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Notes

Evaluation of gastrointestinal behaviour in the rat of amphiphilic β-cyclodextrin nanocapsules, loaded with indomethacin

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Abstract

With a view to investigating the role of encapsulation of indomethacin within modified β-cyclodextrin (β$CD-C_6$) nanocapsules, we prepared nanocapsules with indomethacin as a model drug. These particles had an average size of 194 nm and encapsulated approximately 99% of indomethacin. The encapsulation of indomethacin within orally administered β$CD-C_6$ nanocapsules protected against both gastric and intestinal ulceration is compared with an oral administration of an aqueous solution of Indocid®. The relative bioavailability was also increased by this encapsulation.

Keywords: Amphiphilic cyclodextrins; Nanocapsules; Indomethacin; Bioavailability; Gastro-intestinal tolerance

The use of non-steroidal anti-inflammatory drugs (AINS), such as indomethacin, is limited by their ulceronecrotic effects on the gastro-intestinal mucosa. The origin of this toxicity is believed to be a local inhibition of prostaglandin secretion resulting in a deficiency of bicarbonate secretion which normally protects the epithelial surface against gastric acidity. However, a systemically-mediated effect has also been evoked.

The search for formulations which could reduce the toxicity of this class of drugs has constituted a major field of research. Colloidal carriers, such as alginate dispersions (Shiraishi et al., 1991) or nanocapsules prepared from biodegradable polymers (Andrieu et al., 1986; Ammoury, 1989; Ammoury, 1990) have demonstrated an effective protection, without reducing the pharmacological effects of indomethacin. Inclusion complexes of various AINS within cyclodextrins have also been tested, with very satisfactory results (Nambu et al., 1978; Santucci et al., 1990; Lin et al., 1994).

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Our laboratory has recently developed a new concept in drug targeting (Skiba et al., 1993a,b, in which the colloidal carriers, nanocapsules and nanospheres, are prepared from chemically-modified cyclodextrins. It therefore seemed interesting to evaluate these systems as carriers for indomethacin, particularly with respect to gastrointestinal tolerance.

Indomethacin (Indo) and benzylbenzoate (BB) were purchased from Sigma Ltd (Saint Louis, MO, USA), the chemically-modified β-cyclodextrin (βCD-C_6) was synthesized in Laboratoire de Chimie Organique (CNRS ER 45) according to the method described by Zhang et al., 1991 and reported by Skiba et al., 1995, injectable indomethacin was the commercial product Indocid® (MSD-Chibret, Paris, France), and Pluronic PE F68® and Span 85® were obtained from ICI France (Clamart, France). All other ingredients and solvents used were purchased from Prolabo (France). Acetone was chosen as the organic water-miscible solvent.

Nanocapsules were prepared according to the method described by Skiba et al., 1994 (c). Briefly, indomethacin, a lipophilic phase of 20 μl of benzylbenzoate, and βCD-C_6 (25 mg) were dissolved in acetone (12.5 ml) and added under mechanical stirring to an aqueous sodium acetate buffer phase (25 ml) containing the non ionic surfactant Pluronic PE F68® (31 mg). Indo-NC were formed immediately. The colloidal suspension obtained was concentrated by evaporation under vacuum and the final volume of 20 ml filtered through a sintered glass filter (9–15 μm). A second batch of nanocapsules were also prepared in the same manner, using a mixture of lipophilic surfactant (10.33 mg of Span 85®) and hydrophilic surfactant (31 mg of Pluronic PE F 68). This combination of surfactants allowed stable nanocapsules to be obtained which could be concentrated to 5 ml, 7.5 times less than the initial volume without breakage or aggregation.

The size of the nanocapsules was estimated by laser light scattering using a monochromatic laser ray diffusion counter (Nanosizer, Coultronics France SA, Margency, France). The drug loaded and drug lost in the aqueous suspending medium were assayed by an HPLC technique.

The indomethacin content of the various nanoparticles was analyzed using a Waters HLPC system (division Millipore, St-Quentin-en-Yvelines, France) equipped with a variable wavelength ultraviolet detector Waters 484, a Waters integrator 746 and a 25-cm, 4.6-mm i.d.. reverse phase column, and SFCC ultrabase C_{18}, 10 μm. The column was eluted with acetonitrile/sodium acetate 0.25 10^{-3} M (55/45 by volume), at a rate of 1 ml/min, the pH being adjusted to 3.5 with acetic acid. The column eluent was monitored at 280 nm and the chromatograph was operated at a pressure of 2000 psi at room temperature. The calibration curve was constructed using freshly prepared samples of standard solutions containing 0.025–0.2% indomethacin in methanol. The nanoparticles were dissolved (1/10) in acetonitrile and injected into a 20 μl loop.

Experiments were carried out on fasted Wistar® male rats (Iffa Credo, Arbresle, France) weighing between 190 and 210 g. They were placed in single cages which had wire-net floors to prevent coprophagy. Eight animals were randomly assigned to each of six groups (A–F) as follows.

(1) rats received Indocid® solution (5 mg/kg)
(2) rats received Indo-NC supension (5 mg/kg)
(3) rats received Indocid® solution (10 mg/kg)
(4) rats received Indo-NC suspension (10 mg/kg)
(5) rats received nanocapsules free of indomethacin

Animals were divided into homogeneous groups of 8 animals. The formulations were given orally as an aqueous dispersion of nanocapsules containing a dose of 5 or 10 mg/kg of indomethacin for 3 days consecutively. Two batches were similarly given nanocapsules without indomethacin in a similar volume and acted as a control.

The animals were weighed before and after treatment, as described by Ammoury, 1989 and Lin et al., 1994.

The rats were fasted for 18 h before the first administration, but allowed free access to water and food after treatment.

Twenty four hours after the experimental period, the animals were sacrificed and their intes-

tines and stomachs removed and opened along the greater curvature. Any lesions were examined macroscopically. The number of erosions per intestine and stomach was assessed for severity according to the scoring system described by Lin et al., 1994. The Gastric Lesion Index (GLI) induced by indomethacin was calculated using an arbitrary score (AS):

AS = 0 no lesions
AS = 0.5 one or more haemorrhagic ulcers length < 1 mm
AS = 1 one or more haemorrhagic ulcers length between 1 and 2 mm
AS = 2 one or more haemorrhagic ulcers length > 2 mm

The mean scores for each group were calculated and expressed as the ulcer index (GLI)

$$GLI = AS \times number\ of\ lesions$$

Values are given as arithmetic mean ± S.D. The significance of differences between means was evaluated by Student's t-test for paired data.
Finally, the preparation of biological sample were carried out as follows: A quantity of 200 μl of plasma (from 6 groups of rats) was added to 100 μl of acetonitrile. Samples were mixed thoroughly and centrifuged. The clear supernatant was used for HPLC analysis.
The results of a typical light scattering experiment are given in Table 1A.

Analysis of the particle size distribution indicated no difference in mean diameter size between NC (214 ± 52nm) and NC-Indo (209 ± 81 nm).

Table 1
(A) Size distribution of nanocapsules made from βCD-C_6. NC = without indomethacin. NC-Indo = with indomethacin

	Size (nm)	Dust (%)	P.I*.
NC	214 + 52	0	0.074
NC-Indo	209 + 81	0	0.0089

*polydispersity index.
(B) Degree of injury of stomach of male rats produced by oral administration of Indocid® and NC-indo. Minimum number value per group n = 8

		Amount of Indomethacin administered[a]	
		5 mg/kg	10 mg/kg
Gastric Ulceration (GLI)	Control	0 ± 0	0 ± 0
	Indocid®	0 ± 0	1.03 ± 0.01[b]
	N C-Indo	0 ± 0	0.08 ± 0

[a]Average ± S.D. [b]Significant by t-test at 5% level.

The relation ship between the indomethacin encapsulated in nanocapsules and the total quantity of drug incorporated into the medium is linear and positive (Fig. 1a). The maximum encapsulation values were determined by the solubility of indomethacin in benzylbenzoate. Above 15 mg, precipitation of indomethacin in the continuous phase could be seen.

These observations are in agreement with the results of Ammoury, 1986 who found that at an indomethacin loading of 5% (w/v) in the oil phase (benzylbenzoate), drug incorporation into nanocapsules was not complete. As long as the maximum solubility in benzylbenzoate was not exceded, the encapsulation yield was 99%.

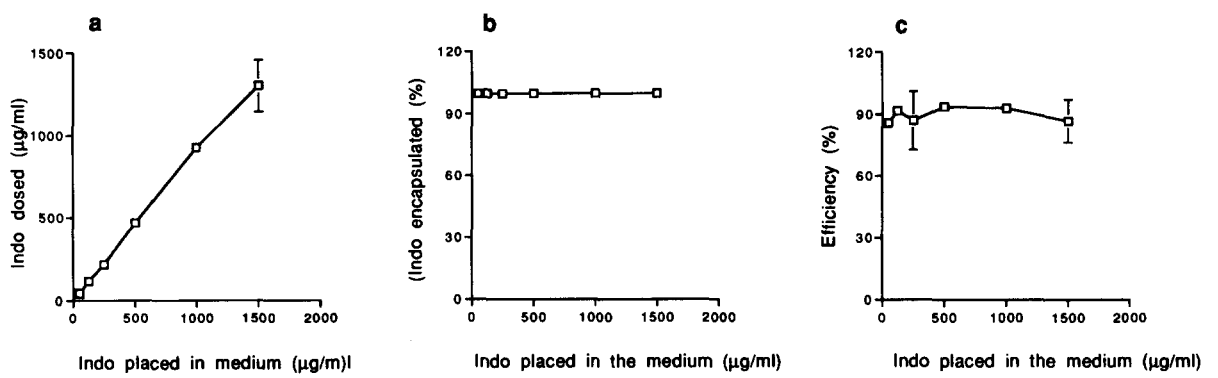

Fig. 1. Indomethacin content in βCD-C_6 nanocapsules and encapsulation yield.

For gastro-intestinal tolerance studies in the rat, only nanocapsules prepared with a mixture of Span 85® and Pluronic PE F 68® were used.

The ulcerative effects observed after oral administration to rats of various dosage levels of free indomethacin, or indomethacin encapsulated within βCD-C_6 nanocapsules, are shown in Table 1B. The gastric ulcerative effects were minimal, and no significant difference was observed between the two dosage levels of indomethacin.

In order to facilitate evaluation of the protective effect of nanocapsules in the rat in comparison with an aqueous solution of Indocid®, we examined the intestinal tract, where the ulcerative effects of Indocid® are more pronounced. The protection afforded by NC-$Indo_{5\ mg\ kg}$ (82%) was significant compared with NC-$Indo_{10\ mg\ kg}$ (53%), when intestinal lesions were quantified after dosing for 3 days (Fig. 2A).

Fig. 2B summarizes the results of the bioavailability studies. Administration of nanocapsules led to higher plasma concentrations than with the free drug. When this result is compared with the results of gastro-intestinal tolerance, it appears that the toxicity of indomethacin seems not systemic, since the formulation which gives higher circulating concentrations is the least toxic. We can therefore assume that the toxicity was due to exposure of the mucosa to the drug.

In the case of the carriers, the amount of free indomethacin in the digestive lumen is considerably reduced, especially in the case of the 5 mg/kg dosage. These results resemble those obtained by Ammoury, 1986 with polymeric nanocapsules. These authors obtained an even lower damage index than the one reported here. This could be related to the stability of the nanocapsules made from βCD-C_6

The explanation for the increased bioavailability of indomethacin entrapped in βCD-C_6 nanocapsules is not yet fully elucidated. There is evidence, mainly qualitative, that some colloïdal particles can be adsorbed intact from the digestive tract. Various routes have been proposed: intercellular, intracellular and transcytosis through the specialized M cells of Peyer's patches. The Pattes route seems to be important for evoking a mucosal immune response to tigens associated with

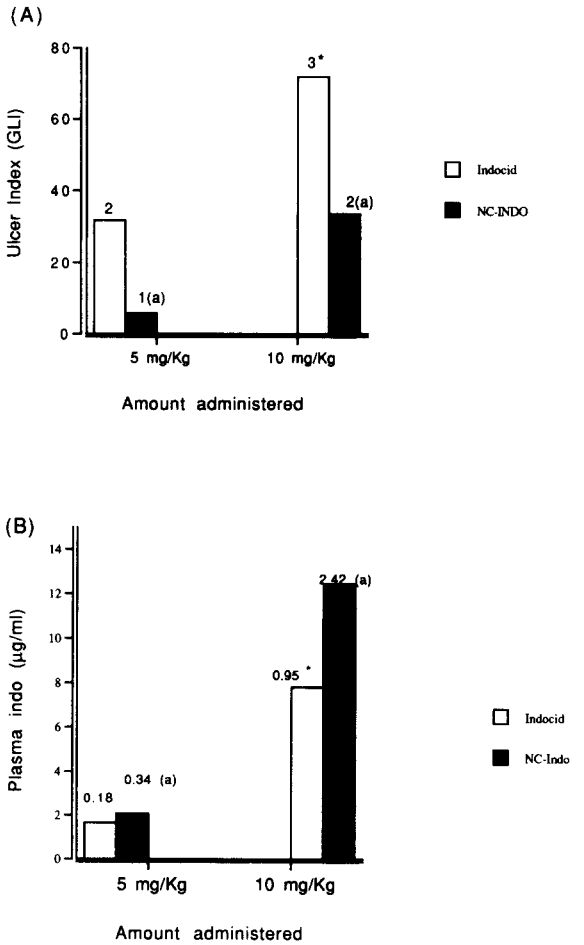

Fig. 2. (A) Comparison of the intestine protective action of NC-$Indo_{5\ mg\ ml}$ and NC-$Indo_{10\ mg\ ml}$. (B) Indomethacin plasma levels. *Average ± S.D. [a]Significant by t-test at 5% level.

nanoparticles or nanospheres (Ammoury, 1989) However, it seems unlikely that this absorption is sufficient to explain the pharmacological action of encapsulated drugs. The uptake of βCD-C_6 nanocapsules has not yet been studied: radiolabeled particles would be a way of studying this. Another possibility is that individual amphiphilic cyclodextrin molecules are adsorbed together with the indomethacin guest after disruption of the nanocapsule structure. β-cyclodextrins have been shown to be poorly absorbed by the intestine (Uekama and Irie, 1987; Szejtli, 1992). The absorption of the amphiphilic modified βCD-C_6

might be expected to be increased compared with the native molecules, because the amphiphilic characteristics of βCD is conducive to their transmembranal passage.

Although βCD-C_6 nanocapsules are probably not adsorbed to a great degree, they may possess bioadhesive properties (Durrer et al., 1994) and thus prolong the stay of indomethacin in the digestive tract. The drug is protected from hydrolysis and released slowly, allowing its absorption without exposing the mucous membrane to large amounts of the free drug. Thus, the ulcerogenic effect is reduced whilst absorption is increased.

The aim of this work was the formulation of indomethacin nanocapsules with modified β-cyclodextrins (βCD-C_6) and the study of their digestive tolerance. Further assays of in vitro release could give more information about the mechanism release of the drug from nanocapsules.

The study in the rat showed that repetitive oral administration of the unloaded colloidal suspension was perfectly tolerated. βCD-C_6 nanocapsules of indomethacin seemed to protect the gastro-intestinal mucus against the ulcerogenic effect of the drug, especially at the local level. Other studies, i.e autoradiography, are necessary to explain more precisely the mechanism of this protection.

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