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In vivo evaluation of indomethacin/cyclodextrin complexes Gastrointestinal tolerance and dermal anti-inflammatory activity

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Abstract

Inclusion complexes of indomethacin in β-cyclodextrin and hydroxypropyl-β-cyclodextrin were prepared and evaluated in vivo. With respect to the gastrointestinal tolerance assessed in the rat, it appears that the inclusion of indomethacin in these cyclodextrins results in a decrease in its irritation power, confirming that this undesirable effect has at least a local origin. With respect to the dermal anti-inflammatory activity assessed on healthy volunteers by the methyl nicotinate test, the results seem to demonstrate that inclusions in either of the cyclodextrins investigated have a higher activity than free indomethacin.

Key words: Indomethacin; β-Cyclodextrin; Hydroxypropyl-β-cyclodextrin; Inclusion; Gastrointestinal tolerance; Anti-inflammatory activity; Dermal route

1. Introduction

The analgesic and anti-inflammatory properties of indomethacin when administered by the oral route have been well established for several years. More recently, its dermal administration has been proposed for traumatology and rheumatology, and also for its effects on solar erythema. However, this molecule presents numerous drawbacks which can limit its possible uses. Like many non-steroidal anti-inflammatory products, indomethacin has a potent irritant effect on the gastro-intestinal mucosa. Furthermore, from a physicochemical standpoint, indomethacin, which is slightly acidic, is poorly water soluble (≈ 0.01 mg/ml), and soluble only at pH 12, a pH at which significant hydrolysis of the molecule occurs. Such quasi-insolubility in water for the skin physiological pH leads to poor bioavailability. Still on the physicochemical level, indomethacin is unstable under UV light. For these reasons, it appeared to us that indomethacin was a good candidate for inclusion into either β- or hydroxypropyl-β-cyclodextrin.

Despite the fact that various authors have described the formation of an inclusion complex of indomethacin in β-cyclodextrin (Hamada et al., 1975; Sumimoto Chemical Co., 1981; Szejtli and

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Szente, 1981), and hydroxypropyl-β-cyclodextrin (Müller and Backensfeld, 1990; Backensfeld et al., 1991), it is rather difficult to obtain a solid inclusion complex with a good yield. For our part, we compared several methods of preparing complexes of indomethacin in β- and hydroxypropyl-β-cyclodextrin (Lin et al., 1991, 1992). We report here the in vivo evaluation of the spray-dried complexes, in order to determine their ulcerous effect after oral administration to the rat, and cutaneous anti-inflammatory activity in human volunteers.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (Kleptose®) and hydroxypropyl-β-cyclodextrin (MS = 0.40) were obtained from Roquette Frères (Lestrem, France). Indomethacin (free acid form) was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). All other materials were of analytical reagent grade.

The indomethacin complexes with β-cyclodextrin and hydroxypropyl-β-cyclodextrin were prepared by spray-drying a solution of 37.2 mmol of indomethacin and 24.8 mmol of β-cyclodextrin (or hydroxypropyl-β-cyclodextrin) in ethanol/water (50:50) as described previously (Lin et al., 1991). The products obtained were rapidly washed with cold acetone in order to eliminate free indomethacin.

2.2. Gastrointestinal tolerance

Hydroxyethylcellulose hydrogels were prepared with indomethacin (acid form) and its cyclodextrin complexes according to the following formula: indomethacin (free or complexed), 0.1; hydroxyethylcellulose, 0.5; distilled water, q.s.p. 100.

The product was administered orally to male Wistar® rats, weighing about 200 g (Iffa Credo, Arbresle, France) in a dose of 5 mg/kg IM for 3 days consecutively. The rats were fasted for 18 h before the first administration, but allowed free access to water.

24 h after the last administration, the rats were killed, and a visual examination was carried out. The lesion index (LI) induced by indomethacin was calculated using an arbitrary score (Sc): diameter < 1 mm Sc = 0.5, between 1 and 2 mm Sc = 1, > 2 mm Sc = 2:

LI = Sc × number of lesions

The indomethacin concentration in plasma (for eight rats) at the moment of killing was determined by an HPLC method. The weight increase in rats was followed throughout the experiment.

2.3. Anti-inflammatory activity

Hydroxyethylcellulose hydrogels were prepared with indomethacin (acid form) and its cyclodextrin complexes according to the following formula: indomethacin (free or complexed), 1; hydroxyethylcellulose, 2; diazolidinyl urea, 0.2; distilled water, q.s.p. 100.

The anti-inflammatory power of the above-mentioned hydrogels was assessed on the forearm of 8–10 healthy and informed adult volunteers using a non-invasive technique (Poelman et al., 1989). Clinical approval was obtained for the experiments.

The anti-inflammatory activity of indomethacin hydrogels was investigated on the inflammatory vasodilatation induced by methyl nicotinate. Since the activity of methyl nicotinate is much more rapid than the anti-inflammatory activity of indomethacin, a prior occlusion of 1 h with the hydrogel of indomethacin was performed. After the occlusion period, each zone was washed with water and allowed to dry. A dose of 2 mg/cm^2 of 0.5% aqueous methyl nicotinate solution was then applied.

The vascular response to methyl nicotinate on the pretreated sites was quantified by laser Doppler velocimetry (LDV), using a Periflux PF 2B laser Doppler flowmeter (Perimed KB, Stockholm, Sweden). The anti-inflammatory activity of indomethacin can be expressed as the inhibition of the induced vasodilatation which can be calculated according to the following formula:

$$\text{inhibition (\%)} = \left[(AUC_{(C)} - AUC_{(D)})/AUC_{(C)}\right] \times 100$$

where $AUC_{(C)}$ is the area under the response-time curve in the control-treated site, and $AUC_{(D)}$ denotes the area under the response-time curve of the drug-treated site.

The significance of the results was tested by paired Student's *t*-test.

3. Results and discussion

3.1. Gastrointestinal tolerance

In this work, only the ulcerations in the intestine were scored. Under the experimental conditions (5 mg/kg IM), no significant ulcerations in the stomach were noted.

As shown in Table 1 and Fig. 1, the average lesion index is higher for free indomethacin (45.7 ± 24.1) than for the IM/βCD complex (8.5 ± 11.2) and for the IM/HPβCD complex (18.6 ± 19.2). The standard deviations in the lesion index of the groups treated with indomethacin were very high due to the great individual variations. However, the difference between the lesion index of hydrogels containing free indomethacin or complexed indomethacin is significant ($p < 0.01$), when the difference between the complexes (IM/βCD and IM/HPβCD) is not significant.

Larger lesions, such as perforations, were almost absent in the case of the IM/βCD complex (one out of 12 rats), whereas there were four perforations for the IM/HPβCD complex and 28 for free indomethacin.

Table 1
Lesion index after administration of hydrogels containing free or complexed indomethacin

Gel	Number of rats	Lesion index [a]	Number of perforations [b]
Control	6	0.3 ± 0.4	0
βCD	6	0.7 ± 0.5	0
HPβCD	6	0.8 ± 1.0	0
IM	12	45.7 ± 24.1	28
IM/βCD	12	8.5 ± 11.2	1
IM/HPβCD	12	18.6 ± 19.2	4

[a] Expressed as average per rat.
[b] Total perforation number.

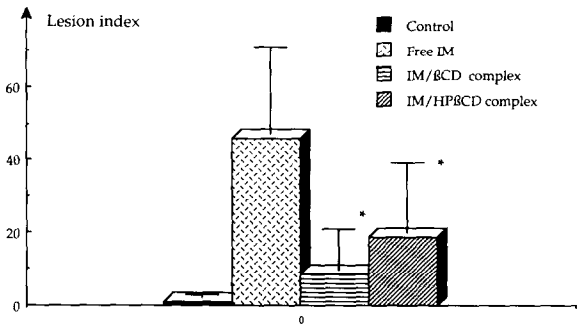

Fig. 1. Gastric lesion index after administration to the rat of indomethacin, free or included in cyclodextrins.

The weight increase (Fig. 2) of rats is in the order: control = IM/βCD > IM/HPβCD > IM, logically inversely proportional to that of the lesion index.

The indomethacin concentrations in the plasma (Fig. 3) were in the order: IM (1.56 ± 0.67mg/ml) > HPβCD/IM (1.37 ± 0.29) > βCD/IM (1.05 ± 0.47), which are reasonably comparable and demonstrate the real absorption of indomethacin from the complexes.

These results appear to be in contradiction with the assertion of Djahanguiri (1969), according to whom the ulcerous effect of indomethacin is due to a systemic, not local, effect, which lowers the secretion rate of mucus as a consequence of the inhibition of the synthesis of prostaglandin by indomethacin. Here, compara-

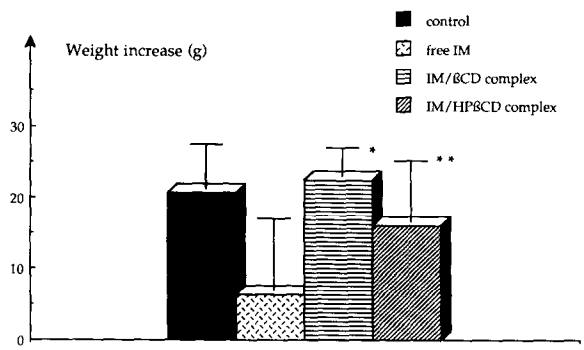

Fig. 2. Average weight increase per rat during experimentation (* $p < 0.001$ according to *t*-test vs free indomethacin; ** $p < 0.05$).

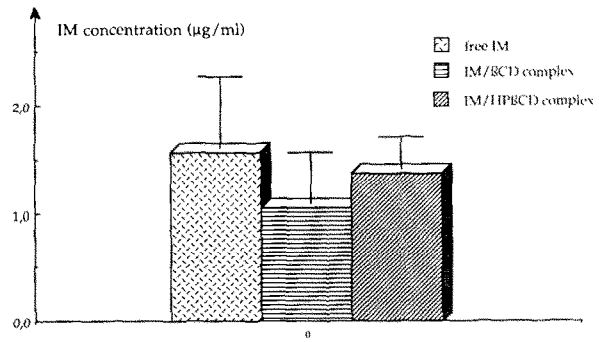

Fig. 3. Indomethacin plasma concentration at the moment of killing the rat (no significant difference in any case).

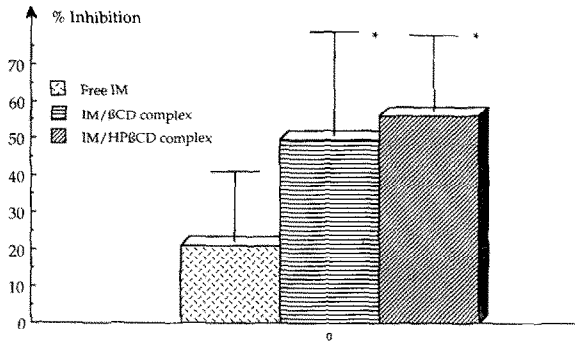

Fig. 5. Inhibition of methyl nicotinate vasodilation by hydrogels containing indomethacin, free or included in cyclodextrins.

ble indomethacin blood concentrations do not result in comparable ulcerations. On the other hand, our results confirm the work of Bianchi et al. (1987), who showed that the contact between indomethacin and the mucous membrane is an important factor in the ulcerous effect. Inclusion of indomethacin, in either β-cyclodextrin or hydroxypropyl-β-cyclodextrin, leads to, at least, a partial coating of indomethacin molecules, thereby preventing them from making direct contact with the gastrointestinal mucosa. However, due to the low stability constant of both complexes (370 and 414, respectively, for β-cyclodextrin and hydroxypropyl-β-cyclodextrin at pH 6.5 (Lin 1993)), the protection is not complete because of the permanent equilibrium between free molecules of indomethacin and cyclodextrin and supermolecules of inclusion complex as shown in Fig. 4.

This explanation is in agreement with the work of Shiraishi et al. (1990), who showed a reduction in stomach irritation of indomethacin by alginate dispersions, this effect being attributed to the smaller extent of contact of the drug molecules with the gastric mucosa.

However, the protection resulting from inclusion in cyclodextrin remains lower than that obtained by encapsulation of indomethacin in polylactic acid nanocapsules (Ammoury, 1990).

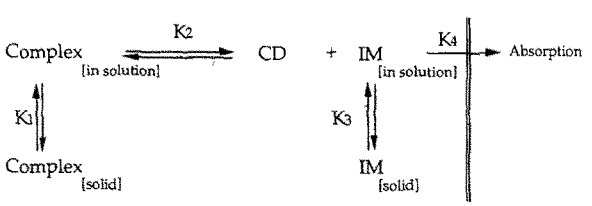

Fig. 4. Schematic behaviour of cyclodextrin complexes in the gastrointestinal tract after oral administration.

3.2. *Anti-inflammatory activity*

Despite the low number of subjects under investigation and the high interindividual variations, the results of inhibition of methyl nicotinate induced vasodilatation (Fig. 5) showed that after 1 h of occlusion, the anti-inflammatory activities of indomethacin/cyclodextrin complexes are significantly greater than that of free indomethacin (50–55% of inhibition for the complexes, 20% for free indomethacin; $p < 0.01$). There is no significant difference between the two complexes.

This increase in anti-inflammatory activity of indomethacin when included in a cyclodextrin can be explained by the fact that the amount of indomethacin dissolved in the form of a complex in the hydrogel is larger than that of indomethacin itself, due to the respective water solubility of the products. The permanent equilibrium between free indomethacin (and cyclodextrin) molecules and supermolecules of complex results in the permanent disposal of in-

domethacin available for dermal absorption and activity.

4. Conclusion

The inclusion of indomethacin either in β-cyclodextrin or in hydroxypropyl-β-cyclodextrin appears to be very interesting from a therapeutic standpoint.

After oral administration to the rat, the gastrointestinal ulcerous effect of indomethacin is significantly reduced by inclusion, despite the same blood concentrations.

Via the dermal route, the methyl niconitate-induced inflammation, assessed by evaluation of vasodilatation, is reduced significantly more by hydrogels containing indomethacin complexes with both cyclodextrins than by those containing free cyclodextrin.

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