Poly(D,L-Lactide) Nanocapsules Containing Non-Steroidal Anti-Inflammatory Drugs: Gastrointestinal Tolerance Following Intravenous and Oral Administration

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INTRODUCTION

Like other non-steroidal anti-inflammatory drugs (NSAIDs), diclofenac and indomethacin are known to exhibit gastrointestinal side-effects such as irritation and mucosal damage (1). NSAIDs may cause gastrointestinal inflammation and ulceration by a direct "barrier-breaking" effect, due to the local contact between the mucosæ and solid drug particles, and by an indirect effect, due to the local and/or systemic inhibition of enzymatic systems capable of producing protective substances, such as PGE_2 and prostacyclin (2).

While drug formulation has little or no effect on inherent systemic adverse effects of drugs, topical irritation can be decreased by appropriate formulations and dosage forms (3). The effectiveness of colloidal drug delivery systems such as liposomes (4) and nanocapsules (5) in reducing gastrointestinal ulceration following oral administration has been demonstrated.

The aim of the present study was to assess the potential of nanocapsules prepared from poly(D,L-lactide) to improve the gastrointestinal tolerance of NSAIDs in rats folloging repeated intragastric and intravenous administration.

MATERIALS AND METHODS

Chemicals

Diclofenac as its sodium salt, indomethacin, ketamine and phenylbutazone were obtained from Sigma (St Louis, Missouri USA); indomethacin as its sodium salt (Indocid®) was purchased from Merck, Sharp & Dohme (Riom, France); clomethacin was supplied by Laboratoires Cassenne (Osny, France); poly(D,L-lactide), molecular weight 88,000, was purchased from Boehringer (Ingelheim, FRG); phospholipid mixture (Epikuron 170®) was supplied by Lucas Meyer (Hamburg, FRG) and poloxamer (Synperonic PE/F 68®) by ICI (Clamart, France). Caprylic/capric triglyceride (Miglyol 810®) was obtained from Hulls (Puteaux, France). All other chemicals and solvents were of an appropriate grade and purchased from Prolabo (Paris, France).

Production of the Free Acid Form of Diclofenac

An aqueous solution of sodium diclofenac was acidified to pH 4.0 with acetic acid and was extracted three times with chloroform. After washing with water and filtering through sodium sulphate, the solvent was removed by evaporation. The diclofenac obtained in this way was characterized by infrared spectroscopy (Perkin Elmer 16 PC, Saint-Quentin en Yvelines, France) and ^{1}H-NMR (Bruker 80 MHz, Connecticut, USA).

Preparation and Characterization of Nanocapsules

Nanocapsules containing diclofenac (formulation A, Table I) or indomethacin (formulation B, Table I) were prepared by deposition of the poly(D,L-lactide) polymer following solvent displacement as previously described (5,6). Diclofenac and indomethacin concentrations in nanocapsules were determined by HPLC using phenylbutazone and clomethacin as internal standards, respectively. The HPLC equipment (Millipore, USA) consisted of a Waters TM 600 Controller, a Waters 600E Multisolvent Delivery System, a Waters TM 717 Autosampler, a Waters 484 Detector and a prepacked Nova-Pak® 3.9×150 mm C_{18} column. The mobile phase was acetonitrile-water (45:55, v:v), the pH being adjusted to 3.5 with acetic acid. A maximum of 20 μg/ml of indomethacin or diclofenac was injected. Detection was performed at 280 nm.

Animals

Experiments were carried out on male Wistar rats weighing about 350 g (Elevage Charles River, Saint-Aubin-les-Elbeuf, France).

Gastrointestinal Tolerance

The animals were divided into groups of eight. The groups were kept in separate cages and the rats were allowed to eat and drink freely. The diclofenac formulations (aqueous solution or nanocapsule formulation A, both at 3 mg/ml) were given at a dose of 20 mg/kg by the intravenous or intragastric route. The indomethacin formulations (aqueous solution and nanocapsules formulation B, both at 1 mg/ml) were given at a dose of 5 mg/kg by the intravenous or intragastric route. For intravenous administration, a catheter was implanted in the left jugular vein under anæsthesia (ketamine 150 mg/kg) and for intragastric administration, a tube was used. The formulations were administered daily for 3 consecutive days.

Twenty-four hours after the third administration, the rats were lightly anesthetized with ether and gross macroscopic examination was carried out following laparotomy. Animals were then sacrificed by a longer exposure to ether.

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Table I. Characteristics of the Diclofenac-Loaded Nanocapsules (Formulation A) and Indomethacin-Loaded Nanocapsules (Formulation B)[a]

Formulation	A	B
Diclofenac (mg/ml)	3.0	—
Indomethacin (mg/ml)	—	1.0
Miglyol 810® (mg/ml)	105.5	105.5
Size (nm)	180 ± 63	248 ± 83
pH	4.7	3.4

[a] Poly(D,L-lactide) (12.5 mg/ml), Synperonic PE-F 68® (19.2 mg/ml), and Epikuron 170® (19.2 mg/ml) were present in all formulations. Encapsulation of diclofenac and indomethacin was close to 100%.

In order to quantify gastrointestinal lesions, the stomach was opened along the greater curvature and the intestine (duodenum, jejunum and ileum) was slit open opposite the attached mesenteric tissue. The organs were washed with normal saline (0.9% NaCl) to remove luminal contents and the mucosal surfaces were examined for lesions using a magnifying glass. Lesions were scored for each organ according to an arbitrary scale (Table II) as previously reported (5). The mean organ lesional index was calculated for each group by adding the various index scores observed for each organ in all animals of the same group and then dividing the total lesional score sum by the number of animals in each group.

Experimental data were analysed according to the Kruskal-Wallis analysis of variance by rank (7), the aqueous solutions being used as the reference.

RESULTS

Tables III and IV show the mean lesional scores in different parts of the rat gastrointestinal tract after administration of diclofenac or indomethacin formulations respectively. At the chosen doses, considerable toxicity could be observed after administration of the solution form, whatever the route. As far as intravenous administration was concerned, no significant differences were observed between the drug solution and nanocapsules. In contrast, after intragastric administration, for both NSAIDs, the nanocapsules evoked lower lesional scores in all sections of gastrointestinal tract. This reduction was statistically significant in all regions except the duodenum, where the score was low even with the solution.

Table II. Scale for Visible Macroscopic Mucosal Irritation (× Number of Lesions).

Localized hemorrhages <1 mm	:	0.5
Ulcers <2 mm	:	1
Ulcers >2 mm	:	2
Perforations	:	3

DISCUSSION

The doses of NSAIDs (20 mg/Kg for diclofenac, 5 mg/Kg for indomethacin) and the protocol (3 consecutive days) were chosen after preliminary experiments because they yielded levels of toxicity after administration of the drug solution which were sufficiently marked to allow any protective effect of nanocapsules to be easily evaluated. The scoring scale (Table II) was chosen in order to compare with previously published data (5).

Gastrointestinal toxicity of NSAIDs after intravenous administration has already been demonstrated (8,9). The enterohepatic circulation has been implicated (10). If was recently been shown that, because of their capture by Küpffer cells in the liver, nanocapsules increased the enterohepatic circulation of indomethacin in the rabbit after intravenous administration (11). It might therefore be expected that the nanocapsule would increased toxicity by this route; however, no significant differences between the solution and the nanocapsules were noted.

In contrast, nanocapsules afforded a spectacular protection by the oral route, confirming previous observations (5). In fact, under the acidic conditions encountered in the stomach, diclofenac and indomethacin are mainly present in their unionized form and their partition is largely in favor of an oily phase rather than an aqueous phase. It could therefore be deduced that they were slowly released from the internal lipophilic core of nanocapsules, thus preventing direct contact of high concentrations of the free drug with the gastric mucosa. The importance of direct contact of indomethacin with the mucosa in the production of gastric lesions has been suggested by the finding that this drug is more ulcerogenic by oral than by parenteral administration (12). The same expla-

Table III. Mean (± SD) Gastrointestinal Lesional Indexes in Rats (n = 8) Following Three Consecutive Daily Doses of Diclofenac (20 mg/kg) Given Intravenously and Intragastrically Either as an Aqueous Solution or as the Nanocapsule Formulation A.

	Intravenous route		Oral route	
Organ	Solution	Nanocapsules	Solution	Nanocapsules
Stomach	3.8 ± 2.4	3.9 ± 2.6	5.2 ± 2.9	0.3 ± 0.7[a,b]
Duodenum	4.2 ± 3.1	0.9 ± 1.5	1.4 ± 1.8	0.4 ± 1.1
Jujenum	19.4 ± 10.2	4.2 ± 6.7	21.0 ± 17.8	1.5 ± 3.4[a]
Ileum	71.6 ± 28.9	72.8 ± 21.0	43.2 ± 27.5	8.3 ± 7.1[a,b]
Total	98.2 ± 31.9	81.7 ± 24.2	70.9 ± 38.4	10.7 ± 7.0[a,b]

[a] Significant difference with respect to solution by the same route, $P < 0.05$.
[b] Significant difference with respect to the intravenous route with the same dosage form, $P < 0.05$.

Table IV. Mean (± SD) Gastrointestinal Lesional Indexes in Rats (n = 8) Following Three Consecutive Daily Doses of Indomethacin (5 mg/kg) Given Intravenously and Intragastrically Either as an Aqueous Solution or as the Nanocapsule Formulation B.

Organ	Intravenous route		Oral route	
	Solution	Nanocapsules	Solution	Nanocapsules
Stomach	3.5 ± 2.2	6.4 ± 5.0	3.7 ± 1.0	0[a,b]
Duodenum	4.4 ± 3.6	2.7 ± 3.2	2.5 ± 1.9	0.2 ± 0.4
Jejunum	55.5 ± 15.1	69.0 ± 31.7	39.6 ± 20.1	0[a,b]
Ileum	113.1 ± 34.4	130.0 ± 46.3	77.0 ± 27.6[b]	3.8 ± 2.5[a,b]
Total	176.5 ± 32.9	198.9 ± 81.9	120.8 ± 47.7	4.0 ± 2.3[a,b]

[a] Significant difference with respect to solution by the same route, $P < 0.05$.
[b] Significant difference with respect to the intravenous route with the same dosage form, $P < 0.05$.

nation could apply to the duodenum as it can be assumed that both drugs remain essentially unionized in this proximal segment where the luminal conditions are still acidic.

It is unlikely that the reduced toxicity of nanocapsule-associated NSAIDs is due to reduced bioavailability leading to lower plasma concentrations and systemic effects, since it has been shown for both diclofenac (unpublished data) and indomethacin (13) that the two forms are bioequivalent in rats.

Another hypothesis is that the acid form of diclofenac and indomethacin in nanocapsules caused less mucosal irritation than the sodium salt of the aqueous solution. This hypothesis is supported by data from Fara and Myrback (3) who have shown, in an in-vitro model of rabbit colonic mucosa, that NSAIDs, such as naproxen, indomethacin and diclofenac, are less irritant than their sodium salts. In the present study, this hypothesis is supported by the fact that, regardless of whether the dosage form was the aqueous solution or nanocapsules or whether it was administered by the intragastric or the intravenous route, the toxicity of diclofenac and indomethacin was always more marked in the intestine, especially in the ileum where the pH allows ionization of the two drugs, than in the proximal segments where the acidic form could prevail.

In conclusion, the systemic or local origin of the untoward effects of NSAIDs on the gastrointestinal tract has been extensively debated and no clear conclusion has emerged. In the present study we have compared aqueous solutions of diclofenac and indomethacin with their nanocapsule formulations following an intravenous or intragastric administration. We have shown that the ulcerative effects of systemic origin following the intravenous injection are not related with the dosage forms. Despite the uptake of the colloidal carrier by the Küpffer cells and the resulting enhancement of the biliary excretion of the drugs, no evidence of the influence of enterohepatic circulation on the mucosal damage was obtained. In contrast we have clearly shown that, following the intragastric administration of nanocapsules, a spectacular protection of the gastrointestinal mucosæ was obtained. As both dosage forms of the two drugs are bioequivalent by intragastric route, similar plasma concentrations should lead to similar effects of systemic origin. Thus the protective effect observed with nanocapsules demonstrates the major influence of the local irritative effects of NSAIDs on the gastrointestinal tract.

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REFERENCES

1. P. A. Todd and E. M. Sorkin. Diclofenac sodium. A reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy. *Drugs*, 35 : 244–285 (1988).
2. A. J. Collins and L. J. Notorianni. The acute action of fenbufen and piroxicam on the gastric mucosa as measured by gastric microbleeding. *J. Int. Med. Res.*, 12 : 179–183 (1984).
3. J. W. Fara and E. Myrback. Formulation and dosage form design in drug-induced topical irritation of the gastrointestinal tract. *Pharm. Res.*, 7 : 616–620 (1990).
4. E. C. Soehngen, E. Godin-Ostro, F. G. Fielder, R. S. Ginsberg, M. A. Slusher and A. L. Weiner. Encapsulation of indomethacin in liposomes provides protection against both gastric and intestinal ulceration when orally administered to rats. *Arthritis and Rheumatism*, 31 : 412–421 (1989).
5. N. Ammoury, M. Dubrasquet, H. Fessi, J-P. Devissaguet, F. Puisieux and S. Benita. Indomethacin-loaded poly(D,L-lactide) nanocapsules : protection from gastrointestinal ulceration and antiinflammatory activity evaluation on rats. *Clin. Mater.*, 13 : 121–130 (1993).
6. S. S. Guterres, H. Fessi, G. Barratt, J-P. Devissaguet and F. Puisieux. Poly (D,L-lactide) nanocapsules containing diclofenac : I. Formulation and stability study. *Int. J. Pharm.*, 113 : 57–63 (1995).
7. S. Bolton. *Pharmaceutical Statistic—Practical and Clinical Applications*. Marcel Dekker, New York (1984).
8. D. A. Brodie, P. G. Cook, J. Bauer and G. E Dagle. Indomethacin-induced intestinal lesions in the rats. *Toxicol. Appl. Pharmacol.*, 17 : 615–624 (1970).
9. K. J. Levy, D. B. Paone and W. J. Krause. Acute effect of systemic aspirin on gastric mucosa in man. *Digestive Diseases and Sciences*, 25 : 97–99 (1980).
10. D. E. Duggan, K. F. Hooke, R. M. Noll and K. C. Kwan. Enterohepatic circulation of indomethacin and its role in intestinal irritation. *Biochem. Pharmacol.*, 25 : 1749–1754 (1975).
11. F. Fawaz, F. Bonini, M. Guyot, A. M. Lagueny, H. Fessi and J-P. Devissaguet. Influence of poly(D,L-lactide) nanocapsules on the biliary clearance and enterohepatic circulation of indomethacin in the rabbit. *Pharm. Res.*, 10 : 750–756 (1993).
12. V. Cioli, S. Putzolu, V. Rossi, P. Scorza-Barcellona and C. Corradino. The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats. *Toxicol. Appl. Pharmacol.*, 50 : 283–289 (1979).
13. N. Ammoury, H. Fessi, J. P. Devissaguet, M. Dubrasquet and S. Benita. Jejunal absorption, pharmacological activity and pharmacokinetic evaluation of indomethacin loaded poly(D,L-lactide) and poly(isobutyl-cyanoacrylate) nanocapsules in rats. *Pharm. Res.*, 8: 101–105 (1991).