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Ibuprofen Nanoparticles for Oral Delivery: Proof of Concept

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Abstract

Background: Using nanoparticles to improve non-steroidal anti-inflammatory drugs therapeutic profile is an interesting approach, especially concerning their gastric toxicity.

Objective: The aim of this work was to present a proof of concept for a nanoparticulate formulation composed of a biodegradable polymer poly (DL-lactic acid) (PLA) meant for oral delivery of ibuprofen (IBU) to systemic circulation with reduced gastric toxicity.

Materials and methods: IBU-loaded nanoparticles composed of PLA and poloxamer 188 were prepared by an emulsion/solvent diffusion method. The particles obtained were characterized for size, zeta potential and morphology, as well as encapsulation efficiency. Nanoparticles were given to *Wistar* rats at an equivalent dose of 12 mg/kg (t.i.d.) of ibuprofen for a period of 10 days. Both concentration of IBU in the plasma and toxicity in different tissues were evaluated.

Results: Nanoparticles displayed a size of 281.1 ± 66.7 nm with a zeta potential of -4.3 mV. Scanning electron microscopic images showed spherical shape particles with low polydispersity index. IBU concentration in blood samples indicated that nanoparticles were able to deliver IBU to systemic circulation. A significant reduction in toxicity was observed for nanoparticles in gastric mucosa compared to free ibuprofen. This may be due to controlled release of IBU from the nanoparticles, which decreases the mucosal contact to IBU. In summary, we designed a proof of concept for PLA nanoparticles as suitable carrier for IBU allowing reduced gastric toxicity of the drugs. This strategy can eventually be applied to other non-steroidal anti-inflammatory drugs.

Introduction

Ibuprofen, *2-(4-Isobutylphenyl)propionic acid*, is a NSAID that inhibits the cyclooxygenase system. It is generally used as analgesic and antipyretic for a variety of inflammatory pathologies [1]. IBU can be used as short duration therapy (e.g. headache) or as chronic therapy, such as in osteoarthritis and rheumatoid arthritis. Although IBU has poor water solubility (~ 1 mg/mL) [2], when given orally it is well absorbed (approximately 100%, with a peak plasma concentration around 1-2 hours after ingestion [3,4]. However, it is rapidly eliminated from systemic circulation displaying a relatively short half-life (1.7-2 h), and therefore, requiring several dosages for an effective and prolonged pharmacological activity [5]. Furthermore, when taken for chronic diseases, the maintenance of analgesic effect is more important than the rapid onset of action. Considering all this, different exposures to IBU are expected and consequently, are associated to different risks for adverse effects. Despite being better tolerated in comparison to other NSAIDs [1], it has demonstrated NSAIDs-related gastric toxicity, including gastric irritation and bleeding, abdominal pain and ulcers [6]. Hence, to achieve effective and prolonged drug levels for an extended period without having related gastric effects, IBU is a potential candidate for a new formulation based on controlled and sustained release.

Various strategies have been explored to avoid NSAIDs-related toxicity, including concomitant administration of gastric protectors (as free-drugs or coupled) [7], use of rectal drug delivery systems [8], or modified release formulations [9]. The development of oral controlled and sustained release offers a potential benefit for NSAIDs.

The rationale behind it is allowing the release of the drug at a desired rate, providing sustained levels (fewer doses required) and reducing the contact with gastric mucosa (reduced gastric damage). To this end, polymeric nanoparticles have raised increased attention due to their properties and benefits for drug pharmaceutical performance (site-specific targeting and controlled release) [10].

Nanoparticles are solid colloidal delivery systems capable of releasing optimum amounts of drug, while avoiding premature release. Also, due to their small size, they are able to be absorbed through the oral mucosa to reach systemic circulation [11]. The possibility of using biocompatible and biodegradable polymers is another advantage, since the vehicle itself is then removed naturally without promoting toxicity [12].

Poly(lactic acid) (PLA) is a biocompatible and biodegradable linear aliphatic polyester that is regarded as safe [12]. PLA has been extensively

studied for drug delivery applications [13-15]. Due to its insolubility in water, it provides a good platform to easily produce nanoparticles, and is suitable for the encapsulation of poor water soluble molecules, such as IBU. As a result, it is expected that the IBU-loaded PLA nanoparticles will exhibit high loading capacity with a controlled release of IBU and a decrease in gastric irritation.

The aim of the work herein described was to develop IBU-loaded PLA nanoparticles as a proof of concept of the value of this formulation as a delivery systems for NSAIDs, in general. In order to do so, we assessed, through *in vivo* studies, its ability to reduce gastric toxicity and other NSAIDs-related toxicity and verified if IBU is able to reach systemic circulation.

Materials and Methods

Materials

Ibuprofen and Poloxamer (POLX) were obtained from Sigma-Aldrich (Steinheim, Germany). PLA (Purasorb PDL02) was obtained from PURAC (Barcelona, Spain). Benzophenone was obtained from Scharlau (Barcelona, Spain). All chemicals or solvents were of analytical grade.

Nanoparticle preparation

Ibuprofen nanoparticles were prepared by a method of spontaneous emulsification/solvent diffusion (SESD), as generally described in the literature [16]. Briefly, 4 g of PLA and 50 mg of IBU were dissolved in ethyl acetate (2%, v/v) and saturated with water (for a total of 200 mL). The emulsification was produced by the addition of the previous solution to 400 mL of a saturated aqueous solution of ethyl acetate with POLX at 5% (w/v) under stirring for 5 minutes at 11,000 rpm. Then, an excess of water was added to the emulsion under moderate stirring (400 rpm) for 5 minutes. The solvent was evaporated under reduced pressure at 40°C. The remaining solution was then centrifuged and the obtained pellet was used for further experiments.

Characterization of IBU-loaded PLA nanoparticles

Determination of particle size and zeta (ζ) potential: The particle size and zeta potential were measured in Delsa™ Nano C (Coulter Beckman, USA) by photon correlation spectroscopy (PCS) and electrophoretic mobility, respectively. Both measurements of size and zeta potential were performed after centrifugation and re-suspension of the nanoparticles in distilled water. Each analysis was carried out at 25°C with at a scattering angle of 90° and in triplicate. The shape and surface morphology of IBU-loaded PLA nanoparticles were also evaluated using scanning electron microscopy (SEM) analysis. Metal grids were coated with double-faced adhesive tape and re-suspended nanoparticles were deposited on the sticky surface. The samples were then dried and coated with gold before examination in the microscope.

Encapsulation efficiency: The encapsulation efficiency was evaluated by quantification of the unbound drug in the supernatant. Two milliliters of sodium hydroxide were added to 1 mL of the clear supernatant obtained from centrifugation, in order to remove any interference from PLA and solubilize IBU [2,17]. The quantification of IBU was performed at 264 nm by spectrophotometry. Standard solutions with concentrations ranging from 1 to 500 μg/mL in a mixture of 2:1 water: ethyl acetate were produced and used to construct a standard curve ($R^2>0.999$). Values are expressed as mean ± standard deviation of three independent experiments.

In vivo study design

In vivo studies were performed to evaluate the ability of

nanoparticles to deliver IBU to systemic circulation and their ability to prevent IBU-related gastric toxicity. *Wistar* rats (male with 200-300 g with two months old) were divided into 3 groups: (i) one group dosed with ibuprofen loaded-nanoparticles (n=7); (ii) the second group dosed with free ibuprofen aqueous solution (n=3); (iii) the third group dosed with phosphate buffer pH 7.4 (control) (n=2). A single rat receiving water was kept as normal control. Rats were weighted on a daily basis and allowed free access to water and food on a 12 h light/dark cycle. All formulations were given orally and studies were conducted in conformity with the animal ethics guidelines for care and use of laboratory animals and approved by ethics committee.

Animals received either 12 mg/kg (3 times a day) of IBU (suspended in aqueous medium), or an equivalent dose of IBU encapsulated into PLA nanoparticles. Following 10 days of treatment after the last administration, rats were sacrificed and blood samples were collected. The blood was transferred to eppendorf tubes with 10 μL of heparin sodium salt to prevent blood clotting. Samples were then centrifuged at 4000 rpm for 5 minutes and the plasma was transferred into another eppendorf tube and kept in the cold until HPLC analysis. On the other hand, stomachs, esophaguses, livers and kidneys were isolated and rinsed with saline, measured, weighted and prepared for histological analysis. Therefore, the organs were cut into transverse fragments (5 mm) and immersed in a 10% formaldehyde solution for 24 h. The pieces were processed into paraffin and stored at -20°C. The fragments were cut in 4 μm pieces in a SHANDON (AS 325) microtome and stained with Hematoxylin and Eosin. The resulting images were obtained and observed using a NIKON (Eclipse E600) microscope.

For evaluation of the type of lesions in the esophagus and gastric mucosa, a criterion was established, as shown in Table 1. Each tissue sample was scored based on found lesions, according to an arbitrary grading system. The ulcer index (U.I.) for each stomach and esophagus was the sum of scores of all lesions and reported as median (minimum-maximum). The significance of differences between groups was assessed and $p<0.05$ versus control was taken as significant.

Determination of IBU in rat's plasma by HPLC: IBU concentrations in plasma were analyzed by HPLC [18], solely to demonstrate the formulation's ability in allowing quantifiable systemic drug concentrations. From the blood samples taken, 0.5 mL of plasma was treated with 20 μL of benzophenone (internal standard reference) and methanol (to a final volume of 2 mL). The mixture was centrifuged at $300\times g$ for 10 minutes and then analyzed. HPLC separation was performed with a 20 μL injection volume on a LiChroCART 250-4 RP18 column using a mobile phase composed of methanol/phosphoric acid (80:20, v/v). The eluent was monitored at a wavelength of 264 nm and the samples were eluted at a flow rate of 1 mL/min. The elution was performed under isocratic conditions at room temperature. The method was linear in a range of 0.1-50 μg/mL using the same conditions above.

Type of lesion	Score of lesion severity
Normal tissue	0
Inflammation	0.5
Loss of mucosal folds	1
Hemorrhagic areas	1.5
Ulcers/necrotic areas	2
Perforation	3

Table 1: Criterion to evaluate toxicity in different organs.

Results

Characterization of the formulation

The IBU-loaded PLA nanoparticles showed homogeneous distribution (polydispersity index of 0.1), without any noticeable aggregation from the PCS analysis and SEM, with a mean particle size of 281.1 ± 66.7 nm and a zeta potential of -4.3 mV. Such surface charge is expectable due to the high POLX content comparatively to the PLA content. The zeta potential is a function of the surface charge, and therefore, is a predictor value of the stability of the particles. Although particles had a low zeta potential which is normally associated with instability (aggregation due to Van Der Waals inter-particle attraction). SEM analysis (Figure 1) showed well-defined smooth surface and spherical particles, without noticeable aggregation, caused possibly by the presence of POLX as a steric stabilizer [19]. Furthermore, since zeta values close to zero may be undesirable, we could argue the possibility of lowering the amount of POLX used, in order to obtain higher negative zeta potential. However, even PLA particles with stronger surface charge (ranging from -40 to -55 mV) have also been associated to gastric instability due to flocculation [19], thus this alternative scenario would also be undesirable. Moreover, no free drug crystals have been identified and this particulate system achieved high encapsulation efficiency, being able to retain 84 ± 5% of the initial amount of IBU.

In agreement to our results, various methods have been reported to produce PLA nanoparticles with small size and low polydispersivity [16,20,21]. Among them, the SESD method, as used here, is a choice methodology since it is simple to perform, has high batch-to-batch reproducibility and low-energetic input to form nanoparticles [22]. The encapsulation by SESD is based on the dissolution of the polymer with subsequent precipitation of the same, by diffusion of the solvent to the aqueous phase [16]. The POLX acted as stabilizer of the nanoparticulate system, in order to modulate the size and surface properties, as well as stability of nanoparticles.

In vivo study in rats-toxicity assessment

The amenability of PLA nanoparticles as a carrier for IBU was further investigated *in vivo* after oral administration. The IBU levels were evaluated in plasma and NSAIDs-related toxicity was evaluated in the different organs macro- and microscopically. As observed by HPLC analysis (Figure 2), IBU was detected in blood samples taken from rats receiving IBU-loaded PLA nanoparticles (1 from Figure 2) and free IBU (2 from Figure 2), while controls did not show any peak of IBU (3 from Figure 2). However, the concentration peak in blood from rats receiving IBU-loaded PLA nanoparticles was smaller than that reached with free IBU. This difference could be attributed to the polymeric network that could have retarded the release of IBU, therefore suggesting a controlled
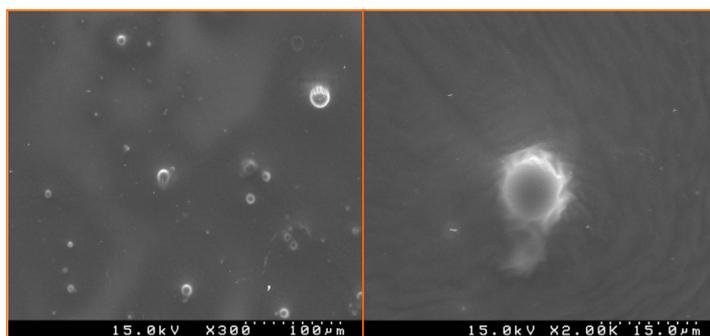

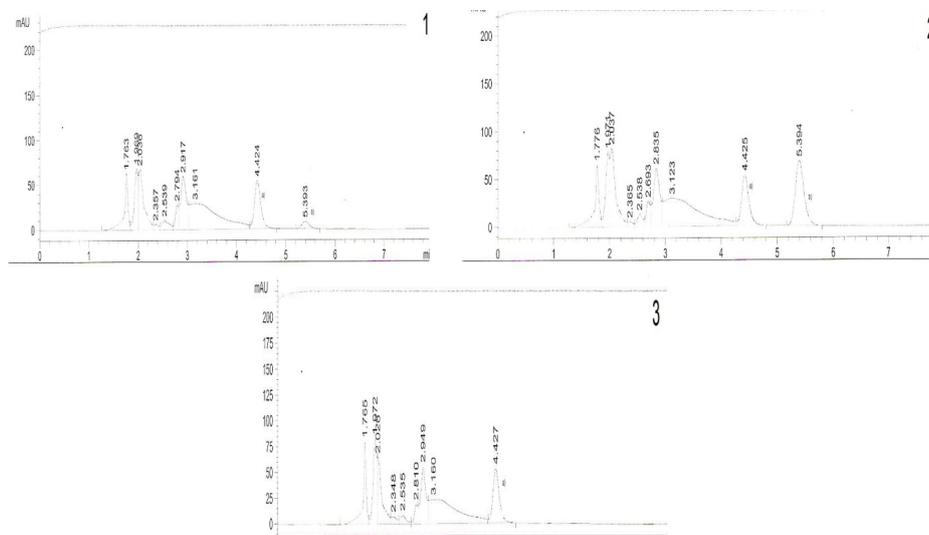**Figure 1:** SEM analysis of IBU-loaded PLA nanoparticles.

Figure 2: HPLC analysis of blood samples; 1: Group taking IBU-loaded PLA nanoparticles; 2: Group taking Ibuprofen; 3: Control group (phosphate buffer); Peak a) Benzophenone (Time retention =4.4 min); peak b) Ibuprofen (Time retention=5.4 min).

Experimental group	Product(s) administered (p.o.)	Dose	Ulcer index (U.I.) [median (min-max)]	
			stomach	esophagus
IBU-NP group (n=7)	IBU loaded-nanoparticles	12 mg/kg	1.6 (4.5-0.0)	0.9 (2-0)
IBU group (n=3)	IBU in aqueous solution	12 mg/kg	2.0 (2.5-1)	1.0
Control group (n=2)	PBS 7.4	0	0.0	0.0
Normal rat (n=1)	water	0	0.0	0.0

Table 2: Ulcer index in the stomach and the esophagus in the different groups studied.
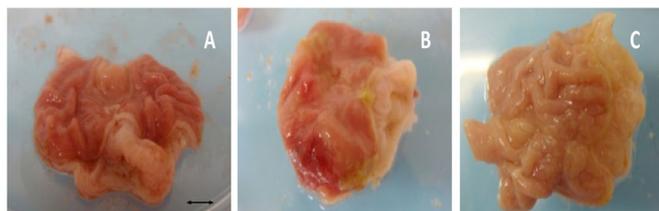

Figure 3: Stomach morphology after intake of (A) IBU-loaded PLA nanoparticles; (B) free IBU and (C) Control.
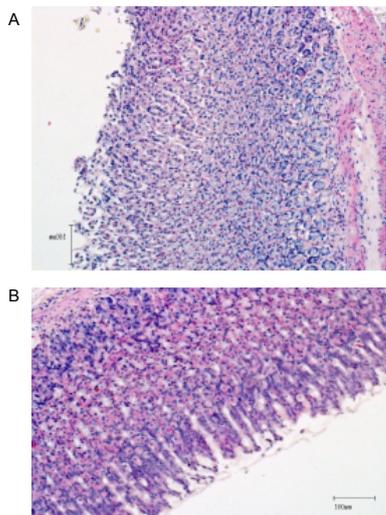

Figure 4: Microscopic analysis of stomach after intake of (A) IBU-loaded PLA nanoparticles; and (B) free IBU. Scale bars indicate 5 mm.

release. Moreover, due to their small size, nanoparticles are expected to be uptake to systemic circulation. In fact, the size of the particles obtained is in the range for good oral absorption, especially by M cells in Peyer's Patches [11]. Since IBU is poorly soluble in water at physiological pH, nanoparticles could have resulted in a reservoir from which IBU would slowly diffuse.

The various organs were examined and no differences were observed in weight and size. However, slight differences in esophagus and stomach morphology could be observed between groups receiving free IBU and IBU-loaded PLA nanoparticles (Table 2), revealing a slightly higher U.I. in the group receiving IBU. NSAIDs-related toxicity occurs mainly at the levels of the gastric epithelium. As it can be observed (Table 2 and Figure 3A), major macroscopic alterations were observed

in the stomach. For rats receiving IBU-loaded PLA nanoparticles (Figure 3A), the macroscopic morphology was maintained to some extent. However, for rats receiving free IBU, the structural morphology was completely altered (complete loss of gastric mucosal surface), with evidence of bleeding hemorrhagic focus, as well as ulcers (Figure 3B). In some cases, all mucosal surfaces showed to be edematous with inflammatory infiltrates. Histological analysis of stomach (Figure 4A and 4B) corroborates the decrease of IBU-related toxicity in the stomach mucosa when IBU was administered in nanoparticle dosage form. High amounts of infiltrated red cells were observed for free IBU, while with IBU-loaded PLA nanoparticles, these infiltrations were very low. Moreover, the latter showed no evident hemorrhage and the maintenance of gastric pits was observed, which is a sign of substantially reduced IBU toxicity. The same pattern was observed for the esophagus, although to a lesser extent. Complete loss of the epithelium was observed for all rats receiving IBU, while in rats receiving IBU-loaded PLA nanoparticles, this effect was slightly decreased.

Figure 5 shows the histological images of liver and kidney for control animals and animals treated with free IBU and IBU-loaded PLA nanoparticles. No differences were observed between the control and test group. The images from the liver show well shaped hepatic cells, with no signs of blood stasis in the extracellular medium or necrotic cells (Figure 5B). Also, the kidney's histological analysis reveals no differences between control and test group (Figures 5D-5E). Together, these results reveal the absence of liver and kidney toxicity of IBU-loaded PLA nanoparticles.

Results provide evidence of cause-effect relationship between IBU uptake and gastric injuries, observed by the absence of damage in the control group, and that PLA nanoparticles could offer an advantage of protection against NSAIDs-related gastric toxicity. The lack of statistical significance between I.U. of free and encapsulated drug can probably be explained by the fact that one single rat in the IBU-NP
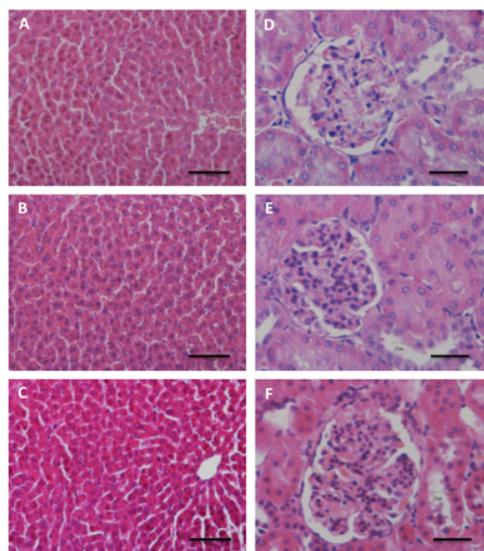

Figure 5: Histological study of liver and kidney. Staining with hematoxylin (cell nuclei-blue) and eosin (cytoplasm-pink/red). A-C: Microscopic analysis of liver: Negative control group (A); Animals treated with IBU-loaded PLA nanoparticles (B); Animals treated with free IBU (C); 20x magnification in the original, Bar=50 µm. D-F: Microscopic analysis of kidney: Negative control group (D); Animals treated with IBU-loaded PLA nanoparticles (E); Animals treated with free IBU (F). 40X magnification in the original, Bar=25 µm.

group showed much higher tissue damage than the rest of the group. However, it should be noted that the IBU-NP group presented animals without any lesion both in the stomach and the esophagus, contrarily to the IBU group, which is indicative of the gastro-protective potential of this formulation.

We hypothesize that PLA allowed the reduction of gastric toxicity by avoiding the direct contact between gastric mucosa and the drug by retardation of its release. Since IBU belongs to the class II drugs of the BCS, this means that the limiting step for its uptake will be the release from the polymeric matrix. The reduced uptake of IBU in the stomach by nanoparticles could, therefore, have a major impact in patients taking chronic therapeutics. The fact that PLA is hydrolysable in aqueous environment and the degradation products are biocompatible [23], represents an extra advantage, which was confirmed by the fact that no toxicity non-related to IBU was found. Other polymers have been reported to encapsulate NSAIDs to alter their delivery. As an example, IBU-loaded PLGA nanoparticles [24] and Eudragit L100 nanoparticles [4] allowed the controlled release of IBU. Others [2] reported diethylaminoethyl-dextran IBU nanoparticles allowed a pH sensitive, burst release. Furthermore, nanocapsules with indomethacine effectively prevented intestinal lesions [9].

As intended from the established goals, this study is in fact a proof of concept for a formulation that will allow IBU administration with reduced gastric toxicity. This would ultimately increase IBU efficacy and safety, offering a major advantage over conventional formulations in improving clinical outcome. Furthermore, these results offer the possibility of using PLA nanoparticles for an extended number of drugs of the same group.

Declaration of Interest

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