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Lipid-core nanocapsules restrained the indomethacin ethyl ester hydrolysis in the gastrointestinal lumen and wall acting as mucoadhesive reservoirs

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ABSTRACT

The aim of this work was to investigate if the indomethacin ethyl ester (IndOEt) released from lipid-core nanocapsules (NC) is converted into indomethacin (IndOH) in the intestine lumen, intestine wall or after the particles reach the blood stream. NC–IndOEt had monomodal size distribution (242 nm; PDI 0.2) and zeta potential of −11 mV. The everted rat gut sac model showed IndOEt passage of 0.16 μmol m^{-2} through the serosal fluid (30 min). From 15 to 120 min, the IndOEt concentrations in the tissue increased from 6.13 to 27.47 μmol m^{-2}. No IndOH was formed *ex vivo*. A fluorescent-NC formulation was used to determine the copolymer bioadhesion (0.012 μmol m^{-2}). After NC–IndOEt oral administration to rats, IndOEt and IndOH were detected in the gastrointestinal tract (contents and tissues). In the tissues, the IndOEt concentrations decreased from 459 to 5 μg g^{-1} after scrapping, demonstrating the NC mucoadhesion. In plasma (peripheric and portal vein), in spleen and liver, exclusively IndOH was detected. In conclusion, after oral dosing of NC–IndOEt, IndOEt is converted into IndOH in the intestinal lumen and wall before reaching the blood stream. The complexity of a living system was not predicted by the *ex vivo* gut sac model.

1. Introduction

Polymeric nanoparticles have been proposed to increase the apparent solubility of lipophilic drugs, protect them from enzymatic attack, control drug release, and improve activity or even to reduce their side effects (Couvreur et al., 2002; Schaffazick et al., 2003). The term nanoparticles refers to nanospheres or nanocapsules that are, respectively, matricial (Pohlmann et al., 2007) and vesicular structures (Jäger et al., 2007) varying in size from 10 to 1000 nm (Soppimath et al., 2001; Torchilin, 2006). The polymeric nanoparticles promote drug selective passage through biological barriers when their distribution sizes range from 100 to 600 nm.

The encapsulation of diclofenac or indomethacin, non-steroidal anti-inflammatory drugs, in polymeric nanospheres and nanocapsules has been reported as a strategy to reduce their gastrointestinal toxicity (Ammoury et al., 1993; Chasteigner et al., 1995; Guterres et al., 1995; Müller et al., 2001). Indomethacin has also been used as a drug model in several studies concerning nanoparticulated systems (Calvo et al., 1996; Guterres et al., 2000; Kim et al., 2001; Vinogradov et al., 2002; Pohlmann et al., 2002). More recently, comparative studies have been performed in order to propose the nanoencapsulation mechanisms for indomethacin and its ethyl ester derivative (Pohlmann et al., 2004, 2007, 2008; Cruz et al., 2006a; Poletto et al., 2008), as well as to determine the different drug release behavior from nanospheres, nanoemulsion and nanocapsules (Cruz et al., 2006b). The nanoencapsulation of indomethacin ethyl ester within semi-crystalline polyester nanocapsules (NC) has been used to simulate a perfect sink condition by releasing the drug after its interfacial alkaline hydrolysis creating a concentration gradient in the system (Pohlmann et al., 2004). Regarding the biological activity, the indomethacin-loaded lipid-core nanocapsules (NC–IndOH) and the co-encapsulation of indomethacin and indomethacin ethyl ester in nanocapsules showed selective cytotoxicity after treating glioma cell lines (U138-MG and C6) (Bernardi et al., 2008). Furthermore, after intraperitoneal administration, the NC–IndOH were

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able to increase the intratumoral penetration of the drug reducing the growth of the glioma (C6) implanted in rats (Bernardi et al., 2009a). In addition, the effects of systemic treatment with NC–IndOH were evaluated in rat models of acute and chronic edema. The drug nanoencapsulation increased the anti-inflammatory effect in long-term models of inflammation, allied to an improved gastrointestinal safety (Bernardi et al., 2009b). Previously, the *in vitro* release experiment using gastric or intestinal simulated fluids containing enzymes (pepsin and pancreatin) did not show any indomethacin ethyl ester release or degradation (Cruz et al., 2006a; Pohlmann et al., 2007). The experiment showed that polymeric nanocapsules and nanospheres containing indomethacin ethyl ester are stable *in vitro* even in the presence of pepsin and pancreatin. However, indomethacin ethyl ester-loaded lipid-core nanocapsules (NC–IndOEt) showed antiedematogenic activity in acute treatment in rats, indicating that the drug was released *in vivo* from the nanocapsules (Cruz et al., 2006a). That *in vivo* experimental protocol was not designed to show which substance, indomethacin or indomethacin ethyl ester, was responsible for the biological effect. In this way, we performed a pharmacokinetic study using the same formulations to evaluate the plasma levels of each substance after i.v. and oral dosing (Cattani et al., 2008). In the study, we demonstrated that the indomethacin ethyl ester acts as a pro-drug. However, if indomethacin ethyl ester is converted into indomethacin after oral dosing of nanocapsules, either at the intestine lumen, intestine wall or after the nanocapsules reach the blood stream, remains to be elucidated.

After oral administration of nano- and microparticles the main uptake route has been considered the follicle-associated epithelium (FAE) in small intestine due to the presence of gut-associated lymphoid tissue (Peyer's patches). There are three possible transport routes for macromolecules, microorganisms and particles in FAE: (1) paracellular transport; (2) transcellular uptake across enterocytes by endocytic process; and (3) endocytosis by M cells (Carreno-Gómez et al., 1999). The literature described several experimental models to investigate the interaction between particles and gastrointestinal tract (Hussain et al., 2001; Ponchel and Irache, 1998). Among the *ex vivo* models, the everted intestinal sac model (Wilson and Wiseman, 1954) has been used in the past 50 years to study bioadhesive properties and substances uptake and transport in the intestine (Chen et al., 2003; Carreno-Gómez et al., 1999; Mainardes et al., 2006). Furthermore, bioadhesion and uptake of particles were previously studied *in vivo* (Jani et al., 1990; Limpanussourn et al., 1998; Jani et al., 1996; Damge et al., 1996; Florence and Hussain, 2001) and *in vitro* (Hussain et al., 1997; Santos et al., 1999; Santos et al., 2003; Mainardes et al., 2006).

Recently, we prepared lipid-core nanocapsules varying the concentrations of sorbitan monostearate and oil (capric/caprylic triglyceride), both constituents of the core, to investigate if the sustained release from those carriers could be a consequence of the variation of the core viscosity and the particle surface area (Jäger et al., 2009). The augmentation in the sorbitan monostearate concentration enhanced the resistance to the pro-drug diffusion due to the core viscosity increase. On the other hand, after increasing the oil concentration, despite the possibility of diminishing the core viscosity, the pro-drug release was delayed due to the decrease in the surface area of the colloidal system as a consequence of the increase in the particle mean size.

Taking those considerations into account, the aim of this work was to investigate if indomethacin ethyl ester released from the lipid-core nanocapsules is converted into indomethacin in the intestine lumen, intestine wall or after the particles reach the blood stream. First, the everted rat gut sac model was used to verify the transport of the pro-drug and the bioadhesion of the nanocapsules. Secondly, after oral administration of the pro-drug-loaded lipid-core nanocapsules to rats, the concentrations of indomethacin ethyl ester and indomethacin were determined in stomach, small intestine, liver, spleen, as well as in plasma from peripheric and portal vein blood.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (PCL) (M_n = 42,500 and M_w = 65,000 g mol^{-1}) was supplied by Aldrich® (Strasbourg, France). Dicyclohexylcarbodiimide, 4-(*N*,*N*-dimethyl)aminopyridine and indomethacin were obtained from Sigma (St. Louis, USA). Caprylic/capric triglyceride (density 0.94 g ml^{-1}), sorbitan monostearate and polysorbate 80 were acquired from Delaware® (Porto Alegre, Brazil). TC 199 Earle® intestinal simulated medium was obtained from Sigma–Aldrich (Strasbourg, France).

o-Aminophenol, 1,2-phenylenediamine, 5-aminosalicylic acid were acquired from Aldrich® (reagent grade) and used without purification. Diethyl β-ethoxymethylenmalonate and polyphosphoric acid were purchased from Acros Chemicals®. Methyl methacrylate, acquired from Aldrich®, was purified before polymerization by passing it through activated alumina (Merck®). 2,2′-Azo-*bis*-isobutyrylnitrile (AIBN) was obtained from Merck® and purified before use by recrystallization from methanol and maintained under vacuum. Silica gel 60 (Merck®) was used for preparative chromatographic column separations. All solvents were used as received or purified using classical standard procedures. Spectroscopic grade solvents (Merck®) were used for fluorescence and UV–vis measurements and chromatographic grade solvents (Merck®) were used for liquid chromatography (HPLC) measurements.

^{1}H NMR analyses were performed at room temperature on a Varian® (model VXR-200, 200 MHz) using tetramethylsilane as internal standard and DMSO-d_6 (Aldrich) or $CDCl_3$ (Merck) as solvents. The UV–vis and fluorescence analyses were carried out using a Shimadzu® UV-1601 PC spectrometer and a Hitachi spectrofluorometer (model F-4500), respectively. Fluorescence spectrum correction was performed to enable measuring a true spectrum by eliminating instrumental response such as wavelength characteristics of the monochromator or detector using Rhodamine B as standard (quantum counter).

2.2. Synthesis and nanocapsule preparation

2.2.1. Synthesis of indomethacin ethyl ester

The synthesis of indomethacin ethyl ester (IndOEt) was carried out under argon by preparing a solution of indomethacin (5 mmol) in ethanol (20 ml) that was added of 4-(*N*,*N*-dimethyl)aminopyridine (0.2 mmol) (Pohlmann et al., 2004). The solution was stirred for 10 min at 0 °C following the addition of dicyclohexylcarbodiimide (5 mmol). After 30 min at 0 °C and 16 h at room temperature, the solvent was evaporated under reduced pressure. The residue was added of dichloromethane (30 ml) and the suspension was filtered. The filtrate was extracted with saturated $NaHCO_3$ aqueous solution (3 × 10 ml) to remove the unreacted indomethacin. Then, the organic phase was dried with anhydrous $MgSO_4$, filtered and evaporated. The product was purified by column chromatography (Silica gel 60, 70–230 mesh) using ethyl acetate and cyclohexane (1:1, v/v) as eluent. The isolated product was obtained as a solid (80% of yield) presenting a melting point (uncorrected) of 82–83 °C.

^{1}H NMR 200 MHz (δ, ppm) $CDCl_3$: 7.66 and 7.46 (AB system, 2H and 2H, ArH *p*-chlorobenzoyl), 6.97 (d, 1H *J* = 2.5 Hz, H-4), 6.87 (d,