilities would also lead to a better understanding of the biological interaction of mesoporous silica.
In summary, although more studies are needed, the MSP (produced by EM Silicon Nanotechnologies) particles can be considered as an interesting carrier for protein delivery as they constitute a system of protection against degradation. Results presented here do not suggest that these particles can permeate through the intestinal wall.

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O_27

INACTIVATION OF ENCAPSULATED CELLS AND THEIR THERAPEUTIC EFFECTS BY MEANS OF TK-GFP-LUCIFERASE PLASMID

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INTRODUCTION

Cell microencapsulation comprises the immunoisolation of non-autologus cells to supply the lack of functional organs or target the sustained delivery of therapeutic factors. Nowadays, biosecurity of these biosystems is clearly one of the prime concerns among scientist in this field. The possibility to inactivate the implant once the therapy reaches its final goal and/or in case of undesirable deleterious effects represents an attractive and necessary feature for cell microencapsulation to be included. Recently, we proposed TGL triple reporter gene, which codifies for green fluorescence protein (GFP), Firefly Luciferase and Herpes Simplex virus type 1 thymidine-kinase (HSV1-TK), as a promising alternative for such aim.[1,2] However, its efficacy to control the therapeutic effects of enclosed cells has not been assessed yet. GCV mediated apoptosis has been well described for cells plated onto 2D surfaces, emphasizing its dependence on cell proliferation and defining in detail the time intervals required for each phase.[3] Nevertheless, due to the remarkably different behaviour shown by cells within three-dimensional scaffolds, such inactivation process must be re-characterized in order to understand and validate its use within these platforms. The aim of this work is to confirm the inclusion of TGL triple reporter gene as a suitable strategy to inactivate encapsulated cells and their therapeutic effect, following a comprehensive characterization of this process both in vitro and in vivo.

RESULTS AND DISCUSSION

Myoblasts genetically engineered to secrete erythropoietin (EPO) were retroviraly transduced with the $SFG_{NES}TGL$ plasmid to further characterize their ganciclovir (GCV)-mediated inactivation process. GCV sensitivity of encapsulated cells was 100-fold lower when compared to 2D-plated cells. However, the number of cells per capsule and EPO secretion decayed to less than 15% at the same time that proliferation was arrested after 14 days of GCV treatment in vitro. In vivo, ten days of GCV treatment was enough to restore the increased hematocrit levels of mice implanted with encapsulated TGL-expressing and EPO-secreting cells. Altogether, these results show that TGL triple-fusion reporter gene may be a good starting point in the search of a suitable biosafety strategy to inactivate encapsulated cells and their therapeutic effects.

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O_28

ENHANCE OF HYALURONIC ACID RELEASE: DEVELOPMENT OF THE TRANSFORMATION OF LIPOSOMES INTO PLANAR LIPID BILAYERS

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INTRODUCTION

Liposomes are colloidal vesicles formed basically by phospholipids. Liposomes are capable to encapsulate hydrophilic molecules in their internal aqueous space as well as lipophilic and amphiphilic molecules embedded into the phospholipid bilayers[1]. The use of liposomes as drug delivery systems has been developed for many years. At present several commercial formulations are available and, hence its potential rediscovered. Specifically, transdermal delivery of drugs has shown to reduce the skin main barrier: the stratum corneum[2].

Although a large number of transdermal studies have used liposomes with a variety of therapeutic agents, there is no experimental evidence explaining how the liposomes release the encapsulated drugs and which underlying mechanism can be related with the carrier structure[3].

Hyaluronic acid is present in the intercellular matrix of most vertebrate connective tissues, especially in the skin. Its biocompatibility facilitates its use in medical and pharmaceuticals applications, as a supplemental for fluid arthritis patients and regeneration of surgical wounds[4].

We have used the concept of supported lipid bilayer systems[5] to enhance hyaluronic acid release by promoting the transformation of liposomes into planar structures onto the skin. In this presentation we show how different formulations of elastic liposomes have been prepared with the aim of enhancing skin drug delivery through the formation of planar lipid bilayers. These formulations were formed from LUVs loaded with hyaluronic acid and small amounts of various enhancers like Tween 65®, Tween 80® and Transcutol®. The formation of planar structures on the clean skin surface was confirmed by Atomic Force Microscopy (AFM).

RESULTS AND DISCUSION

Understanding the kinetics of drug release is a prerequisite to improve or design topical drug delivery formulations. In this study, we prepared liposomes containing hyaluronic acid with different surfactants. These surfactants were incorporated to the liposomes with the aim to slightly destabilize the lipid membrane and to promote the formation of supported lipid bilayer systems when spread onto the skin surface. To verify the formation of these supported planar bilayers on the skin, AFM images with and without liposomes were achieved. Although liposomes spread reasonably well on the skin, the addition of the surfactants facilitated the formation of the lipid planar structures.

According with the results from the release study the incorporation of surfactants to the liposomes promotes a sustained release of the hyaluronic acid to the receptor media. The highest percentages of hyaluronic acid delivered were obtained from the formulation with Transcutol® with 84 %. The values obtained were fitted to different kinetic models. Best results were found fitting the hyaluronic acid release to a Korsmeyer-Peppas kinetic model. Because the release exponent values were below 0.43, diffusion process was the principal mechanism of drug release from our formulations.

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O_29

A METABOLOMICS PERSPECTIVE OF CONTROLLED DRUG DELIVERY

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INTRODUCTION

Metabolomics encompasses the systematic study of metabolic profiles corresponding to the different cellular processes taking place at a given time1. Metabolites are the end products of all physiological transformations and offer a snapshot of the state of a biological system. In preclinical and clinical studies, metabolomics provides information about key factors, including those related to the toxicity or mode-of-action of a given treatment.

Nuclear Magnetic Resonance (NMR)-based metabolomics has proven a fast, effective, nondestructive method of obtaining good quality structural and quantitative information about metabolic processes.2 With this technique, a great variety of samples can be analyzed, including biological fluids, tissues and cultured cells, allowing the study of the metabolic profile of samples from *in vitro* as well as *in vivo* models.

In this study, a HPMA conjugate of doxorubicin for the treatment of breast cancer3 has been chosen as a model system to prove the potential of metabolomics for characterizing the effect of controlled drug delivery. To this end, the metabolic fingerprint of samples from cell cultures and animal models after drug-treatment was determined by NMR.

RESULTS AND DISCUSSION

Characteristic metabolic profiles were associated with the treatment of the conjugate and the free drug in *in vivo* and *in vitro* preclinical models. Metabolite levels could be related to key parameters such as efficacy, toxicity and mode of action, and associated with the different forms of drug release.

Results show that NMR-based metabolomics is a powerful tool for studying controlled drug delivery. Together with gene expression data and proteomic analyses it could be implemented on a regular basis for the evaluation of drug therapies, and especially for the development of new drug delivery systems on a preclinical stage.

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O_30

IMPROVING THE PRE-CLINICAL TO CLINICAL TRANSLATABILITY OF NANOMEDICINES: RE-INVESTIGATING THE EPR EFFECT ACROSS SOLID TUMOURS

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INTRODUCTION

Many nanomedicinal anti-cancer drug formulations have shown substantial therapeutic gains in pre-clinical models, but these successes have not translated to significant improvements in therapeutic effect in the clinic1. Nanomedicines passively accumulate in solid tumours via the enhanced permeability and retention (EPR) effect2; however, it has been recognized that the EPR effect is highly variable in solid tumours in the clinic3, and may be heavily influenced by the tumour microenvironment. Typical pre-clinical tumour xenograft models do not accurately recapitulate the complexities of human disease, and as such, may lead to an over-simplification and over-estimation of the EPR effect compared to the clinical situation. The distribution and density of the tumour vasculature (CD31), stroma (alpha-smooth muscle actin), lymphatics (LYVE-1), and level of immune infiltrate (CD68 or F4/80) are postulated to be key contributors to the EPR effect in human solid tumours; we will study the effects of these features on the intratumoural distribution of a liposomal formulation. We aim to use clinically relevant patient-derived tumour explant (PTX) models to improve our understanding of the EPR effect to identify whether certain solid tumour phenotypes are more (or less) amenable to treatment with nanomedicines, and compare these to typical xenograft models that are often used to test the pre-clinical efficacy of nanomedicines.

RESULTS AND DISCUSSION

Our tumour characterisation data have revealed substantial differences in the key EPR-related structural features between various human clinical tumour types (and subtypes), typical pre-clinical xenograft tumours, and pre-clinical PTX tumours. Compared to typical xenografts, PTX tumours more closely recapitulated the complexities of the clinical tumour samples. Preliminary work has been completed in typical xenografts and relevant PTX models, and the intratumoural distribution of liposomes that was predicted for each model based on the tumour characterisation studies showed a strong correlation with the distribution we observed *in vivo*. We will present further analysis of different tumour models, assessing whether clinical tumour phenotypes are recapitulated by pre-clinical disease models, and how the key EPR-related structural features influence the intratumoural distribution of liposomes. Understanding how key tumour features affect the intratumoural distribution and therapeutic effect of drug delivery systems is required to identify more relevant pre-clinical models for the evaluation of nanomedicines for clinical use and to improve their clinical translatability.

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ABSTRACTS OF POSTER PRESENTATION ONLY

P_1

LIPID NANOCAPSULES: PHYSICAL CHARACTERISATION AND EVALUATION OF THE ROLE PLAYED BY DIFFERENT FACTORS INVOLVED IN THEIR FORMULATION

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INTRODUCTION

Lipid nanocapsules have been claimed to represent a promising platform for the controlled delivery of lipophilic drugs, enabling them, by means of their efficient encapsulation, to be administered intravenously. Moreover, these systems overcome some drawbacks of former nanocarriers, since they are, unlike any other nano-cargo, obtained according to a solvent-free process and have both a higher drug loading capacity and a longer term stability due to their inner structure, when compared to liposomes. The lipid nanocapsules, prepared by an expanded phase inversion-based method, are composed of a liquid core surrounded by a rigid shell of surfactants. The aim of the present study is to evaluate the influence, on the physical characteristics of these systems, of factors involved in their formulation as their composition and the temperature cycling. Furthermore, the reproducibility of the technique and the aptness to freeze-drying are also assessed. Lipid nanocapsules are characterized in terms of size, by dynamic light scattering, and are observed both by atomic force microscopy (AFM) and transmission electronic microscopy (TEM).

RESULTS AND DISCUSSION

Varying constituents ratio lipid nanocapsules were obtained ranged in size from 18 nm to 90 nm with narrow size distributions. These nanocapsules were observed by both AFM and TEM, obtaining the best results by TEM when 1% phosphotungstic acid was used as dye, instead of uranyl acetate, and without using p-formaldehyde as fixer. By increasing the amount of hydrophilic surfactant, the nanocapsules average diameter is strongly decreased; on the contrary, by increasing the amount of lipid, so does the average diameter. The temperature cycling did not prove to expand the feasibility domain of the *classical* phase-inversion-temperature method. In all cases, reproducibility was insured, being that higher, the smaller the size. A comparative study of freeze-drying conditions revealed that the sample characteristics were preserved in the absence of any additive, hence the use trehalose as cryoprotectant agent does not involve any significant advantage.

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P_2

Optimization of quantitative tools to study cellular fate of Polymer Therapeutics

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INTRODUCTION

The full understanding of polymer conjugate pharmacokinetics and fate at cellular level is crucial in order to allow progress from bench to bedside. These macromolecules can interact with the cellular membrane allowing entry into the cell by endocytosis. In this process most nacoconjugates are transported through the endosomes, which are able to sort their content towards different destinations based on its intrinsic characteristics[1]. Classically, the intracellular trafficking of polymer therapeutics has been carried out qualitatively, mainly, by flow cytometry and confocal fluorescence microscopy. However, quantitative methodology is now needed to achieve the complete knowledge of the therapeutic used in specific cell models. Not only cell uptake and distribution is required but also the percentage of conjugate uptake in key organelles is highly desired to fully understand the internalization(s) mechanism used and consequently the most suitable application for the studied conjugate[2,3]. For this reason, we have been working towards the optimization of semi-quantitative and more importantly, quantitative techniques that could clearly complement the traditional knowledge on conjugate trafficking and that will allow moving a step further on the design of more advanced polymer conjugates. Summarising, the goal of the present work was to establish a battery of validated techniques (from qualitative to quantitative methods) to be used in order to fully understand conjugate cellular fate even up to subcellular level. Poly-L-glutamic acid (PGA) conjugates, HPMA copolymers and human breast cancer cells MCF-7 were chosen as model.

RESULTS AND DISCUSSION

Classical approaches to single-cell analysis have been used to determine the cellular trafficking of HPMA copolymer-oregon green (OG) and PGA conjugates in MCF-7 cells. In addition, semi/quantitative techniques as multispectral image-in-flow cytometry (MsIFC) and subcellular fractionation (SCF) have been established and optimised in order to improve systems' characterisation and to validate the more traditional approaches. *MsIFC* combines the features of conventional flow cytometry and fluorescence microscopy allowing analyzed the polymer cellular uptake and more interestingly, to quantify its intracellular localisation. Lysosomal co-localisation of PGA-OG and HPMA-OG was corroborated with this system by the lysosomal marker dextran. IDEAS software was used to perform the semi-quantitative analysis (BDS score) [4,5]. *SCF* is a very demanding technique that requires a careful standardisation and validation, however, up to now is considered the most reliable technique to achieve quantitative data. Herein cell breakage and organelle fractionation with MCF-7 cells was optimised. Differential centrifugation allowed the purification of organelles according to their size. Enrichment of DNA in the nuclear fraction and lactate dehydrogenase (LDH) in the soluble fraction indicate an optimal protocol standardisation. Succinate dehydrogenase (SDH) activity was chosen as a mitochondrial marker and Hex A as a lysosomal marker; as it was expected, both were enriched in the adequate organelle. Procedure standardisation was also supported by electron microscopy. Having established the procedure for MCF-7 cells, intracellular trafficking of the conjugates was determined. After incubating MCF-7 cells the fluorescence recovery in the lysosome was significantly higher than in other fractions. In parallel, deuterated PGA as resonance probe was also used to corroborate and validate the data obtained by fluorescence techniques. Preliminary data indicates that this novel approach is highly promising as a robust and highly selective technique to allow quantification of conjugate trafficking overcoming fluctuations induced by changes in intracellular pH or dye concentration.

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P_3

DEVELOPMENT OF MULTIPARTICULATE SYSTEMS OF CELECOXIB FOR GLIOBLASTOMA

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INTRODUCTION

Glioblastoma multiforme (GBM) comprises a heterogeneous group of neoplasms that differ in their location within the CNS; it is responsible for 51% of all primary gliomas in adults and represents the second cause of cancer death in adults less than 35 years old [1]. A high level of COX-2 expression is associated with clinically more aggressive tumours, such as GBM being a strong predictor of poor survival. Considering the selectivity of celecoxib (CXB) as COX-2 inhibitor [2], the aim of this work is to develop 2 types of controlled delivery systems: microparticles for intracranial administration when biopsy is practised, in order to reduce the inflammation and angiogenesis [3] and, nanoparticles aimed to cross the BBB during the treatment period. Microspheres were prepared by the solvent evaporation technique from an o/w emulsion using 40 mg of CXB and 200 mg of PLGA Resomer® 503, and nanoparticles prepared by a nanoprecipitation method using 5 mg of CXB and 50 mg of PLGA Resomer 502®. Particles were characterized and aliquots from the in vitro release tests were used for cell culture studies. The human glioblastoma cell line U373 was exposed to increasing concentrations of CXB, ranging from 60 to 120 µM, for a period of 24 h in order to determine the antiproliferative effect of CXB by flow cytometry.

RESULTS AND DISCUSSION

All CXB micro- and nanospheres were spherical with smooth surfaces and particle sizes around 60 µm and 173±44.90 nm, respectively. Mean EE were 74.57±2.15% for microspheres and 79.55±11.6% for nanospheres. In vitro release profiles obtained from CXB-loaded PLGA 503 microspheres showed constant release during 34 days with K_0 = 37.90 µg/day/20 mg microspheres. For CXB-loaded PLGA nanoparticles, the in vitro release profiles showed an initial burst release (first day) higher than 20%. From this time on, two release kinetics occurred. In the first one 45% of CXB was released within 30 days. The second phase was faster, with a drug release higher than 90% after 54 days. Zeta potential for the nanoparticles was -14.1 ±0.6 mv. After 24 h, a decrease in cell viability was obtained in U373 cells incubated with increasing concentrations of CXB. This cell viability decrease is dose-dependent.

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P_4

A NEW CONTROLLED RELEASE SYSTEM FOR ROPINIROLE

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INTRODUCTION

Parkinson's disease (PD) is one of the most prevalent neurodegenerative diseases which affects around 2% of adults over the age of 60 years with an estimated 7-10 million people affected worldwide (1). Levodopa coupled with a peripheral decarboxylase inhibitor remains the gold standard of symptomatic treatment of motor features of PD (2). Dopamine agonists such as ropinirole (RP) can be used as monotherapy to improve symptoms in early PD or as adjuncts to levodopa in patients who are experiencing motor fluctuations. Oral bioavailability of RP is 50% with a short elimination half-life (6 hrs) (3). A once-daily prolonged release formulation of RP has shown comparable efficacy and tolerability to immediate release RP in early PD patients, with significantly greater compliance (4). In our work we develop a new biodegradable controlled release system of RP. Microspheres were prepared using two poly D,L-lactic-co-glycolic acid resomers (PLGA 502® or PLGA 502H®) by the solvent evaporation technique from an O/W emulsion. Ratios of RP/PLGA used were 40 mg/400mg, 80mg/400mg and 120mg/400mg. Three batches of each formulation were prepared. Particles were characterized by SEM, process yield, drug loading and *in vitro* release at pH 7.4. Quantification of RP was performed by spectrophotometry at 254 nm.

RESULTS AND DISCUSSION

All RP microspheres were spherical with smooth surfaces. Particle sizes ranged between 10-50 μm. Mean process yields ranged between 64.92-77.07% and 63.15-71.16 for RP-loaded PLGA 502 and 502H microspheres, respectively. Mean encapsulation efficiencies were higher than 82% for all formulations. In vitro release profiles obtained from RP-loaded PLGA 502 microspheres showed a low initial burst release (24 hrs) equivalent to less than 1% RP followed by a constant drug release between days 1 and 21. Mean values of the zero-order release rate constants were 33.29 μg/day, 51.73 μg/day and 78.23 μg/day for the different RP:PLGA 502 ratios used. Complete drug release occurred after 25 days. Regarding RP-loaded PLGA 502H microspheres, initial burst release was lower than 20%, followed by constant release of RP for 11 days. Mean values of the zero-order release rate constants calculated were 57.33 μg/day, 111.48 μg/day and 159 μg/day, respectively. Complete drug release occurred after 14 days. RP-loaded PLGA 502 microspheres showed the most promising results being the formulation destined to further investigation in an animal model of PD.

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P_5

IN VITRO ASSESSMENT OF AZONE® AND OLEIC ACID ENHANCED TRANSDERMAL DELIVERY OF NADOLOL AND PROPRANOLOL HYDROCHLORIDE FOR THE PROPHYLACTIC TREATMENT OF MIGRAINE

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INTRODUCTION

Nadolol and propranolol are drugs used frequently in preventive migraine treatment but they have a low bioavailability after oral administration[1]. For this reason, the development of new pharmaceutical formulations for alternative routes of administration, as the transdermal route, becomes a target of interest. To this end, the dermatopharmacokinetics (DPK) approach suggested by the FDA proposes to evaluate the level of topically applied drug in the stratum corneum (SC) during its uptake so as to calculate classic pharmacokinetc parameters[2]. The objective of this study was to evaluate the effect of two chemical enhancers on the skin transport of two beta-blockers drugs with different lipophilicity and molecular size. Skin transport parameters were obtained via tape stripping and transepidermal water loss (TEWL) measurements. *In vitro* experiments were performed using dermatomed porcine dorsal skin and side-by-side diffusion cells. The permeation of nadolol and propranolol hydrochloride across skin pre-treated with Azone® and oleic acid [5% (w/w) in ethanol] was investigated. After 3h of drug permeation the stratum corneum was sequentially stripped off with adhesive tapes. TEWL was measured along the tape-stripping procedure to normalize the SC thickness. The "drug concentration-SC depth" profiles built were fitted mathematically to the appropriate solution of Fick's second law. The SC-vehicle partition coefficient (K) and the diffusivity parameter (D/L^2, where L=SC thickness) were derived from the fitting, and the extent and rate of drug absorption across the skin were also calculated. Integration of the concentration profiles yielded the total drug amount in the SC at the end of the application period.

RESULTS AND DISCUSSION

Pre-treatment of the skin with Azone® and oleic acid resulted in significantly enhanced fluxes of nadolol and propranolol with respect to control experiments. However, *in vitro* data suggested that Azone® is the most effective, with a value 7.18-fold ($p < 0.01$) (nadolol) and 1.74-fold (propranolol) that of the control. The tape-stripping experiments typically involved 15 tape-strips which removed efficiently most of the SC as shown by the TEWL measurements. Two times more propranolol was retained in the SC (5.26 ± 1.42 µg/cm^2) as compared to nadolol. Pre-treatment of the skin significantly enhanced the SC accumulation of both beta-blockers as well as their SC-vehicle partitioning of both drugs ($p<0.05$). Research evidence also suggests strongly that the partitioning parameter of propranolol hydrochloride by passive diffusion has a high value (4.37 ± 0.62) but was in the presence of oleic acid when it reached a higher value of K and amount of drug in the stratum corneum than other conditions ($p<0.05$) and similar to Azone®. However, the transport parameter (D/L^2) was only increased for nadolol ($p<0.05$). This could be related to the different lipophilicity of the drugs, with nadolol (log P=0.93) being more hydrophilic than propranolol (log P=3.21)[3]. Meanwhile, the accumulated amounts of both beta-blockers in donor compartments after *in vitro* transdermal absorption studies were compared and these were found to be lower in the experiments with nadolol. Tape-stripping provides mechanistic understanding about the effect of chemical enhancers on the skin permeation of different drugs with different physico-chemical properties. In the case of nadolol, the dermato-pharmacokinetic profile indicates that nadolol is efficiently delivered into the stratum corneum of skin pre-treated with oleic acid. In the case of propranolol, an increased accumulation of the drug into the SC is observed. Therapeutic concentrations of nadolol could be attained based on these data and the drug clinical pharmacokinetic properties; however the effect of the skin model and of the possible irritation caused by the enhancer should be considered.

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P_6

POLYETHER-BASED RESPONSIVE DENDRITIC NANOGELS WITH TUNABLE SIZES AND PHASE TRANSITION TEMPERATURES

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INTRODUCTION

Nanogels are water swollen nanosized networks composed of hydrophilic or amphiphilic polymer chains. They are developed as carriers to transport small molecules as drugs and dyes, or biomacromolecules like polynucletides and proteins. Thermoresponsive polymers are water soluble systems which undergo a phase transition at a certain temperature, in aqueous media. They can change their aggregation state, exhibit conformational change and undergo shrinking, swelling, or micellization upon a thermal trigger.[1] The combination of nanogel properties and thermoresponsiveness generates promising candidates for the development of smart nanocarrier systems, which can reveal high loading capacity, can improve drug stability, and thus can be used for stimuli-controlled release in drug delivery.[2]

RESULTS AND DISCUSSION

Herein we present the synthesis of thermoresponsive dendritic nanogels (dNG) through a precipitation approach recently developed by our group.[3] The methodology affords nanogels with tunable sizes over the range of 50 – 400 nm and adjustable phase transition temperatures in the range of 23–74 °C. Acrylated dendritic polyglycerol (dPG-Ac), as water soluble cross-linker, and oligo ethylene glycol (OEG), as thermoresponsive monomer, were chosen as building blocks to combine their inherent multifunctionality, responsiveness, and biocompatibility.[4,5] Free radical dispersion/precipitation polymerization was used for the formation of dNG in a single reaction step. The variation of the monomer mol ratio for a long-chain OEG (OEG475) and a short-chain OEG (OEG188), both different in their hydrophilic/hydrophobic behavior, yielded adjustable phase transition temperatures for the dNGs. Different cross-linker concentration and cross-linker functionalization were used to tune the dNG sizes. Both dynamic light scattering (DLS) and ^{1}H-NMR studies showed that decreasing the cross-linker density resulted in increased dNG sizes. The simplicity of the synthetic approach enabled the tailoring of dNG properties, as size and transition temperature, which highlights their strong potential for pharmaceutical and biomedical application.

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P_7

IN VITRO OCULAR BIOCOMPATIBILITY OF NOVEL HP-β-CD ACETAZOLAMIDE COMPLEXES

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INTRODUCTION

Cyclodextrins (CDs) are a group of natural products formed during bacterial digestion of starch. These cyclic oligosaccharides consist of (α-1,4)-linked α-D glucopyranose units with a hydrophilic outer surface and a lipophilic central cavity[1]. CDs are able to solubilize many lipophilic water-insoluble drugs otherwise is hard to formulate in aqueous eyedrop solutions[2,3].

Acetazolamide (ACZ) is a carbonic anhydrase inhibitor used orally for the reduction of intraocular pressure (IOP) in patients suffering from glaucoma[4]. The two major problems that hinder the topical effectiveness of ACZ are its poor aqueous solubility (w0.7 mg/ml) and a low corneal permeability coefficient of $4.1x10^{-6}$ cm/s[5]. In order to enhance the ocular bioavailability of ACZ, a multicomponent complex with hydroxypropyl-ß-cyclodextrin (HP-ß-CD) and triethanolamine (TEA) was prepared for ocular topical application.

The aim of this work was to evaluate the *in vitro* ACZ delivery performance and the bio-compatibility between ocular surface cells and ACZ complexes as a first step in the design of a topical DDS for ocular administration.

RESULTS AND DISCUSSION

Cell viability: Human corneal cells exposed to ACZ complexes (ACZ-HP-ß-CD and ACZ-HP-ß-CD-TEA) in concentrations of 0,1%, 0,5% and 1% (ACZ), for 24 h exhibited viabilities around 100% in all cases. Morphological details of exposed cells remained intact. Viability was not reduced when cells were exposed to higher concentrations of complexes solutions.

Proliferation: Proliferation rate of human corneal cells exposed to 0,1% or 0,5% ACZ-CD complexes was equivalent to that of controls. Cells exposed to ACZ-HP-ß-CD 1% solutions showed a marked reduction in proliferation rate. Cells exposed to ACZ-HP-ß-CD-TEA 1% solutions also showed an important reduction in proliferation through the first 24 h, although they recovered their normal proliferation rate in the following 24 h.

Drug delivery: CD-free formulations showed a slower ACZ release profile than those of HP-ß-CD-containing formulations, being the drug release from the physical mixture the lowest. This decrease in drug release is probably a result from the formation of an ion pair between ACZ and TEA, whose hydrophilicity and relatively larger size than the free drug might be responsible for its poor membrane permeability.

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P_8

EVALUATION OF POLYMERIC MICELLAR CARRIERS FOR MEGLUMINE ANTIMONIATE

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INTRODUCTION

The mainstays of therapy for leishmaniasis are the pentavalent antimony (Sb^V) compounds sodium stibogluconate and meglumine antimoniate (MA). Parenteral administration of pentavalent antimony organic compounds remains as the first choice drug in all leishmaniasis froms. In the case of Cutaneous leishmaniasis (CL) pentavalent anitmonials can be administrated intralesionally. However, resistances and frequent side effects are still relevant problems associated with this treatment[1]. Topical treatment represents an interesting alternative. Formulations can be applied either to thickened lesions (then the drug must be able to target the *Leishmania* parasites in the deep dermal layer of the skin), or to open lesions, in which the epidermis have been completely lost (then re-epithelization of open lesions must be achieved). In this way, poloxamer micellar carriers have attracted particular interest in the design of dermal nanogels, with a view to promoting drug permeation and possessing healing properties[3]. The present study is therefore aimed at developing a novel nanogel composed of poloxamers and MA in order to build a new and efficient dermal drug delivery system.

RESULTS AND DISCUSSION

The direct dissolution method was used to prepare the MA-nanogel. Results of drug content uniformity test for the formulation indicated that the drug was properly and uniformly dispersed. pH of formulations was 6.77, reflecting no risk of skin irritation. The resulted micelles had an average diameter about 20 nm and narrow distribution. To corroborate the particle size and to examine the morphology, the formulation was observed using transmission electron microscopy. The temperature not significantly affected the size of the micelles. Rheological properties of the nanogels exhibited an exclusive temperature-sensitivity. MA entrapment in the nanogel slowed down the drug release showing a sustained release for about 5 h. Model fitting showed that formulation followed a first order model, with a total release drug ($\%Q_\infty$) of 100 %. The overall results obtained corroborated that nanogel could be an interesting carrier of MA and a candidate for the treatment of CL.

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P_9

MAGNETIC LIPOSOMES AS CONTROLLED RELEASE DELIVERY SYSTEMS OF 5-FLUOROURACIL

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INTRODUCTION

Colloids responsive to magnetic fields are different types of nanoplataforms designed for drug delivery with potential applications in biomedicine[1]. In cancer therapy, they can be based on biocompatible, biodegradable, non-toxic, and non-immunogenic nanosystems [2], consisting of superparamagnetic iron oxide embedded into a lipid vesicle, obtaining nanoparticles so-called magnetic liposomes or magnetoliposomes. The present research aims to develop a formulation of magnetoliposomes as nanoplataforms for specific delivery of 5-fluorouracil (5-FU), an antimetabolite of the pyrimidine analogue type which has recently exhibited limited activity against advanced colorectal cancer (probably due to drug resistance). Hence, it could be expected that the loading of 5-FU magnetic liposomes will optimize its therapeutic efficacy, thanks to a controlled delivery at the cancer tissue, the reduction of the severe associated toxicity, and the optimization of the poor pharmacokinetic profile (short biological half life, and extensive biodistribution)[3].

RESULTS AND DISCUSION

Magnetoliposomes, composed of magnetite (Fe_3O_4) particles and phosphatidylcholine (PC), Fe_3O_4:PC (3:4 mass ratio), were obtained through thin film hydration modified method [4]. Geometry analysis of magnetoliposomes was performed Transmission Electron Microscopy and Photon Correlation Spectroscopy. The magnetic properties of magnetoliposomes were evaluated through hysteresis cycle and visual observation of the particles suspension behavior under influence of a permanent magnet. A positive effect of 5-FU concentration on the absorption efficiency into magnetoliposome particles was clearly appreciated by UV-Vis spectrophotometry[5]. Compared to 5-FU absorption, the incorporation of the chemotherapy agent exclusively onto the surface of magnetoliposome particles (adsorption procedure) resulted in a dramatic reduction of the entrapment efficiency (%). *In vitro* drug release under the physiological conditions (pH 7.4 ± 0.1 and 37.0 ± 0.5 ºC) showed a rapid desorption of 5-FU adsorbed onto magnetoliposomes. The release of 5-FU entrapped into magnetic liposomes followed an initial fast burst effect in drug release in 6 h and provided sustained drug release for 5 days.

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P_10

DEVELOPMENT AND CHARACTERIZATION OF POLY (D, L LACTIDE-CO-GLYCOLIDE) MICROPARTICLES LOADED WITH CANNABIDIOL. INFLUENCE OF POLYMER MOLECULAR WEIGHT.

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INTRODUCTION

Cannabidiol (CBD) is the mayor nonpsychotropic cannabinoid constituent in the plant Cannabis sativa[1,2,4]. Because of its antioxidative, anti-inflammatory, neuroprotective [1] and antitumor [2,3] properties it may prove to have therapeutic utility in several conditions including Parkinson disease, Alzheimer disease, diabetes, rheumatoid arthritis[1] and cancer[2,3]. However despite its potential clinical interest it is difficult to develop an effective formulation with CBD because it has a low aqueous solubility and different stability problems[2,4]. Systems as microparticles (MP) may resolve these questions[2]. The aim of this work was to develop biodegradable microspheres loaded with cannabidiol for parenteral administration. Several resomers of PLGA with different molecular weight have been employed to this purpose. The obtained formulations were characterized in terms of morphology, particle size, drug loading and in vitro drug release, evaluating the influence of the polymer molecular weight in these characteristics.

RESULTS AND DISCUSSION

Spherical, nonporous and uniform MP were obtained with resomers 502 and 503; whereas microspheres obtained with the resomer 504 showed a rough surface. No differences in particle size distribution could be appreciated among PLGA 502, 503 and 504 formulations, with a mean diameter range of 20-28μm. Drug loading and entrapment efficiency were significantly lower in the resomer PLGA 504 microspheres than in PLGA 502 and 503 formulations, probably because that has a minor affinity for CBD. Microspheres prepared from all PLGA resomers exhibited an alike initial burst effect (around 10% of the drug was released in the first 2h). Dissolution profiles from PLGA 503 and 504 MP were very similar, showing more than 85% of drug released at day 14. In the case of PLGA 502 formulation, 85% of drug released was reached at day 21. A zero-order kinetic could be achieved by mixing the different PLGA resomers. A mathematical model was designed to determinate the percentage of each polymer that conducts to stated release rates.

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P_11

Controlled intramyocardial delivery of neuregulin-1 and fibroblast growth factor-1 from biodegradable microparticles in a large preclinical myocardial infarction model

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INTRODUCTION

Growing evidence suggests that growth factor therapy is a promising approach to treat myocardial infarction (MI). Among therapeutic cytokines, neuregulin-1 (NRG1) and acidic-fibroblast growth factor (FGF1) are involved in cardiac repair after MI[1,2]. However, limited protein stability is a major challenge in growth factor therapy. The combination of proteins along with drug delivery systems (DDS) represents a new therapeutic approach. Microparticles (MP), one of these DDS, could protect proteins from degradation and ensure sustained release among time. Recently, our group assessed the efficacy of biodegradable poly(lactic-co-glycolic acid) NRG1-MP and FGF1-MP in a rat MI model demonstrating that MP promoted cardiac repair and improved cardiac performance[3]. In order to further validate this clinically applicable platform for drug delivery, we now propose to test the beneficial effect of NRG1-MP and FGF1-MP in a large preclinical ischemia-reperfusion model.

RESULTS AND DISCUSSION

Growth factor loaded MP used in rats were successfully scaled-up to be tested in an ischemia-reperfusion porcine model. MP were prepared by double-emulsion solvent evaporation technique using the total recirculation one-machine system. The mean particle size measured by laser diffractometry was 7.2±1.9 µm which is compatible with an intramyocardial administration. Growth factors were efficiently encapsulated reaching values of 84.05±15.5% for FGF1 and 83.5±13.25% for NRG1. The released cytokines bioactivity was evaluated *in vitro* by determining H9c2 cardiomyocyte proliferative capacity following growth factor treatment. A 1.3 and 2.1-fold increase in cell density was observed when stimulated with FGF1 and NRG1 respectively. Proliferation rates of cells treated with the free-cytokines were similar to the ones released by microparticles, indicating that both cytokines retained its biological activity after microencapsulation. NRG1-MP, FGF1-MP and non-loaded MP were next injected locally into the infarcted myocardium using NOGA® XP Cardiac Navigation System, a highly accurate electro-guided methodology which creates precise 3-D heart images, 1 week after the infarct. Preliminary results showed that intramyocardial growth factor-MP injection has therapeutic effects and improves cardiac function. Histological studies are currently been performed to fully characterize treatment effectiveness. The validation of this therapeutic approach could make significant progress for patients with ischemic heart disease.

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P_12

IN VITRO TRANSDERMAL ABSORPTION STUDIES WITH SOME IBUPROFEN FORMULATIONS USING DIFFERENT MODEL MEMBRANES

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INTRODUCTION

Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug (NSAID) that has demonstrated high efficiency in the treatment of muscle and joint pain as well as in rheumatoid and osteoarthritis therapy. The inherent side effects of orally administered IBU justify the vast amount of marketed formulations intended for topical use. Its skin permeability is intrinsically poor due to its solubility properties (log P ≈ 4)[1]. In the present study, the in vitro penetration properties of four commercially-available topical formulations of IBU were compared: Ibuprofeno-Pharmacia® (Pharmacia Labs. – Pfizer Spain), Ibufen® (Cinfa Labs. Spain), Ibuprofeno-Farmasierra® (Farmasierra Labs. Spain) and Solvium® (Omega Pharma Inc. Spain). All formulations were presented as 50 mg/g Ibuprofen gels. Two inert synthetic membranes were selected to study the *in vitro* penetration of IBU in order not to introduce biological variations inherent to natural skin[2]: a non-porous polydimethylsiloxane (PDMS) membrane (Silmax® – Pillar surgical Inc. CA USA) and a new porous membrane (Strat-M™) Merck KGaA, Darmstadt, Germany) which is reported to imitate the diffusional characteristics of natural skin for a wide variety of compounds[3].

RESULTS AND DISCUSSION

The diffusion of all formulations was studied using an automated in line flow-through equipment with seven cells (PermeGear Inc. USA). PBS solution (pH 7.4) including a surfactant to ensure sink conditions was used as receptor medium. Flux values (J), the permeation rates at steady state (μg/cm^2 h) for each individual diffusion experiment were calculated by monitoring the cumulative amount of drug diffused at each time by HPLC using a previously validated method.

Big differences were found between the four formulations using the PDMS membrane: it was found that IBU disposed onto the membrane with Solvium®, was able to diffuse to the donor chamber in a percentage close to 40% in 24 hours whereas the IBU diffused ranged from 2 to 13% with the other formulations. These findings contrast to the obtained with Strat-M™ where no statistically significant differences were found over 16 hours. A comparative analysis of the J data showed that the non-porous PDMS membrane is adequate to detect differences between the studied compositions. The resulting values range from 14 to 98 μg/cm^{2}h, what correlates with the calculated data by different authors for IBU with different kinds of skin and under different conditions[4-6]. Strat-M™ provided significantly higher values of J with all the formulations studied.

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P_13

MUCOADHESIVE PARTICLES WITH ENHANCING INTESTINAL ABSORPTION CAPACITY AS DRUG DELIVERY PLATFORM FOR VITAMIN E

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INTRODUCTION

M-cells are the specialised antigen sampling sites of the intestinal immune system and represent a potential portal for mucosal drug and vaccine delivery since they possess a high transcytotic capacity and are able to transport a broad range of materials including particulates. To increase bioadhesion to enterocytes in the gastrointestinal tract, targeting ligands (such as lectins) are of special interest, as they mediate highly specific binding to epithelial cell subpopulations, effected by interaction of carbohydrate-binding sites of lectin with sugar residues in the glycocalyx of epithelial cells. Exploiting lectins for bioadhesive drug delivery purposes, this cell-specific interaction can result in active receptor-mediated endocytosis and/or transcytosis of the drug delivery system.

Vitamin E is a class of lipid-soluble antioxidant molecules widely used in food, cosmetic and pharmaceutical fields. Unfortunately due to its high sensitivity to external stimuli such as light, heat and oxygen, it rapidly degrades and this limits its applications.

The aim of this work is to formulate mucoadhesive and biodegradable polymeric particles intended for oral use to enhance the intestinal absorption of γ-tocopherol. Poly(lactic-co-glycolic acid) (PLGA) based carriers have been produced with the classic nanoprecipitation method, which is an easy, reproducible and scale up method. The biocompatible polysaccharide chitosan have been added to the formulation to increase the mucoadhesion of the particles, which is exert due to its polycation nature and ability to interact with mucus producing surfaces. Moreover the particles surface has been covalently modified with lectins obtained from *Sambucus ebulus* L., to further increase mucoadhesiveness and cell interactions.

RESULTS AND DISCUSSION

Different formulations have been performed to select the optimal conditions and components percentages to be used in the carrier system, particularly regarding the kind of surfactants/stabilizers (poly(vinyl alcohol) (PVA), Poloxamer 407, Tween 80 or PEGylated poly(lactic acid) (mPEG-PLA)). The γ-tocopherol delivery system has been optimized regarding the encapsulation efficacy, the particles morphology and the cell penetration ability. Spherical particles have been produced and characterize with dynamic light scattering (< 1 μm) and microscopy techniques.

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P_14

DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD BY RP-HPLC TO SIMULTANOUSLY QUANTIFY ACETAZOLAMIDE AND TIMOLOL FROM TRANSETOSOMES. APPLICATION OF QUALITY-BY-DESIGN TO DESIGN AND DEVELOPMENT OF OPTIMIZED FORMULATION

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INTRODUCTION

The instrumental technique of HPLC-DAD has been widely used in the pharmaceutical field. The development of a selective analytical method allows us to separate the components of the sample to subsequently identify and quantify them. When an analytical method has been developed, it is important to confirm that it is suitable for its intended purpose. Therefore, the method validation according the ICH Q2R1 guidelines is today an essential concern in the activity of analytical chemistry laboratories.

In this work, it is important to develop a specific analytical technique for quantifying acetazolamide (ACZ) and timolol (TML), which were encapsulated in transetosomas, to subsequently improve pharmaceutical properties.

Furthermore, the variability in the manufacturing process leads to a quality problem; results are not reproducible batch to batch. Therefore, we must have a more comprehensive approach, strict and necessary in terms of safety. It is necessary to ensure quality through Quality-by-Design (ICH Q8) which is defined as designing and developing formulations and robust manufacturing processes and reproducible to ensure that our drug is safe and effective for their objective.

RESULTS AND DISCUSSION

The chromatographic conditions were optimized using an octadecylsilyl column C18 (*Merck, LiChrospher 100 RP-18*, 125x4 mm). The column temperature was 45 °C (column oven L-2350, Elite LaChrom). The mobile phase consisted of acetonitrile-sodium acetate buffer pH 4.1 (ACN:SA).

A linear gradient of 10:90% of ACN:SA for 2.2 min at a flow rate of 1.5 mL/min and 40:60% of ACN:SA for 5 minutes at a flow rate of 2.0 mL/min. ACZ and TML were detected with a diode detector at 286 nm and the injection volume was 10 µL.

The method has been validated. Selectivity was demonstrated qualitative and quantitatively. The accuracy of the method revealed recovery values around 99%. Instrumental precision was satisfactory (RSD < 2%) and method repeatability was considered valid, as the RSD values were ≤ 2%. Adequate system and method linearity were obtained in the range of the study (0.25-0.75 mg/mL). LOD and LOQ were found to be $8x10^{-4}$ and $3x10^{-3}$mg/mL, respectively. Finally, the robustness assay was demonstrated by applying the Taguchi method.

Then, the principles of QbD were applied to get the optimized formulation, which was characterized in terms of encapsulation efficiency for ACZ = 22.03 ± 2.53% and TML = 29.66 ± 3.12%, vesicular size: 385.92 ± 65.6 nm, zeta potential: -30.8 ± 11.3 mV, permeation flux: 3.41×10^{-4} and 2.74×10^{-4} mg/cm^2/h for ACZ and TML, respectively. Formulation containing PC: 0.0954-0.10498 mmol, Cho: 0.0812-0.08933 mmol, 0.00724-0.00796 mmol, ethanol 1.5%, showed the best characteristics for the intended purpose of these transethosomes to be potentially administered by ophthalmic route.

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P_15

APPLYING THE MOLECULAR TROJAN HORSE TECHNOLOGY TO DESIGN IMMUNOLIPOSOMES FOR BRAIN DRUG DELIVERY

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INTRODUCTION

The central nervous system (CNS) is protected by the blood-brain barrier (BBB). Unfortunately, the same mechanisms that protect it against intrusive chemicals can also frustrate therapeutic interventions. Drug delivery to the brain is limited due to the BBB. Only small molecules (molecular weight lower than 600 Da) can pass the BBB paracellularly or transcellularly, depending on their lipophilicity. However, high molecular weight drugs, such as proteins, peptides or DNA, cannot cross it.

The most versatile and attractive approach for delivery of drugs in their native state into the brain parenchyma involves the use of drug carriers, such as liposomes, as "Molecular Trojan Horses" (MTH). The MTH bind to a receptor (e.g., for transferrin or insulin) on the BBB and brain cell membrane, triggering receptor-mediated transcytosis of the Trojan Horse Liposome (THL) across the BBB in vivo, and receptor-mediated endocytosis into brain cells beyond the BBB.

In this study, we have developed immunoliposomes as drug delivery carrier. Immunoliposomes are liposomes in which an antibody has been attached to the liposome allowing the drug to be targeted to the brain cells.

RESULTS AND DISCUSSION

The molecular structure of immunoliposome involves the preparation of liposomes by TLE technique. Liposomes contained phosphatidylethanolamine (PE) as phospholipid, which included maleimide (SMPB) as functional group. Maleimide residues allow the liposome conjugation with the monoclonal antibody (Ig G). Inside liposomal structure, the drug was included. Functionalized liposome binding to the antibody occurs after thiolation of the antibody with the terminal ester group of N-succinimidyl S-acetylthioacetate (SATA) obtaining a stable amide linkage. Once developed various synthetic steps, the characterization of the resulting structure has been realized.

After monoclonal antibody attached to liposomes, the synthesis of immunoliposomes occurred satisfactorily. These functionalized vesicles were characterized in terms of mean diameter and size distribution by laser scattering technique (Zetasizer Nano ZS). Shape and surface characteristics were determined by fluorescence optical microscopy (Olympus BX61). A confocal laser scanning microscope (CLSM) (Leica DM-IRE2) was used to know the presence and the distribution of Ig G around the immunoliposomes.

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P_16

DEVELOPMENT AND CHARACTERIZATION OF BUCCAL BILAYER TABLETS CONTAINING MICROPARTICLES OF IBUPROFEN

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INTRODUCTION

Bilayer compacting technology has gained more popularity in recent years, because bilayer tablets offer several advantages over conventional tablets (1). Buccal delivery of drugs using mucoadhesive polymers has been the subject of interest since the early 1980s and several formulations have been developed earlier like ointments, patches, films, tablets and gels containing different classes of drugs including antimicrobials, corticosteroids, antiepileptics, local anesthetics, etc. (2, 3).

The aim of this study was to develop and characterize buccal bilayer tablets containing microparticles with ibuprofen to obtain sustained release. Ibuprofen is an non-steroidal anti-inflammatory drug used in the treatment of inflammatory diseases and for pain relief of oral mucositis, and was used as a model drug of class II Biopharmaceutical Classification System (BCS), characterized by high permeability but poor solubility.

Three types of bilayer tablets containing three bioadhesive polymers, namely polycarbophil, Carbopol® 974 and Carbopol® 980 were prepared as follows: the first layer with a weight of 300 mg, responsible for the tablet retention on the mucosa, was obtained by compaction of each one of the bioadhesive polymers; the second layer with a weight of 338 mg, responsible for buccal drug delivery, was obtained by compaction of lipid microparticles, prepared using the emulsion/chilling method, corresponding to 100 mg of ibuprofen. The drug encapsulation efficiency was previously determined before tablets production.

Weight variation, friability, hardness and "in vitro" drug release study, by two different methods (A and B), were evaluated in the manufactured tablets. In method B the tablets were attached at a height of 7.0 cm to the inside of the dissolution vessel, and in method A, the tablets were positioned on the bottom of the dissolution vessel (4). Calculation of similarity factor (f_2) was also carried out (5).

RESULTS AND DISCUSSION

Buccal bilayer tablets with acceptable physical properties were produced. The result of the determination of the ibuprofen content in the lipid microparticles was 87.4%.

"In vitro" dissolution tests, performed during 8 hours, have demonstrated a sustained release of ibuprofen (maximum drug released: 30.0-45.1%). According to f_2, both dissolution methods (A and B) and the three polymers studied showed similar dissolution profiles ($f_2 > 50$).

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P_17

HUMAN SERUM ALBUMINE LOADED MICROPARTICLES TO MODULATE DEXAMETHASONE DRUG RELEASE

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INTRODUCTION

Biodegradable and biocompatible polymeric microparticles are of special interest to avoid the inconvenients related to surgical insertion of implants[1]. Among the methods employed to prepare microparticles, the emulsion solvent extraction-evaporation technique is one of the most employed. PLGA (poly lactide-co-glycolide) is widely used to prepare biodegradable drug delivery systems. Many researchers have investigated the modulation of the drug release profiles by different strategies. One of the most widely employed is to incorporate additives into the formulation[2-3].

RESULTS AND DISCUSSION

A family of dexamethasone loaded microparticles have been obtained using a double emulsion S/O/W extraction-evaporation method[4]. The additives employed in this study have been vitamin E (Vit E) and Human Serum Albumine (HSA). Physico-chemical characterization of the different formulations has been accomplished. Dexamethasone – vitamin E - human serum albumine (HSA) microparticles show no difference in encapsulation efficiency assays compared to those only including dexamethasone. In order to evaluate the influence of the different additives, release assays of dexamethasone from the different formulations were performed in PBS buffer. The release of the drug resulted slower in the vitamin E-HSA microparticles compared with dexamethasone and dexamethasone-vitamin E microparticles. The initial phase characterized by a burst effect that was clearly higher in the dexamethasone loaded microparticles. The profile obteined in both formulations show the typical shape of PLGA systems.

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P_18

INCREASED ANTIPARKINSON EFFICACY BY THE COMBINED ADMINISTRATION OF VEGF- AND GDNF-RELEASING NANOSPHERES IN A PARTIAL LESION MODEL OF PARKINSON'S DISEASE

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterised by the progressive degeneration of the nigrostriatal dopaminergic pathway[1]. Current research efforts are being focused in the use of neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF), as neuroprotective and neuroregenerative strategies that will halt the neurodegenerative process. However, the critical problems for the clinical application of GDNF and VEGF are their rapid degradation rate and their difficulty in crossing the blood-brain barrier[2]. To overcome these problems, in a recent successful study published by our research group, VEGF and GDNF were encapsulated in poly-lactic-co-glycolic (PLGA) microspheres. This strategy made possible the administration of these two factors in the brain, getting a continuous and simultaneous drug release[3]. Continuing with the study mentioned, the aim of this novel work was to evaluate the combination of lower doses of VEGF and GDNF PLGA nanospheres in a partially lesioned rat model and analyze the neuroregenerative potential of these factors individually and in combination.

RESULTS AND DISCUSSION

The nanospheres particle size was about 200 nm. The simultaneous addition of VEGF-NS + GDNF-NS resulted in a significant protection of PC12 cell line against 6-OHDA induced cell death. Once the PLGA-NS were implanted in the striatum of 6-OHDA partially lesioned rats, *in vivo* efficacy of VEGF-NS, GDNF-NS and VEGF-NS + GDNF-NS was assessed during 12 weeks using an amphetamine rotation behavior test. Our results showed that VEGF-NS+GDNF-NS significantly reduce the number of rotations induced by amphetamine. In addition, TH+ inmunohistochemical analysis in Substantia Nigra demonstrated a significant enhance of neurons in VEGF-NS+GDNF-NS treatment group. Taking all results together, it may be concluded that the synergistic effect of lower VEGF and GDNF-NS dose may be a neuroregeneration-neuroreparation strategy to treat PD.

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P_19

IN VITRO DISSOLUTION/PERMEABILITY ASSAYS WITH NIMESULIDE NANOPARTICLES

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INTRODUCTION

Nimesulide (NS) is a non-steroidal anti-inflammatory drug with preferential inhibition of the COX-2 isoform and less renal and gastrointestinal adverse effects than other non-selective NSAIDs (1). It has been shown that the COX-2 isoform is over-expressed in different epithelial cancers such as the prostate (2) and several *in viv*o experiments have shown nimesulide is a promising lead compound for anti-cancer drug discovery (3). However, the nimesulide concentrations used in these studies are ranged from 200 to 500 mM, which greatly exceed the concentration necessary to inhibit COX-2 activity. These facts suggest that nimesulide inhibits cancer cell growth and induces cancer cell apoptosis independent of COX-2. Although intratumoral administration is a promising approach for the treatment of various solid tumors by achieving high local drug concentrations with minimal systemic toxicity, its efficacy is strongly dependent on the timing and frequency of the drug injections because of its rapid clearance from the tumor site. To achieve this goal we have formulated poly lactide-co-glycolide (PLGA) nanoparticles (NPs) loaded with NS. In recent studies, surface coating by hydrophilic polymers such as chitosan (CS) has been also evaluated (4). This study focused on the *in vitro* release and permeation of the NS loaded in PLGA NPs which were prepared by emulsification-solvent evaporation method.

RESULTS AND DISCUSSION

NPs showed average sizes of 378.7 ± 58.9 and 392.9 ± 66.1 nm when using CS as an external coating. Free NS in solution passed through a simulated biological membrane (adapted PAMPA) following apparent zero order kinetics (0.42 %/min). NS in suspension at concentrations comparable to NP formulations showed non linear kinetics with a first faster step lasting about 100 min followed by a slower permeation rate. In all cases NPs retarded the permeability of NS from 50 to 100 min. CS-coated NP did not modify the apparent zero order kinetics but NS permeability rates decreased to 0.15 and 0.11 %/min for 1 and 0.25% PVA, respectively. Uncoated NP showed biphasic permeability curves like the one obtained with drug crystals but the initial release and permeability rates were slower. However, these initial release rates were faster than the ones achieved with CS coated NP (30% *vs.* 10% released in 200 min).

These experiments would simulate the situation occurring after intraprostatic injection of NSNP. At first, a deposit of NS (*non-sink* conditions) will be formed but, later, the initially released drug could be swept along by the blood flow causing additional NS dissolving from the NP and permeating through biological membranes.

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P_20

PHARMACOKINETICS AND PHARMACODYNAMICS OF HYDROPHOBIC GENTAMICIN-LOADED NANOPARTICLES IN *BRUCELLA*-INFECTED MICE

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INTRODUCTION

Brucellosis is a worldwide zoonosis that remains widespread and neglected in many areas despite notable advances in science, technology, and management. Specific therapy is based on long-lasting combined drug treatments that generate toxicity and are difficult to achieve, therefore, relapse currently presents a significant problem[1]. On the other hand, the intracellular location of the pathogen in phagocytic cells is also involved in therapeutic failure[2]. As an alternative to current therapy, the use of biodegradable and biocompatible poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) have been studied for the intracellular targeting of a hydrophobic gentamicin (GEN-AOT)[3]. A sustained release of the antibiotic at the site of infection would make it possible a higher accumulation of the drug in the infected tissues, thus improving the effectiveness of the antibiotic, and may make it possible to reduce both the required number of doses and the frequency of administration, and thus the treatment-associated toxicity.

RESULTS AND DISCUSSION

GEN-AOT PLGA NPs prepared by the single emulsion-solvent evaporation method presented mean diameters of 300 nm, 100% of encapsulation efficiency and drug loadings of 24 μg of GEN/mg of NP. When tested in *Brucella melitensis*-infected human macrophages GEN-AOT NPs significantly improved the efficacy of the free drug at clinically relevant concentrations (>2-$\log_{10}$ unit reduction at 18 μg/mL). On the other hand, pharmacokinetics studies were performed after the administration of a single intraperitoneal dose equivalent to 5 mg/kg of GEN to Balb/c mice. The concentrations of the antibiotic in the liver and spleen (target organs of *Brucella*) and kidneys (as GEN is nephrotoxic) were determined. It was observed that while no GEN was detected in the liver or in the spleen, NPs efficiently targeted the drug at both tissues and maintained therapeutic antibiotic concentrations for up to 4 days, allowing the design of a therapeutic schedule for NPs with an extended dosing interval. Finally, mice were infected with *B. melitensis* and treated for 14 days with daily GEN (5 mg/kg), the NPs every 4 days (5 mg/kg) and the classical combined therapy of daily GEN (5 mg/kg) and doxycycline (10 mg/kg). Importantly, 3 weeks after the end of the treatment the splenic infection of mice treated with the NPs was reduced by 3.2 logs, and 50% of the infected mice were cured, with no evidence of adverse toxic effect. In contrast, the classical combined therapy of GEN and doxycycline was associated with nephrotoxicity and with a rapid re-emergence of the infection. In summary, encapsulation of gentamicin in PLGA nanoparticles improved the antibiotic therapeutic index and allowed to reduce the required dosing frequency and the treatment-associated side effects, presenting a great therapeutic potential.

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P_21

THERANOSTIC MACROMOLECULAR PRODRUGS: FRET FOR POLYMER THERAPEUTICS

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INTRODUCTION

The basic concept of polymer therapeutics involves the use of polymers as carriers for bioactive molecules. Covalent linking of a payload to a macromolecule leads to certain advantages compared to the free drug alone. The most important abilities of these systems are the passive targeting through the EPR effect of macromolecules and the controlled drug release.[1] Additionally, the reduction of toxicity, elimination of undesirable body interactions, improvement of the solubility, stability, and prolonged blood half-life of the small molecules in the conjugated form, lead to an improved therapeutic efficiency.[2] Such attachments, however, can potentially introduce steric hindrances and prevent association of the drug with its molecular target and thus render it inactive. Therefore, improved therapeutic efficacy is commonly only realized when the active agent is linked to the carrier through a cleavable linker that is stable in circulation but readily hydrolyzed upon entry into its target cell. Intracellular monitoring of drug release is thereby an indispensable ability to understand the mode of action of polymer drug conjugates and to further improve these systems.

RESULTS AND DISCUSSION

Herein we present a FRET-based theranostic macromolecular prodrug (TMP) consisting of dendritic polyglycerol as the carrier, doxorubicin as the drug and fluorescence donor, and a cyanine dye as the florescence acceptor (quencher). Its fluorescence is quenched via intramolecular FRET until the pH-sensitive hydrazone bond between the TMP and the doxorubicin is cleaved at low pH. By measuring its fluorescence, we characterized the TMP cleavage kinetics at different pH values in vitro. The intracellular release of doxorubicin from the carrier was monitored in real time in intact cancer cells, giving more insight into the mode of action of a polymer drug conjugate.

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P_22

DESIGN OF NEW NANOCARRIERS OF HISTONE DEACETYLASE

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INTRODUCTION

The treatment of Leishmaniasis is a complex procedure due to the different clinical forms and the influence on the immune status of the host. In addition, the drugs used in this treatment present major problems including the high toxicity and many side effects to which must be added the emergence of resistant strains. Nowadays, none of them eliminate completely the parasite so the host, end up suffering from a chronic infection and remains infectious for sandflies. Nevertheless the treatments commercially available achieve a reduction/elimination of the clinical signs of disease. One of the paths aimed in this work is focused in the inhibition of histone deacetylase (HDAC). In the present work we will explore this therapeutic strategy in combination with gold nanoparticles as drug delivery systems.

RESULT AND DISCUSSION

In the present work, 20 nm gold nanoparticles synthesized following the citrate thermal reduction method (1), were functionalized with histone deacetylase inhibitors. The adsorption of HDAC is proven by dynamic light scattering measurements, electrophoretic mobility measurements and optical absorbance. The adsorption of HDAC on the gold cores, produce an increase of the size up to 150 nm. In addition, the electrophoretic mobility of the particles tends, in absolute value, to decrease to more positive. By optical absorbance measurements the loading capacity of HDAC by the gold nanoparticles was determined, and it was found that the amount adsorbed is below 10 %. In addition, the release of HDAC was followed up in culture medium at 37 º C, and it was found that the capacity of release is very effective, being the amount released higher than 80 %. Finally, preliminary in vitro test shows that the functionalization of gold nanoparticles with HDAC, is effective.

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P_23

OPTIMISED POLYMERIC NANOPARTICLES LOADED WITH ANTIINFLAMATORY DRUG.

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INTRODUCTION

Biodegradable Polymeric Nanoparticles (NPs) are widely used to enhance the bioavailability of topically administered drugs. Poly lactic-co-glycolic acid (PLGA) is the most extensive biodegradable polymer used due to its biocompatibility, mucoadhesiveness and nonantigenic nature (1,2,3). In addition, PLGA-NPs are mainly absorbed in inflamed areas by the reticulo-endothelial system (RES), resulting in an increased concentration of NPs in the aforementioned areas.
Ketorolac Tromethamine (KT) is a non-steroidal anti-inflammatory drug (NSAID) with a potent and moderate non-opioid analgesic activity. It is currently administered orally and intramuscularly for pre- and postoperative pain treatment (4).
The purpose of this work was to study the release behaviour of developed NPs of KT formulations for ophthalmic administration.

RESULTS AND DISCUSSION

PLGA nanoparticles (6.25 mg/ml, 185 nm, PI 0,040, pH 5.09 and EE 99.72%) were tested in Franz diffusion cell, using a KT solution as a reference formulation. Samples were analyzed by a validated HPLC method with UV detector, and data analysis was carried out by non-linear regression software (WinNonLin) applying mathematical modelling (zeroorder, first order, Weibull, Higuchi and Korsmeyer-Peppas) in order to depict release behaviour. Best fitting profile was selected based on the lowest AIC (Akaike Information Criteria) value, CV% and good-of-fitness. According to these criteria, Weibull was the best model to describe the release profile for both formulations. In this regard, td from solution and NPs were compared (tdNPs > tdsolution) and analysed by statistical software (GraphPad) which demonstrated significant differences ($p<0.05$). Thus, NPs showed a sustained release in comparison to the solution.
According to the results obtained, and taking into account higher mucoadhesiveness of polymeric NPs, PLGA-NPs loading KT should present a major antiinflammatory local effect.

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P_24

Δ^9-THC-BASED NANOACARRIERS FOR ORAL ADMINISTRATION: ANTICANCER *IN VITRO* EVALUATION

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INTRODUCTION

Δ^9-Tetrahydrocannabinol (THC) exhibits antitumor effects on various cancer cell types and in animal models of cancer. Nevertheless, the route of administration for THC requires special attention due to the physicochemical characteristics of drug[1]. Hence, the objective of the present work is to develop biocompatible nanoparticles (NPs) based on PLGA, in order to improve its oral absorption.

PLGA-NPs were produced by the nanoprecipitation technique[2]. To produce surface modified nanoparticles, NPs were incubated in a chitosan (CS) and/or in a polyethylenglycol (PEG) solution. Main physicochemical characteristics, *in vitro* release, hemocompatibility, protein adsorption of NPs and uptake in Caco-2 cell line have been studied. Anticancer activity in A549 cell line (human lung carcinoma) and in non-malignant lung cell line (MRC-5) was also studied.

RESULTS AND DISCUSSION

Plain PLGA NPs presented a size of 320-420 nm with narrow size distribution and negative zeta potential (ZP) (-35.6 mV). Modification with CS turned ZP strongly to positive values (+70 mV). However, PEG-NPs presented neutral ZP (+ 2.90 mV). In all cases, high encapsulation efficiency values were obtained (≈ 80%).

In order to determine protein adsorption for each formulation, THC-loaded NPs were incubated in a 400 μg/mL BSA solution. After this, changes in ZP values were evaluated. For coated-PEG nanoparticles, ZP changes were less than 10 units, meaning a lower NPs clearance in the body[3].

In hemocompatibility studies, all formulations presented high safety *in vivo* behavior. Hemocompatibility of nanoparticles was studied evaluating the potential effects of nanoparticles on different blood component values, such as erythrocyte lysis.

A higher uptake in Caco-2 cell line was obtained in PEG-CS-PLGA, due to mucoadhesive properties of CS. After 6 h incubation, 75% of PEG-CS-PLGA-NPs were uptaked in cells.

NPs cytotoxicity was studied in A549 human lung cancer cells and in MRC-5 human non-malignant lung cells. Statistical significances were found among cell lines at a 50 μM THC concentration which indicate selectivity for cancer human lung cells A549. In A549 cell line, the cytotoxicity was higher for loaded PEG-PLGA NPs than for free THC and the other kind of NPs (PEG-PLGA > PEG-CS-PLGA > PLGA). In MRC-5: THC = PEG-PLGA > PEG-CS-PLGA > PLGA.

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P_25

POLYETHYLENIMINE NANOCONJUTES AS MAGNETIC RESPONSIVE NONVIRAL VECTORS FOR TARGETED GEN DELIVERY

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INTRODUCTION

Last years, gen therapy has emerged as a promising novel strategy, which can give answers and better therapeutic options to a high number of diseases. Specially focused on hereditary diseases at the beginning, its aim has reached many others diseases such as cancer or vascular diseases[1]. The study and application of this new therapeutic strategy require new delivery agents know as "vectors". First vectors were based on viral structures but some problems like autoimmune response suggested the development of another carrying agents. This new vectors were called non-viral vectors and try to solve the main problem of viral vectors. In this area, many polymers have been proposed as possible non-viral vectors, one of them is polyethylene imine (PEI). This polymer has been described as promising gen carrier despise his high transfection efficiency and many other advantages like simple manipulation or functionalization properties[2]. In this work we describe the characterization of magnetite-PEI complexes and we describe the pros and contras of their utility as gene delivery vehicles.

RESULT AND DISCUSSION

Magnetite-PEI complexes were prepared at different ratios of magnetite:PEI. The follow up of the functionalization of the magnetite surfaces was carried out by means of electrophoretic mobility and dynamic light scattering measurements. The consecutive characterization of the non-viral vector obtained as described has shown that the polymeric coating its effective and his size its small enough to work with it as an injectable system. The zeta potential measurements was made by laser Doppler electrophoresis and shown a result of 66,8 ± 8,6 mV for the selected synthesis method. The particle size measurements was made by photon correlation spectroscopy (PCS) and shown a diameter of 110,1 ± 21 nm for the selected synthesis method.

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P_26

NANOPRECIPITATION SYNTHESIS OF ALL GLYCEROL THERMO-RESPONSIVE NANOGELS FOR ENCAPSULATION AND RELEASE OF DOXORUBICIN

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INTRODUCTION

Thermo-responsive nanogels are water soluble/dispersible networks that are able to shrink or swell upon a thermal trigger. Their chemical structure is based on linear thermo-responsive polymers connected to cross-linking points, forming a tridimensional lattice. The linear polymers undergo a process of shrinkage or expansion upon a thermal trigger in aqueous environments, providing a responsive modality to the whole network. In such way, they enable the encapsulation of bioactive molecules with high loading capacities and their release at the site of action after an external thermal trigger [1].

In this work we describe a methodology for the synthesis of a thermo-responsive nanogels based on glycerol trough nanoprecipitation polymerization. A systematic study of the conditions and the polymer composition was performed. The sizes, thermo-responsive properties, encapsulation, and release studies were investigated.

RESULTS AND DISCUSSION

For the synthesis of the nanogels poly(glycidyl methyl ether-co-ethyl glycidyl ether) (poly(GME-co-EGE)) and hyperbranched polyglycerol (hPG) as crosslinker were copolymerized. Nanogels were prepared by nanoprecipitation while the cross-linking points were generated by (1) copper catalized azide alkyne cycloaddition (CuAAC) [2] or (2) strain promoted azide alkyne cycloaddition (SPAAC) [3].

The size of the particles could be finely tuned from 150 to 400 nm by changing the concentration of the polymers in the synthesis, and the transition temperature could be controlled between 18 to 55°C by varying the composition of the co-polymer and the molecular weight of the thermoresponsive polymer.

In order to use these nanoparticles as carriers, the encapsulation of different molecules was studied. Due to the synthetic method, hydrophobic molecules could be in situ encapsulated, as for example nile red. On the other hand amphiphilic drugs could be encapsulated post synthesis (doxorubicin, DOXO). Besides, DOXO release was studied and showed temperature responsive release above the cloud point.

These results suggest that these thermoresponsive nanogels are promising as nanocarriers for biomedical applications and therefore cellular experiments are currently ongoing.

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P_27

PREPARATION AND CHARACTERIZATION OF POLYMERIC MICROPARTICLES CONTAINING CIPROFLOXACIN

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INTRODUCTION

The two major types ofso called inflammatory bowel disease (IBD),Crohn's disease (CD) and ulcerative colitis (UC), are characterizedby chronic, intermittent inflammation of the gastrointestinaltract (both the large and small bowel). The distinction between CD and UC isbased largely on the distribution of inflammation. Quinolonesare used in therapy directed against mycobacteria (one of main causes ofIBD) because are effectives against aerobic gram-positive bacteria inhibiting DNA synthesis by affecting the DNA gyrase[1].Reducing the size of drug delivery systems to micro- ornanometre scale might increase colonic residence time. A size dependent accumulation of micro- and nanoparticles (µP and NP) can be observedspecifically in the inflamed intestinal regions.An increase mucus productionis observed in CD, making mucoadhesion a strategy toincrease targeting and retention of drug delivery systems. Dendritic cells and macrophages are highly activated and increased in numbers in inflamed areas,showing increased phagocytotic activity. As a consequence of inflammation a disruption of the intestinalepithelial structures was found, a phenomenon comparable to the enhanced permeability and retentioneffect. This offers therapeutic potentialas nanoformulations maytarget to the inflamed areas[2].So microparticles with Ciprofloxacin and Eudragit® RS (PO) were prepared in two different proportion drug:polymer and by two different methods, spray drying[3] and solvent evaporation[4]. The aim of our study is to compare the differences on particle size and drug delivery profiles of the prepared microparticles.

RESULTS AND DISCUSSION

For the twostudieddrug:polymer proportions(2:1 and 1:1), microparticles obtained by spray drying method (SDµP) were smaller and than those obtained by emulsion-solvent evaporation (SEµP).

Drug delivery studies at pH 7.4 (PBS) showed that at the same drug:polymer proportion, drug release was slower for SDµp than SEµp. For all formulations the percent of drug released in 24 hours was 80 % whereas in the 2sp samples (1:1 drug:polymer prepared by spray dryer) was only 60 %.

Release data were fitted to zero order, Higuchi, Peppas-Ritger and Higuchi-zero order kinetic equations. The best fit was for the latter equation and in all cases the K_H values were higher than $K_{o,}$ suggesting that the drug release corresponds to a diffusion mechanism. This is confirmed by the high r^2 values in Higuchi equation and the n values in Peppas-Ritger equation ($n<0.5$).

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P_28

NOVEL ZWITTERIONIC SYSTEMS FOR CYTOSOLIC DELIVERY

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INTRODUCTION

Current research focuses on the synthesis of a non-viral gene transfer carrier that will be able to perform successfully the cytosolic transport of siRNA or pDNA *via* endocytosis. Typically, the encapsulation in liposomal envelopes and the complexation of the negatively charged siRNA or pDNA with cationic polymers and peptides (the so called polyplexes [1]) are applied to form non-viral gene transfer vectors [2]. However, up to now almost no conjugates with negatively charged surface delivery system (with completely different behaviour in the pharmacokinetic properties with respect to the cationic carriers) have been postulated.

Polyglutamic acid, PGA, is a negatively charged polypeptide with multiple favorable qualities for drug delivery [3]. It is a multifunctional, biodegradable polymer already in Phase III trials through Opaxio®[4]. Its valuable features make it a good candidate for therapy in chronic diseases or those requiring high doses of drug, among other interesting applications.

The aim of this study was to formulate and to characterize several compounds formed by a well defined structure of PGA (synthesized in Dr. Vicent´s lab) and different percentages of different oligo(ethaneamino)amides developed in Prof. Wagner's lab[5] . These substances are used to produce efficient polymeric nucleic acid carriers with the help of solid-phase assisted synthesis. In our case, the main function of these artificial oligoamino acids is to complex the nucleic acid and to provide an endosomolytic activity through the proton sponge effect. The resulting zwitterionic molecule will then be complexed with the siRNA or pDNA.

RESULTS AND DISCUSSION

In our work, for nucleic acid binding, 20% of W-Stp5 was incorporated in the PGA structure by EDC/sulfo-NHS activation. The primary and secondary amines of this polyamidoamine are partially protonated under physiological conditions and therefore, are able to bind nucleic acids by means of electrostatic interactions. The uptake into the cells is the mediated through endocytosis.

Gel shift assays showed that the modification of PGA with W-Stp5 allows DNA complexation. Conjugates formed by PGA-WStp5 20% are capable to form complex with DNA in contrast to both compounds separately. Therefore this union improves the DNA binding ability of W-Stp5. Moreover, PGA-WStp5 20% efficiently mediated gene transfer. An increasing luciferase expression with increasing N/P values was observed. The obtained RLU values at the highest N/P ratio exceeded those of the "gold standard" linPEI (w/w 0,8) for more than 1 log unit. On the other hand, MTT assay showed no significant reduction in cell viability.

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P_29

Cτ AS A WAIVER FOR STEADY-STATE STUDIES ON ORAL EXTENDED-RELEASE DRUG PRODUCTS

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INTRODUCTION

It is widely accepted that single-dose studies are generally more sensitive than multiple-dose studies to differences in the rate of drug substance absorption, and therefore, the requirements for the use of steady-state studies have been reduced on the new EMA guideline for bioequivalence on oral immediate release drug products [1]. The same rational supports the FDA decision to currently not require multiple-dose studies for the determination of bioequivalence between innovator and generic MR products [2]. However, under the European regulations, its use for the purpose of bioequivalence has been mandatory for these type of drug products [3].The objective of this work is to use a computer simulation in order to test the two approaches and evaluate the possibility of waiving the steady-state studies.

RESULTS AND DISCUSSION

Three pharmacokinetic models, representing different release mechanisms, were considered, and Monte Carlo simulations with intra- and inter-individual variabilities were performed. Five different bioequivalence protocols were used and a new pharmacokinetic metric – Cτ, the concentration at the end of the intended dosing interval obtained in the single-dose study – is proposed in order to avoid the need for steady-state studies while keeping the ability to detect differences between formulations. Results have shown that the European requirements are more capable to discriminate between two potentially different formulations but at the cost of the multiple-dose study and with an increased number of subjects when compared to the FDA requirements. However, the use of Cmax and AUC0–t obtained on a single-dose study with the added Cτ metric equals the discriminatory ability of the current EMA requirements, without the need of a multiple-dose study. This proposed approach results in the reduction in the number of studies and volunteers enrolled in clinical bioequivalence trials, without compromising the quality assurance of a new extended-release oral formulation [4].

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P_30

DIALYSIS OF MEMBRANE OF FREEZE DRYING NANOPARTICLES CARPROFEN

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INTRODUCTION

Carprofen (CP) is non-steroidal anti-inflammatory drug (NSAID), which is used for the treatment of inflammatory diseases due to COX-2 inhibition [1]. CP is a racemic mixture of the *R* and *S* and it is widely used in veterinary medicine where it has been investigated in several species [2]. Nowadays only CP solution for intravenous. Administration is commercially available. Due to its half-life the drug should be administrated frequently; hence it was decided to introduce CP into polymeric nanoparticles, in order to increase the drug residence time into the animal.

Poly (D,L lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) has been widely used because of its biocompatibility and the ability of deliver the drug in a controlled release pattern. Unfortunately, the nanoparticles in solution could lead into short stability of the drug delivery. One approximation performed by researchers in order to increase the stability is the NPs freeze-drying, using different tipes of cryoprotectants. Thus, the aim of this research is to study the biopharmaceutical profile of CP NPs after freeze-drying stabilization.

RESULTS AND DISCUSSION

Optimized NPs with a matrix structure containing CP (0.75mg/ml) were prepared by the solvent displacement technique described by Vega et al. [3]. The mean particle size was 189 ± 0.03 nm, the polydispersion index (PDI) was 0.03 ± 0.007 and the zeta potential (ZP) was - 20 0.001 mV. These results suggest a good stability of the produced NPs. Good values of encapsulation efficiency were obtained (74.7%). CP-loaded NPs were freeze-dried in the presence of 5% (w/v) HPfiCD used as cryoprotectant, according to previous investigations [4]. The final concentration of the cryoprotectant used was chosen in order to maintain the NSs suspension around 300 mOsm/K. Due to the nanoparticles would be administrared intravenous or intraarticular route a fl-irradiation sterilization was performed using 60Co as the irradiation source at dose of 25 KGy, according to Eur. Ph.

Sample reconstitution was performed by adding 3 mL of MilliQ water pH 3.5 to the dried cake followed by manual shaking. The macroscopic appearance was similar and the particle size, PDI and ZP remained almost constant after reconstitution ($p<0.05$), demonstrating that the sterilization and the freeze-dried process did not affect the NPs phisico-chemical properties. Resuspended NPs underwent into a release experiment versus free CP solution using PBS pH 7.4 which ensure that sink conditions were kept along the study. Both formulations exhibit a first-order kinetic release, typical for the PLGA NPs. Solution release was faster than NPs release (K=1.68 h-1 versus 0.15 h-1, respectively) and the maximum percentage achieved at the end of the experiment was 73.37 % versus 45.8%, respectively, demonstration the ability of NPs to controlled the release of CP.

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P_31

DEVELOPMENT OF A NEW COMBINED FORMULATION FOR OPHTHALMIC ADMINISTRATION: ACETAZOLAMIDE AND TIMOLOL LOADED TRANSETHOSOMES

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INTRODUCTION

Glaucoma is the second cause of blindness in the world. Acetazolamide is used orally at high doses to reduce intraocular pressure in glaucomatous patients. However, chronic administration causes important side effects in patients. So, an alternative to minimize these side effects consists in topical drug administration in the eye. Moreover, timolol is considered a first-line drug for the treatment of glaucoma. Nevertheless, after instillation of a solution of timolol to the eye, about 80% of the instilled dose being lost, either through leakage or due to draining towards the nasolacrimal duct, causing cardiovascular and respiratory side effects.

This study aims to design, develop and characterize transethosomes of acetazolamide and timolol, in order to provide a controlled release system, which is suitable for topical administration improving the ocular bioavailability of these drugs.

RESULTS AND DISCUSSION

Phosphatidylcholine, cholesterol, 1.4% ethanol, buffer Hepes pH 7.2 and sodium deoxycholate as edge activator have been used to produce transethosomes. Different techniques to make them, such as thin layer evaporation (TLE), freezing and thawing (FAT), reverse phase evaporation (REV) and sonication (SON), have been employed. Transethosomes characterization was performed according to morphological analysis (optical microscopy and transmission electron microscopy), entrapment efficiency (EE%), size and zeta potential. In vitro permeation properties were evaluated by using artificial membranes from gels incorporating drug transethosomal dispersions. In vitro release was determined using the membrane diffusion technique. Additionally, a solubility assay of timolol and thermal analysis of the components of the formulation were realized.

TLE was the technique that provided the most efficient encapsulation of both drugs and better physical stability. The inclusion of acetazolamide in the aqueous phase and timolol in the oil phase of the vesicles has shown the best results of EE%.

The permeation of drugs was primarily influenced by the size of the vesicles rather than the method used for its preparation. In vitro release study was clearly highlighted the modulatory effect of all transethosomal formulations. Drugs were released from transethosomes by a diffusion mechanism.

Thermal analysis of the components of the vesicles disclosed a significantly interaction between drugs and amphiphilic compounds such as phosphatidylcholine and sodium deoxycholate.

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P_32

TIMOLOL-LOADED TRANSFERSOMES FOR OPHTHALMIC ADMINISTRATION: DEFORMABILITY STUDIES FROM EXPERIMENTAL DESIGN

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INTRODUCTION

Timolol is a non-selective beta-adrenergic receptor antagonist widely used in ophthalmic pathologies to reduce the intraocular pressure in patients suffering from glaucoma.

Topical route is preferred for administration of these drugs. However, this route has certain drawbacks due to the impermeability of ocular barriers. Actually, vesicular systems, in particular, transfersomes, have been developed as drug delivery systems. These lipid vesicles include in their formulation one or more "edge activator" surfactant, showing a highly deformable membrane that allows the intact penetration of liposomes through the membrane.

The technological development of transfersomes involves many factors, which will significantly affect properties such as size, encapsulation efficiency and deformability index of vesicles.

In this work, transfersomes composed of different surfactants (Tween® 20 and sodium deoxycholate) with cholesterol (20 or 27 μmolar) and stearylamine amount (0-5 mg) were formulated.

These factors were selected as variables and their levels were introduced into L16 orthogonal array. The aim was to optimize the manufacturing conditions by applying Taguchi methodology. Response variables were vesicle size, polydispersity index, zeta potential, encapsulation efficiency, phosphate groups and deformability index. The latest parameter was determined from the difference of vesicle size before and after the extrusion process. Also, the influence of the extrusion on these parameters was evaluated. Finally, formulations were optimized by applying the marginal means methodology.

RESULTS AND DISCUSSION

Once analyzed data obtained from the experimental matrix, Taguchi method concluded that all factors exerted a significant influence on the responses. With respect to vesicular size and polydispersity index, as logical, the extruded batches showed lower values compared to non-extruded. In relation to zeta potential and phosphate groups, no significant variation after extrusion was detected, so it can be considered that transfersomes are quite stable during the process.

According to encapsulation efficiency and deformability index data, it was observed that extruded batches which had higher deformability index values, showed higher encapsulation efficiency. This may be related with the ability of liposomes to pass intact through the membrane, so maintaining their integrity.

After analysis, the optimization process was carried out. This optimization methodology of the individual responses, allowed us to select the factor levels which theoretically reach maximum values for the encapsulation efficiency, deformability index and phosphate groups and minimum values for size, polydispersity index and zeta potential. The optimized conditions were checked with the confirmatory tests. However, results of this assay were not satisfactory, because it is a multivariate analysis and it is more difficult to reach only one conclusion. In this sense, we are currently working in the application of alternative optimization techniques to obtain the most advantageous formulation.

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P_33

ROLE OF ENDOCYTIC UPTAKE IN TRANSFECTION EFFICIENCY OF SOLID LIPID NANOPARTICLES-BASED VECTORS

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INTRODUCTION

When developing vectors for efficient gene therapy it has to be kept in mind that the entry routes will influence the overall transfection process. The mode the vector is internalized affects its uptake quantitatively, whereas its intracellular trafficking and final destination (e.g. recycling compartments, late endosomes, lysosomes) will lastly define the efficiency of penetration into the nucleus and finally gene expression. In addition, the components of the formulation itself may have particular features that facilitate the transfection process. Currently, 9 different endocytic routes can be differentiated based on the coat material, lipid microdomains and the requirements for dynamin and accessory molecules[1]. Experimentally, distinct entry pathways can be blocked using pharmacological drugs (so-called endocytosis inhibitors). Therefore, the aim of this work was to explore the cellular uptake mechanisms of three different vectors based on SLN in HeLa cells. We prepared three vectors based on SLN[2] bearing the plasmid that encodes the green fluorescent protein (EGFP): without protamine, with protamine, and with protamine and dextran. The uptake, the percent of transfected cells and the EGFP production were analyzed in the presence or absence of different endocytosis inhibitors (including Pitstop2®, EIPA, dynasore, chlorpromazine and filipin). In addition, colocalization studies using lysosomal markers were carried out in order to determine the influence of the trafficking to late endosomal compartments on the transfection capacity of the vectors.

RESULTS AND DISCUSSION

The uptake and transfection of each vector was differently affected by each endocytosis inhibitors. EIPA did not affect the uptake of the DNA-SLN vector, whereas all the inhibitors affected the transfection. In the case of protamine-DNA-SLN and dextran-protamine-DNA-SLN vectors EIPA affected the uptake, and dynasore did not decreased the transfection. In summary, DNA-SLN vector seems to enter productively by multiple pathways in HeLa cells. In contrast, dynamin does not seem to be essential in the productive entry of protamine containing vectors. In addition, the enhancement of the macropinocytic route increases the EGFP production when dextran is added to the vector. Finally, dextran-protamine-DNA-SLN vector needs to be routed within the late endosomes/lysosomes in order to produce EGFP.

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P_34

RELEASE STUDIES OF DOXEPIN FROM MUCOADHESIVES FILMS

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INTRODUCTION

Toothache is one of the most important causes of the increased demand of drugs for pain treatment. Another alternative to analgesics are tricyclic antidepressants, as doxepin hydrochloride (DH), since there are studies reporting that they may have analgesic local effect due to its biochemical action, as they are able to increase the amount of noradrenaline and serotonin in the synaptic space. Oral administration of DH has also side effects related to its main function as antidepressant, especially when it is systemically administered (e.g. sublingual administration). However, delivery of DH directly to the oral cavity could be an interesting alternative for toothache due to its analgesic local effect[1].

The objective of this study was to develop new DH-based mucoadhesive films indicated for dental pain treatment. For this purpose, we tested three different polymers: chitosan, sodium hydroxypropylmethylcellulose and sodium carboxymethylcellulose. The DH release profiles of different formulations were compared using Franz-type diffusion cells.

RESULTS AND DISCUSSION

As a previous step to the drug release study, membrane selection processes were studied in order to arrive at a reliable assessment of the influence of the films in doxepin hydrochloride release. It is clear that the nylon membrane is that which offers least resistance, allowing a greater quantity of the drug to pass through it.

The percentages of the quantities of released doxepin hydrochloride past the first 6 hours of the test are similar for SCMC and HPMC films being 46.4%, however the release continues for 24 hours with percentages of 63.5% and 58.2% respectively. These two results are very similar, and after being subjected to statistical analysis (ANOVA), the release profiles among two formulations did not show any statistically significant differences ($P > 0.05$). On the other hand, chitosan film release maximum yields 57.8% of the drug to 3 hours of starting the test and this value does not change during the same. This behaviour can be explained by the fact that SCMC and HPMC polymers form hydrophilic matrices that forms viscous liquids when hydrated with aqueous media, as confirmed by previous swelling studies[2-4]. It is therefore possible to make an accurate assessment of the influence of the swelling behaviour on released amounts. On completion of the test, it can be seen that chitosan film are not dissolved and release the drug more slowly.

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P_35

EVALUATION OF A LIPOSOMAL ACTIVE IN THE FORMULATION OF ANTI-AGEING CREAM: PRELIMINARY STUDY "IN VITRO"

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INTRODUCTION

The aim of this project was the evaluation of Emortal ®, a registered liposomal product as active in a cosmetic cream anti-aging. This product is a mixture of peptides derived from pea, whose main claim is to activate epidermal stem cells. The EM has ability to increase the number of fibroblasts in vitro. This would allow to get insight into the actual ability of liposomes to diffuse along the stratum corneum.

RESULTS AND DISCUSSION

The sizes of EM-liposomes were measured be means of a Malvern Z-sizer (U.K.). The measurements were performed at room temperature (25°C) and measurement angle of 173º. A sample of each of the three available batches was measured and fixed to 611.5±127.4 nm. A slightly high degree of polydispersity (PDI) has been measured (around 0.2). To ensure that this was not due to agglomerations of liposomes in the starting solution, serial 1:10 dilutions of each sample were assayed. The last dilution has a very low particle concentration, and yet, the polydispersity remains. This fact shows that liposomes are quite heterogeneous in terms of size and the presence of agglomeration cannot be discarded.

To have an insight in the proteic composition of EM, in order to identify the active, determination by MALDI / TOF mass spectrometry was used at the Proteomics Central Service of U.V. This equipment allows proteins identification by disrupting the amino acid sequence, which can be identified with the help of database. Once identified, a quantification method can be established. To simplify this step, only actives that can permeate through skin are analyzed. For doing so, the Franz diffusion cell set-up was used with human heat separated epidermis as membrane. Samples of permeated active were analyzed. For the analysis a trap column (Column nanoLC, 3C18-CL, 75umx15cm; Eksigen) via an isocratic flow of 0.1% TFA at 2 uL / min for 10 min, a guard column (LC Column, 3C18-CL, 75umx25cm) were used with a gradient 5 to 50% acetonitrile in aqueous 0.1% in 150 min. The mass spectrometer device was a nanoESIQqTOF (5600 TripleTOF, ABSCIEX). The results obtained show that no active permeated through skin from EM liposome solution after 24 h of contact. In this case, we can conclude that liposomes do not cross the epidermal barrier. On the other hand taking into account that the actives must diffuse to dermis to produce an effect “in vivo” on fibroblasts this formulation is not suitable for cosmetic use, even if there is “in vitro” evidence of activity in the fibroblasts culture. However, it is well known that for transdermal permeability of drugs, the vehicle in which the actives are formulated can influence their permeability at a high extent. Therefore, the use of these active is a great challenge that will need to be faced for to the use active for cosmetic purposes.

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MULTIFUNCTIONAL DENDRITIC POLYGLYCEROL SULFATE AS A NOVEL PLATFORM FOR PACLITAXEL DELIVERY

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INTRODUCTION

In the last years, polymer-drug conjugates have emerged as promising platforms to improve the therapeutic value of many low molecular weight drug candidates by increasing their water solubility, bioavailability, and their blood circulation time[1].

Recently, increased attention has been paid to dendrimers as drug carriers because of their monodispersity, multiple sites of attachment and controllable, well-defined size and structure[2]. Thus, the covalent attachment of drugs to dendritic scaffolds is a promising route for controlling the loading and release of the active species. In addition, the inherent multivalency of dendrimers allows the simultaneous incorporation of different molecules of interest, such as imaging agents, targeting ligands, or biocompatible molecules.

In particular, we wanted to explore the potential of dendritic polyglycerol sulfate (dPGS) as a suitable platform for the delivery of the anticancer drug paclitaxel. dPGS is a highly anionic sulfated compound that presented extremely high affinities towards inflammation targets (glycoproteins L- and P- selectins)[3]. In addition, in vivo studies showed that dPGS displayed and efficient anti-inflammatory efficacy in a mouse dermatitis model[3] and could be employed for the molecular imaging of inflammatory diseases[4].

RESULTS AND DISCUSSION

Polymer–drug conjugates derived from biocompatible dendritic polyglycerol (dPG) and paclitaxel have been efficiently synthesized. More specifically, neutral dPG and anionic dPGS were linked to paclitaxel through an ester bond, which could be potentially cleaved at acidic pHs such as those typically found in solid tumors and in the intracellular compartments endosomes/lysosomes. The conjugates were labelled with a fluorescent dye, which allowed the study of their cellular uptake by confocal microscopy. The release of paclitaxel could be evaluated at different pHs and in human plasma by HPLC. Finally, the in vitro activity of paclitaxel was monitored for both dPGS- and dPG-conjugates and compared with the activity of the free drug.

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PGA-BASED COMBINATION THERAPY FOR THE TREATMENT OF BREAST CANCER

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INTRODUCTION

Polymer-drug conjugates are drug delivery technologies in which a bioactive agent is covalently bound to a polymeric carrier, normally via a biodegradable linker. The first generation of polymer conjugates already achieved clinical proof and more sophisticated second-generation polymer conjugates are already being developed[1]. The use of polymer-drug conjugates in combination therapy is seen as an important opportunity to enhance tumour response rates and to reduce the severe side-effects[2]. By conjugating two different drugs covalently to a single polymer chain, the simultaneous or controlled delivery of both drugs can be accomplished. The combination of endocrine therapy with chemotherapeutic agent by simultaneous binding to the polymer could bring significant advantage versus single treatments. We have previously reported the first conjugate of this type: an HPMA copolymer carrying the combination of endocrine therapy (the aromatase inhibitor aminoglutethimide (AGM)) and chemotherapy (Dox), HPMA copolymer-AGM-Dox conjugate[3]. In vivo proof of concept for the combination conjugate has already been achieved in an orthotopic 4T1 murine metastatic breast cancer model and its molecular mechanism of action studied[4].

In order to further improve this construct, we now propose the use of a biodegradable and multivalent carrier poly(L-glutamic acid) (PGA) being able to increase conjugate molecular weight enhancing therefore its tumour targeting by EPR effect.

RESULTS AND DISCUSSION

A novel PGA-AGM-DOX conjugate family has been done synthesised via carbodiimide coupling reaction. Different polymer drug linkers have been explored looking at a possible structure-activity relationship. Conjugate identity by different techniques, (NMR, HPLC, etc) solution conformation by Small Angle Neutron Scattering (SANS), and drug release kinetics in presence of the lysosomal enzyme cathepsin B have been carried out in order to identify the design features required to achieve drug synergism.

Biological evaluation of combination conjugates in comparison with the single counterparts has been also performed in 4T1 and MCF-7ca cell lines looking at drug synergism. The best conjugates were selected for in vivo evaluation in the 4T1 model and PGA-G-AGM10-Dox5 has been selected as the best candidate to move forward. Importantly, we have demonstrated with this PGA conjugate family that conjugate solution conformation is a key feature ruling biological performance, in vitro as well as in vivo.

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