Jejunal Absorption, Pharmacological Activity, and Pharmacokinetic Evaluation of Indomethacin-Loaded Poly(*d,l*-Lactide) and Poly(Isobutyl-Cyanoacrylate) Nanocapsules in Rats

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The jejunal absorption of indomethacin nanocapsules was studied using an *in vivo* infusion technique. Jejunal absorption of indomethacin from the nanocapsules was slightly delayed as compared to a commercial indomethacin solution. The plasma and jejunal mucosa indomethacin concentrations were similar in both cases. However, the nanocapsules protected the rat jejunum from the ulcerating effect of indomethacin, probably by avoiding direct contact of the free drug with the surface of the mucosa. The pharmacokinetic profile of indomethacin nanocapsule formulations was compared to a solution of free drug following oral administration of 5 mg/kg in rats; no difference in the mean concentration–time profiles of the drug was observed. Blood levels of thromboxane showed a sustained biological activity, over a period of 24 hr, of indomethacin-loaded nanocapsules, relative to the drug in solution, following oral administration.

KEY WORDS: indomethacin; polymeric nanocapsules; jejunal absorption; oral pharmacokinetics; thromboxane; intestinal lesions.

INTRODUCTION

Nanocapsules, prepared using an interfacial polymerization process for isobutylcyanoacrylate monomer (PIBCA), were recently proposed as a new type of vesicular colloidal polymeric drug carrier (1). They may present significant advantages over liposomes since they are stable in the gastrointestinal tract (2). In addition, the PIBCA nanocapsules were able to cross the intestinal mucosa (3), suggesting that they may be valuable for oral administration of poorly absorbed drugs.

More recently, Fessi *et al.* (4) have proposed another method of nanocapsule preparation involving the interfacial deposition of a preformed poly(*d,l*-lactide) polymer (PLA). This method would avoid any risk of cross-reaction between the content of nanocapsules, especially the drug molecules, and the acrylic monomer during the polymerization process, as has been described for PIBCA nanocapsules (5).

The aim of the present study was to evaluate the ability of PIBCA and PLA nanocapsule formulations to protect the gastrointestinal mucosa from the ulcerating effect of oral indomethacin in rats. It was anticipated that protection would arise from avoiding direct contact of the free drug with the surface of mucosa, as suggested for indomethacin liposomes (6).

Indomethacin blocks prostaglandin synthesis by inhibiting cyclooxygenase activity (7). A pharmacological evaluation, involving determination of prostaglandin blood levels, was carried out in an attempt to determine the ability of these nanocapsules to release their content in the free pharmacologically active form following oral administration to rats. In addition, the pharmacokinetic profiles of indomethacin dosages in PLA and PIBCA nanocapsules and in solution were compared following oral administration to rats.

MATERIALS AND METHODS

Materials

Indomethacin was obtained from Sigma (St. Louis, MO); poly(*d,l*-lactide), MW 120,000, was purchased from Phusis (LeVersoud, France). Isobutylcyanoacrylate monomer was obtained from Ethnor S.A. (Paris, France). Phospholipid mixture (Epikuron 170) and poloxamer (Synperonic F68) were supplied by Lucas Meyer (Hamburg, FRG) and ICI-FRANCE (Clamart, France), respectively. All other analytical grade ingredients were purchased from Prolabo (Paris).

Preparation of Nanocapsules

Nanocapsules of PLA containing indomethacin were prepared according to the following procedure (4): 125 mg of PLA was first dissolved in acetone. A mixture of phospholipids (250 mg) was also dissolved in this acetone by heating close to the boiling point. One-half milliliter of benzyl benzoate containing 12.5 mg of indomethacin was then added to the acetone solution. The resulting organic solution (25 ml) was poured, under light magnetic stirring, into 50 ml of water containing 250 mg of poloxamer. The resulting mixed phase immediately turned milky with bluish opalescence as a result of the formation of nanocapsules. The acetone, which diffused rapidly into the aqueous phase, was removed under reduced pressure. The colloidal aqueous suspension was then concentrated to the desired final volume (10 ml) by removal of water under the same conditions. The PIBCA nanocapsules were prepared according to the method reported by Al-Khouri *et al.* (1) using the same combination of surface active agents as for the PLA nanocapsules above but with 0.125 ml of isobutylcyanoacrylate monomer in 25 ml of ethanol instead of 125 mg of PLA polymer in acetone.

Nanocapsule Evaluation

Morphological examination of nanocapsules was performed using a transmission electron microscope (TEM) after negative staining with tungstophosphoric acid solution

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(0.5%). Free drug was determined in the clear supernatant following separation of nanocapsules from aqueous medium by a combined ultrafiltration centrifugation technique (Centrisart I 20.000 Sartorius, FRG). Total indomethacin was measured following complete dissolution of the nanocapsules in acetonitrile. The indomethacin content of the nanocapsules was calculated by difference between these measured values. Indomethacin was determined using a modified HPLC technique with clomethacin as an internal standard (8). *In vitro* release kinetics of indomethacin from nanocapsules were examined under various appropriate sink and pH conditions. All these determinations were conducted on both types of nanocapsules. A detailed description of the various analytical techniques used to characterize the nanocapsules formed and their drug content release profiles, as well as the effect of various process parameters on the properties of the two types of nanocapsules, have been recently reported (9).

Indomethacin Jejunal Perfusion Studies

After an overnight fast, male Wistar rats, 250–300 g, were anesthetized by an im injection of urethane (1.5 g/kg). Following laparotomy, the jejunum was exposed and the ligament of Treitz localized. Lengths (equivalent to five venous arcades) of the jejunal segment to be perfused were taken 2 cm distal to the ligament of Treitz. A catheter was inserted in the jejunum and secured with a silk suture. A second catheter of the same type was placed in the same manner 10 cm distal to the first one. The perfused segment was kept inside the rat body without disrupting the blood flow. The temperature of the exposed tissue was kept at 37°C, with a lamp at an appropriate distance. A compress impregnated with saline kept the tissues wet, as previously reported by other authors (10). The cannulated segment was rinsed with saline and connected to an open perfusion system, previously filled with saline to exclude air from the system. The peristaltic pump was operated at a rate of 0.5 ml/min. The colloidal drug suspensions (PLA or PIBCA nanocapsules) or a solution, equivalent all at a dose of 5 mg/kg of indomethacin, were diluted to a final volume of 30 ml with bidistilled water and saline, respectively. The liquid was infused over 60 min. The jejunal segment was further rinsed with saline (20 ml) at the end of the drug infusion. The nonabsorbed indomethacin was assayed from the collected solution in the receptor compartment using HPLC assay. Blood samples were collected in heparinized tubes from the abdominal artery at the end of the experiment. Indomethacin levels in plasma were then estimated by the previously described HPLC technique. The infused jejunal segment was excised, then slit open opposite the attached mesenteric tissue, and the mucosis surface was examined for superficial macroscopic lesion points using a dissecting binocular microscope. The mucosae were removed by scraping with a glass slide, homogenized in saline, and assayed for indomethacin by HPLC. Afterward, the musculosis was also examined in the same manner for interior macroscopic lesion points. In the present study, lesions were scored according to an arbitrary scale: 0 = normal, 1 = ulcerations ≤ 1 mm in diameter, and 2 = ulcerations >1 mm in diameter. Such an approach has been used by other authors who investigated the potential ulcerative effect of indomethacin (11). Six rats were used for each experiment.

Indomethacin Pharmacokinetic Studies

Rats were treated orally using a syringe with either indomethacin solution or indomethacin-loaded nanocapsule suspensions of both types (5 mg/kg). Blood samples were collected in heparinized tubes at various time intervals (0.5–24 hr) from the three groups of rats. Each group consisted of 36 rats. Four rats were sacrificed for indomethacin estimation at each time point, thus the plasma drug levels reported are means of eight measurements (duplicate for each rat). Plasma was separated by centrifugation and frozen prior to further processing. Indomethacin levels in plasma were determined by the previously cited HPLC technique. Peak concentrations (C_{max}) and peak times (T_{max}) were extracted from the experimental data, whereas the half-life of elimination ($t_{1/2}$) was determined from the slope of the terminal post-absorption curves. Areas under the plasma concentration–time curves ($AUC^{0-24\ h}$) were calculated using the trapezoidal rule.

Thromboxane Blood Level Studies

Three groups of 10 rats each were given indomethacin (5 mg/kg) by the oral route in either saline, PIBCA nanocapsules, or PLA nanocapsules. Three groups (10 rats each) were given an identical volume of either saline or aqueous suspension of empty similar nanocapsules. Five animals in each group were sacrificed either 4 or 24 hr after oral administration. Blood samples were collected in nonheparinized tubes, serum was separated following centrifugation, and all samples were frozen at −80°C. The thromboxane serum level was estimated using an enzymoimmunological assay (Laboratoire des Stallergènes; Fresnes, France). The measurement is obtained by competition using constant and limited concentrations of antibodies (anti-rabbit IgG) and tracers (Eicosanoid and conjugated eicosanoid-acetylcholinesterase) (12).

Statistical Analysis

All animal experiments were done sequentially. One-factor analysis of variance (ANOVA) was used to compare experimental data concerning the perfusion experiment: percentage absorbed, plasmatic and mucosal concentrations, severity of the lesional index. A simple paired t test was applied to compare the severity index of intestinal lesions following the perfusion of indomethacin solution between the mucosis and the musculosis of the same animals. Two-way analysis of variance (ANOVA) was used to test the stability and the homogeneity of the thromboxane blood level of the control groups with time and between groups. One-factor analysis of variance (ANOVA) was used to compare the means of the thromboxane blood levels between treated and control groups.

RESULTS AND DISCUSSION

Physicochemical Characterization

Both methods of preparation yielded spherical vesicular nanocapsules, the properties of which have been reported elsewhere (9). Particle size distribution determinations indicated no marked difference in mean diameter between PLA

(229 ± 29 nm) and PIBCA nanocapsules (237 ± 24 nm). The amount of indomethacin in both nanocapsule suspensions reached 0.25% (w/v), corresponding to a 5% solution of indomethacin in benzyl benzoate, and 100% of the drug was encapsulated. *In vitro* results showed that PLA and PIBCA nanocapsules rapidly released indomethacin when the physicochemical conditions were close to physiological environmental conditions (13), whereas drug release was very poor at pH's below 5 as a result of the poor solubility of indomethacin in the acidic conditions which are encountered in the stomach and proximal intestinal tract. Further, the stability of nanocapsules in rat plasma was studied; the morphology was not affected following 4-hr incubations, as previously reported (9).

Animal Studies

The high percentage of drug absorbed by jejunum (70%) during a 1-hr infusion of the aqueous solution (Table I) confirms the complete and prompt absorption of indomethacin from the entire gastrointestinal tract as previously reported in the literature (14). No significant difference was observed between PIBCA nanocapsules and drug solution, while the fraction absorbed was significantly smaller with PLA nanocapsules. This result indicates that the rate of indomethacin jejunal absorption from the PLA nanocapsules was slightly slower than from the aqueous solution and PIBCA nanocapsules, which were quite similar. In contrast, mucosal and plasma concentrations were in the same range and no significant difference was observed between the three dosage forms (Table I).

Nanoencapsulation of indomethacin induced a protective effect on the jejunal tissue as compared to the ulcerative effect observed with the aqueous solution of the drug (Fig. 1). The lesions were noticed on the surface of the mucosis as well as on the surface of the musculosis when the drug solution was used. No lesion of any kind was noticed following the administration of indomethacin-loaded PLA nanocapsules, and only a few superficial lesions were detected with PIBCA nanocapsules. The similarity in the mucosal and the plasmatic concentrations associated with the induced protective effect suggested that nanoencapsulation prevented direct contact of free indomethacin with the surface of the jejunal tissues. Further, a simple o/w emulsion of indomethacin was previously shown to induce a protective effect, but not total protection as observed in the present case with PLA nanocapsules (15). This result suggests that the formation of a thin polymeric film around the oily nanodroplets is needed to prevent immediate release of indomethacin from the dosage form in the gastrointestinal tract.

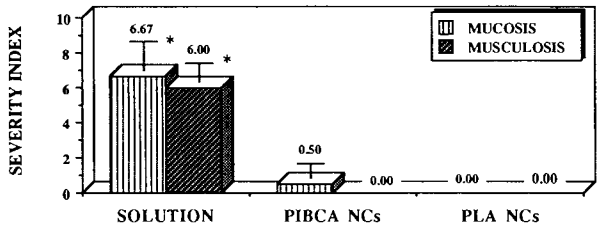

Fig. 1. Comparative ulcerative effect of indomethacin from aqueous solution, PIBCA and PLA nanocapsules at a dose of 5 mg/kg following 1-hr perfusion in the isolated rat jejunum. (*)$P < 0.05$.

Following oral administration, no significant difference was noted between the overall pharmacokinetic parameters (Table II) of the indomethacin solution and both types of nanocapsule suspensions and similar mean plasma concentration–time profiles were observed (Fig. 2). However, the values of AUC yielded by PLA and PIBCA nanocapsules (158.5 and 145.6 μg/ml/hr, respectively) were somewhat smaller than the AUC value yielded by an aqueous solution of indomethacin (189.3 μg/ml/hr), leading to a relative bioavailability of 0.77–0.84 (calculated by ratio of AUC of nanocapsules to AUC of aqueous solution). In addition, the peak plasma concentration value, C_{max}, obtained with the solution was moderately higher (22.04 ± 0.6 μg/ml) than those for PLA and PIBCA nanocapsules (15.89 ± 1.6 and 14.78 ± 1.1 μg/ml, respectively). The absorption rate was slower in the case of the indomethacin PLA nanocapsule suspension, confirming the previous results from the jejunal absorption experiment (T_{max} value of 3 hr, as compared to 2 hr for both the solution and the PIBCA nanocapsule suspension). The absorption of indomethacin from nanocapsules may be expected to be governed by the duration of contact between the colloidal carrier and the gastrointestinal mucosae. It may also be attributed to a different pathway of absorption, confirming previous results reported for PIBCA nanocapsules (16). It should be emphasized that the plasma half-life for the elimination of indomethacin was prolonged with the nanoencapsulated forms (6.32 and 6.28 hr for PLA and PIBCA nanocapsules, respectively), compared to the value found in our study with the indomethacin solution (4.47 hr), which was in agreement with the value reported by Hucker *et al.* in 1966 (14).

The thromboxane blood level determinations, carried

Table I. Mean (±SD) Data Following 1-hr Perfusion of the Isolated Rat Jejunum with Indomethacin (5 mg/kg)[a]

Dosage form	Aqueous solution	PIBCA nanocapsules	PLA nanocapsules
Absorption (%)	69.8 ± 2.4	64.2 ± 9.8	50.2 ± 5.7*
Mucosis conc. (μg/g)	1.8 ± 0.6	1.5 ± 0.4	1.8 ± 0.7
Plasma conc. (μg/ml)	1.3 ± 0.7	1.9 ± 0.7	1.5 ± 0.9

[a] Six animals were used for each time point.
* $P < 0.05$.

Table II. Pharmacokinetic Parameters Following Oral Administration of Indomethacin (5 mg/kg) to Rats

Dosage form	Aqueous solution	PIBCA nanocapsules	PLA nanocapsules
C_{max} (μg/ml)[a]	22.04 ± 0.60	14.78 ± 1.06	15.89 ± 1.57
T_{max} (hr)	2.0	2.0	3.0
$AUC^{0-24\ hr}$ (μg/ml/hr)	189.3	145.6	158.5
$t_{1/2}$ (hr)	4.47	6.28	6.32

[a] Mean ± SD.

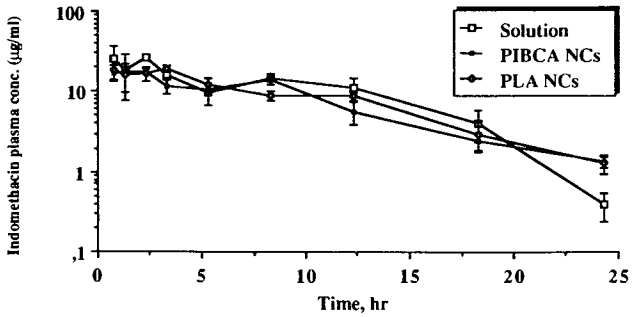

Fig. 2. Plasma concentration profiles following oral administration of 5 mg/kg indomethacin from aqueous solution and PIBCA and PLA nanocapsules.

out 4 and 24 hr after oral administration, were not significantly different in all three control groups, demonstrating that neither type of empty nanocapsules had an effect on thromboxane blood concentration in rats. In contrast, in the treated groups, a marked thromboxane blood level reduction was noted at 4 hr, reflecting the rapid onset of the pharmacological activity of the drug administered in the various dosage forms (Fig. 3). The magnitude of the reduction was similar with indomethacin solution and indomethacin-loaded nanocapsules, and no statistical difference was observed between the various treated groups (Fig. 3). However, at 24 hr, the inhibitory effect was more pronounced with indomethacin-loaded PLA nanocapsules than with PIBCA nanocapsules, and least pronounced with indomethacin solution. The pharmacological activity of indomethacin-loaded PLA nanocapsules was maintained to an extent similar to that after 4 hr, while in the case of indomethacin aqueous solution and PIBCA nanocapsules, the reduction in blood levels of thromboxane was smaller, as shown in Fig. 4. Moreover, the thromboxane blood level values reported at 24 hr were statistically different from those at 4 hr in the case of the solution ($P < 0.05$) and PIBCA nanocapsules ($P < 0.05$); only the animals treated with PLA nanocapsules showed no significant difference between the 24-hr and the 4-hr values. These results suggest that indomethacin release from the nanocapsules was prolonged but not to an extent which would delay the onset of the pharmacological effect. This appears to be in agreement with the pharmacokinetic profile of PLA nanocapsules which showed some kind of sustained release after oral administration to the rat. It should be pointed out that we were unable to distinguish the free and nanocapsule-incorporated drug in the bloodstream. The correlation observed between the pharmacological and the pharmacokinetic evaluation could therefore suggest that at least part of the indomethacin carried by the PLA nanocapsules is retained in the oily core or slowly released through the thin biodegradable polymeric wall, in contrast to rapid indomethacin release from PIBCA nanocapsules.

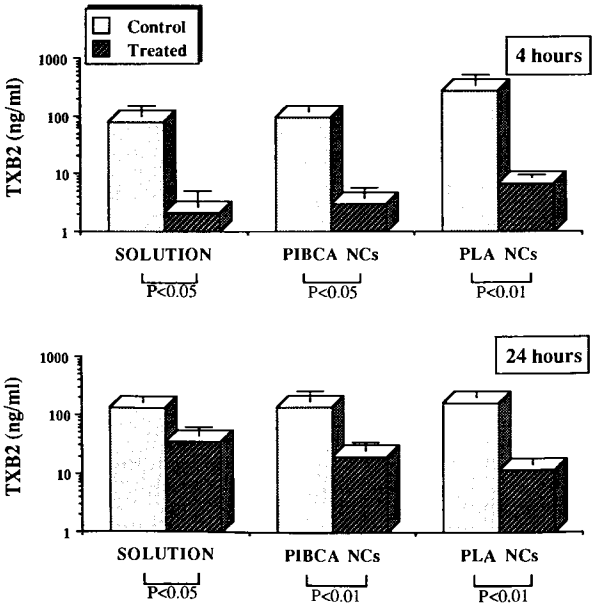

Fig. 3. Thromboxane (TX B2) blood level in rats following oral administration of 5 mg/kg indomethacin.

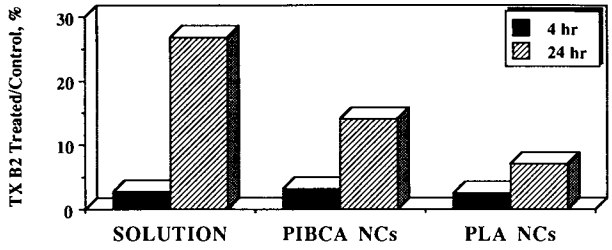

Fig. 4. Thromboxane (TX B2) blood level reduction percentage vs control groups in rats following oral administration of 5 mg/kg indomethacin at 4 and 24 hr.

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