PEPTIDES, PROTEIN AND VACCINE DELIVERY: A BIOPHARMACEUTICAL APPROACH

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Introduction

Proteins/peptides and nucleic acids are bioactive compounds that are poorly stable in biological fluids and unable to pass across phospholipid membrane. To be active and used in therapeutics and immunology, it is necessary to administer large doses of these compounds and to repeat frequently drug administration. The development of polymeric biodegradable delivery systems has allowed to protect these molecules against degradation and to allow their delivery by mucosal sites or in a prolonged fashion. These molecules can be entrapped within implantable or particulate systems made of hydrophobic polymers. Poly (methilidene malonate), Poly(cyanoacrylate) and Poly (lactide-co-glycolide)-will be described after an overview of the major issues concerning pharmacokinetics and biopharmaceutics of these molecules.

Poly(methilidene malonate) delivery system for growth factors

Current strategies to maintain, restore and/or stimulate tissue healing, such as wound healing, cartilage, bone or nerve regeneration seek to (re)initiate endogenous chemotactic, proliferative, differentiation, survival, and maturation phases involved in tissue regeneration. In vivo, these complex processes are controlled by growth factors and cytokines (i.e. polypeptides transmitting signals to modulate cellular activities). If a large variety of growth factors have been yet characterized in terms of their biological activity and potential subsequent therapeutic applications, their delivery is generally unsatisfactory mainly because of their instability in biological fluids and tissues, and their slow tissue penetration (1). Poly(methylidene malonate 2.1.2) (PMM 2.1.2) is a new polyacrylic derivative, structurally designed a few years ago to improve the biocompatibility of this type of polymers. Thanks to side chain-born ester functions, PMM 2.1.2 showed that it could therefore be eroded into soluble polymers, ethyl glycolate, glycolic acid and ethanol (2). Previous studies have suggested that the addition of oligomers to MM 2.1.2 (OMM 2.1.2), acting as plasticizers, may represent a relevant strategy to modulate degradability and physico-chemical characteristics of PMM 2.1.2 implants or particulate systems. It has also been shown that this type of material, in form of nanoparticles, exhibit interesting degradability/erodability properties and low in vitro and in vivo toxicity (3). Therefore, we postulated that mixtures of OMM 2.1.2/PMM 2.1.2 at various ratios could generate versatile biocompatible implants, varying in toughness, or topical formulations suitable for the sustained delivery of growth factors. In the present study, we characterized VL4001 implants, a specifically designed blend of OMM 2.1.2/PMM 2.1.2. Its degradation profile was studied and its cytotoxicity on various

cell types was tested. We demonstrated the great potential of VL4001 implants for the sustained release of single or combinations of bioactive growth factors. Some of them (FGF2 (fibroblast growth factor 2), PDGF (platelet-derived growth factor), TGF-β (transforming growth factor β) stimulate a wide range of biological processes (proliferation, wound healing, neovascularization, etc…), while NGF (nerve growth factor) could greatly impacts the cure of CNS diseases. Moreover, we investigated the use of sphingosylphosphorylcholine (SPC) which can provide a similar cellular effect than the growth factors themselves because they share common downstream transduction components. A selected dose of formulated growth factor was carefully chosen in order to avoid VL4001-induced cytotoxicity while providing optimal stimulatory concentrations of the formulated growth factors. We then first determined the incidence of VL4001 on growth factor activity. Results showed that the MM 2.1.2-based material did not affect growth factor bioactivity in terms of both cell proliferation stimulation and specificity, when compared to the unformulated ones. This was observed for each factor tested. In addition to VL4001, we then investigated other compositions of OMM 2.1.2/PMM 2.1.2. FGF2 free of any MM 2.1.2-based formulation or embedded into 3 different OMM 2.1.2/PMM 2.1.2 blends (40/60, 60/40 and 80/20) was assayed for stimulation of cell proliferation after 0, 7, 15 and 21 days of storage at 4°C. If all compositions seemed equally suitable for FGF2 formulation (similar bioactivity at day 0), they also preserve FGF2 bioactivity over time. On the contrary, non-formulated FGF2 readily lost its activity after one week storage. Formulating growth factors into OMM 2.1.2/PMM 2.1.2 mixtures results not only in bioactive formulations, but also provides a protection of the bioactivity suitable for storage and a prolonged action at injury sites. Finally, we investigated VL4001 formulations of SPC alone or in combination with various growth factors. We showed that formulated SPC was as potent as free SPC in stimulating fibroblast proliferation. It also stimulated the neurite outgrowth of PC12 cells to the same extent than free SPC did. In fact, SPC was as potent as FGF2 and NGF to promote fibroblast proliferation and PC12 neurite outgrowth, respectively.

In conclusion, it may be outlined that mixtures of different OMM 2.1.2/PMM 2.1.2 ratios, with different toughness and implantability or topical application features, have been tested for formulation of peptidic growth factors. None of these formulations affects the loaded-drug bioactivity but, inversely, all of them significantly stabilize and protect the peptides. Thus, compositions of OMM 2.1.2 and PMM 2.1.2 represent potent versatile vehicles available for local peptide delivery. Part of drug delivery device complexes or directly used as implants, this new type of synthetic material could be considered in applications for which controlled delivery of therapeutic biological molecules is a crucial issue.

Poly (cyanoacrylate) nanocapsules for the oral delivery of insulin

Nanocapsules with an oily core made of poly (isobutylcyanoacrylate) entrapping insulin were obtained by interfacial polymerization. When administered orally by force-feeding to diabetic rats, insulin nanocapsules (12.5, 25, and 50 U/kg) decreased fasted glycemia 50-60% by day 2. This effect was maintained for 6 or 20 days with 12.5 or 50 U/kg, respectively. Only the dose of 100 U/kg decreased fed glycemia by 25% in diabetic rats. In normal rats, hyperglycemia induced by an oral glucose load was reduced by 50% with the same dose of oral insulin nanocapsules (4). It was further shown in-vitro that nanocapsules protect insulin against proteolysis from pepsin, chymotrypsin and trypsin (5). Hypothesis about the mechanism of action of nanocapsules were investigated. It was shown that insulin molecule is not chemically modified during

the nanoencapsulation process. In addition, no interaction between the poly(isobutylcyanoacrylate) and the insulin could be observed. Thus the biological activity of the nanoencapsulated peptide and the high efficiency of insulin encapsulation achieved with this nanoencapsulation process cannot be explained by a specific interaction of the insulin with the polymer forming the nanocapsule's wall (6). Therefore, it is more likely that particles uptake by peyers patches is one of the main mechanism but this does not explain the long term effect which is at the moment under investigation.

Poly (Lactide-Co-Glycolide) For Vaccine Delivery

Mucosal admistration of antigens by mucosal routes is able either to induce a mucosal immune response or a suppression of the systemic immune response called tolerance. However, antigens are generally degraded by proteolytic enzymes before reaching the priming site (e.g. Peyers patches in the gut). Nevertheless, encapsulation of vaccines into biodegradable microparticles provides excellent mucosal immunogens with a high potential for immunization or the induction of oral tolerance since it allows both protection against degradation and targeting of the follicle sites. We present here the possibilities of using Poly(lactide co glycolide (PLGA) microparticles to induce oral tolerance against a milk protein.

Allergies to milk proteins are frequently encountered in the new born population. In order to prevent this allergy by inducing oral tolerance, one of the major allergenic milk protein, β lactoglobulin (BLG) was entrapped into biodegradable PLGA microparticles prepared by the multiple emulsion solvent evaporation method and was then orally given to mice.

The goal of the formulation study was to associate large amounts of proteins to the smallest amount of polymer so that a minimal quantity of microparticles would be administered. It was shown that introducing tween 20 in the formulation was able to increase the encapsulation efficiency and to better control protein release reducing the burst release effect (9). Moreover, Oral administration of microparticles containing BLG reduced significantly (by 10, 000) the amount of protein necessary to decrease both specific anti BLG IgE and DTH response. In conclusion, microparticles appear to be optimal systems to induce oral tolerance (10).

Taken together, our results highlight the potential of antigen encapsulation in PLGA microparticles for inducing oral tolerance against a dietary antigen.

Conclusion

These studies highlight the ability of polymer delivery system to improve peptide-protein and vaccine bioavailability through several administration routes.

References

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