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Reduction in the ulcerogenicity of naproxen by complexation with β-cyclodextrin

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Summary

Nonsteroidal anti-inflammatory drugs are largely prescribed for patients with inflammatory conditions, but their efficacy is offset by a significant incidence of gastrointestinal side effects. To investigate the effect of inclusion with β-cyclodextrin on the irritation in the stomach caused by naproxen, the drug and its complex prepared by freeze-drying, were administered to three groups of eight rats, one of which behaved as a control. Damage was evaluated macroscopically. Significantly less gastric irritation was observed for the groups treated with the complex, which did not appear to differ in this respect from the controls.

Introduction

Naproxen [(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid] is a non-steroid antiinflammatory with analgesic and antipyretic properties frequently used to treat rheumatic complaints such as rheumatoid arthritis and osteoarthritis. The drug, when orally administered, forms crystals that coat the digestive mucus due to its acidity and low solubility: these dissolve slowly, irritating and damaging the stomach walls, which for high doses or prolonged treatment, can lead to the formation of ulcerations in the mucus.

From the pharmacological-toxicological point of view, giving drugs in the form of inclusion complexes with cyclodextrins has certain advantages, such as a reduction in the side effects of various active principles (Uekama et al., 1982; Uekama and Otagiri, 1987), and specifically, less local irritancy at the level of the gastric mucus, due to the greater solubility and the increase in pK_a of the drug (Nambu et al., 1978; Fónagy et al., 1981; Neumark et al., 1981; Szejtli and Szente, 1981).

This communication describes the effect of including naproxen with β-cyclodextrin on the gastric irritation, frequently associated with this drug.

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Materials and Methods

Materials

Naproxen was from Sigma, and β-cyclodextrin from Chinoin. All reactants and solvents were of analytical grade.

Solubility diagram

Higuchi and Connors' (1965) method was followed. Naproxen in excess was added to aqueous solutions of β-cyclodextrin at pH 1. After reaching equilibrium the samples were passed through 0.45 μm (of aqueous phase) Millipore filters, diluted to a convenient volume and evaluated spectrophotometrically. K_c was calculated in terms of a 1 : 1 stoichiometry from the diagram obtained.

Preparation of the solid complex

The complex was prepared by freeze-drying. A 1 : 1 mixture of naproxen and β-cyclodextrin in distilled water with a little ammonia, to increase the solubility, was prepared. The solution was stirred for 24 h and freeze-dried.

UV and IR spectroscopy

UV spectroscopy was performed in a Shimadzu UV-240 spectrophotometer. All solutions were prepared at pH 1. The value of K_c was calculated from the Benesi-Hildebrand equation as modified by Scott (1956).

IR spectra were obtained in a Perkin Elmer 1330 IR spectrophotometer using KBr pellets.

Differential scanning calorimetry (DSC)

Thermographs were obtained in a Perkin Elmer DSC-4 apparatus, using aluminium pans for volatile compounds and nitrogen as the purging gas. Samples of approx. 5 mg were employed and heating was at 10 °C/min up to 170 °C.

Administration of the samples and evaluation of ulcers

Male rats weighing 180–210 g were used, divided into three homogeneous groups of eight animals. The formulations were given orally every day over a 4 day period as aqueous suspensions containing 20 mg/ml. In all, 200 mg of naproxen/kg of body weight, or its equivalent of complex were administered per animal. One group was administered distilled water and acted as a control. The animals were put into individual cages and denied access to food and water 3 h before the first treatment and throughout the entire experiment.

On the day after the final dose (day 5) the animals were killed with chloroform fumes. A laparotomy was rapidly performed and the stomachs removed, which were opened along the length of the greater curvature and cleaned of debris. The gastric walls were carefully washed with physiological saline solution, placed on a support and photographed under identical conditions with a Nikon F2 camera with FX extension ring located 30 cm from the stomachs.

Damage was evaluated independently, from photographic slides, by five people not connected with the experiment, following Robert and Nezamis' method (1958) and using the scoring criteria proposed by Cano et al. (1981):

- Changes in the gastric mucus appearing as reddened spots or petechiae, 1–3 points.
- Damage in the form of desquamations, lessening of the consistency of the gastric tissue, 4 points.
- Wounds in the form of ulcers, 5–8 points according to severity.
- More severe ulcers in terms of a larger affected area of gastric mucus, 10–15 points for each depending on the area covered. Compact groups of ulcers varying in severity that were difficult to evaluate separately were also included in this group.

The sum of the scores noted by each observer was used to calculate the ulcerogenic index (UI).

Results and Discussion

The solubility diagram obtained (Fig. 1) can be classified as A_L according to Higuchi and Connors' (1965) terminology, which indicates the formation of a soluble inclusion complex. In keeping with a slope of less than unity for the straight line, we can assume that the observed increase in solubility is due to the formation of a complex of 1 : 1 stoichiometry. The apparent stability constant

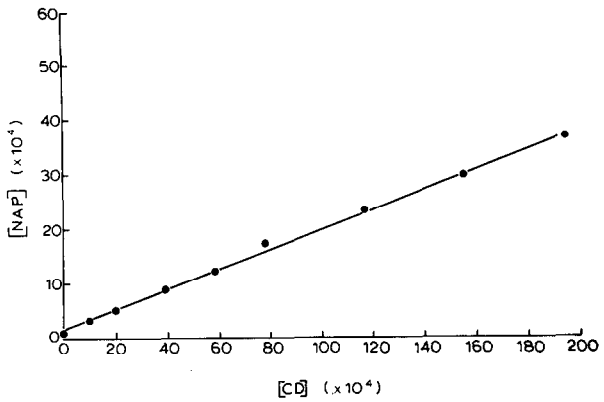

Fig. 1. Phase-solubility diagram of naproxen-β-cyclodextrin inclusion complex at pH 1 and 25 °C.

K_c, calculated from the slope assuming 1:1 stoichiometry, was 1379 M^{-1}, in keeping with the value obtained spectroscopically (1356 M^{-1}) calculated using Scott's method (Fig. 2).

Comparing the IR spectra obtained for naproxen and the inclusion complex (Fig. 3) reveals a strong displacement towards shorter wavelengths of the band corresponding to the naproxen carbonyl group (1728 cm^{-1}), indicating host-guest interactions. Similarly, the thermographs obtained via DSC (Fig. 4), where the naproxen peak of fusion disappears completely when mixed with β-cyclodextrin, corroborate the idea of the formation of an inclusion complex.

The ulcerogenic effects of the drug and the inclusion complex can be compared in schematic representation of the inside of the rats' stomach

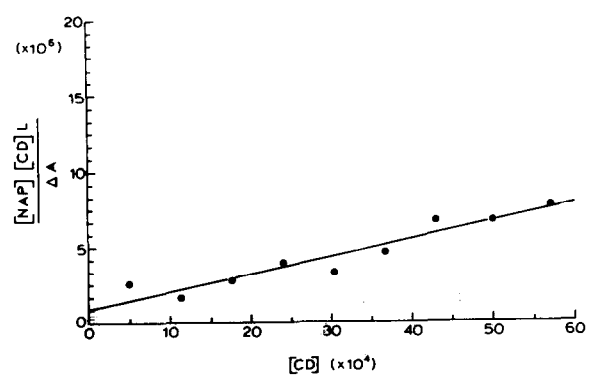

Fig. 2. Scott's plot for the naproxen-β-cyclodextrin 1:1 complex system at pH 1. L: path length of a cell ($L = 1$).

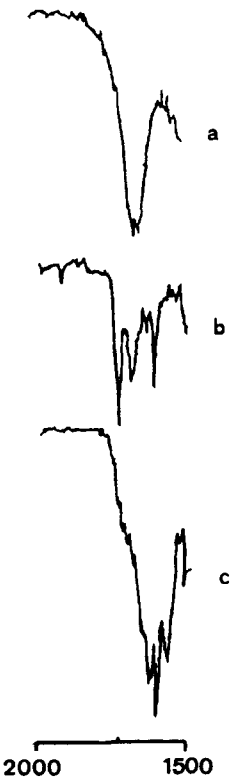

Fig. 3. IR absorption bands in the 1500–2000 cm^{-1} region of: (a) β-cyclodextrin, (b) naproxen and (c) freeze-dried inclusion complex.

(Fig. 5), showing the upper and lower extremes of damage caused by each formulation. It can be seen that most ulceration occurred in rats treated with naproxen accompanied by greater deterioration in the tissue of the gastric wall. The limiting cases, indicating a wide range of response to each formulation, show a variation in the individual response to side effects, that is a common feature of most drugs.

The ulcerogenic index assigned by each observer to each rat is listed in Table 1. It can be seen that the highest values occur for the group treated with naproxen. Table 1 also shows that the scores of each observer do not differ greatly from each other, suggesting that the method used is adequate. Nevertheless, the values obtained were analyzed statistically with the Kruskal-Wallis rank test. A non-parametric test was used since these are most useful in the treatment of categoric values, i.e. values of qualitative pharmacological re-

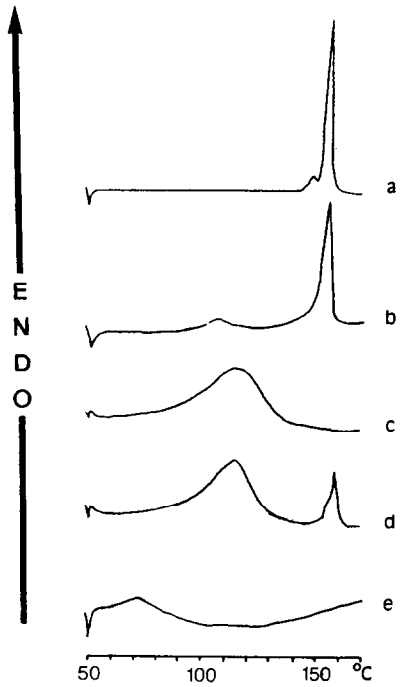

Fig. 4. DSC curves of: (a) naproxen, (b) freeze-dried naproxen, (c) β-cyclodextrin, (d) physical mixture and (e) freeze-dried inclusion complex.

sponses (Bolton, 1984; Siegel and Castellan, 1988).

In the Kruskal-Wallis test each observation is replaced by a rank. The smallest becomes rank 1, the next smallest score rank 2 and so on up to rank N, where N is the total number of independent observations.

The values of the average UI and their ranks are shown in Table 2. The Kruskal-Wallis statistic (H) is calculated as:

$$H = \left[\frac{12}{N(N+1)} \sum_{j=1}^{k} n_j \bar{R}_j^2 \right] - 3(N+1)$$

where k is the number of samples or groups, n_j the number of cases in the j-th sample, N the number of cases in the combined sample (the sum of the n_js), $\bar{R}_j$ the average of the ranks in the jth sample or group and the summation is across the k samples.

The value of $H = 17.54$ was obtained and indicates the existence of statistically significant differences between the groups at the 0.05 probability level.

A simple procedure for determining which pairs of groups are different is based on calculating the differences $|\bar{R}_1 - \bar{R}_2|$ for all pairs of groups and testing them for the condition:

$$|\bar{R}_1 - \bar{R}_2| \geq Z_{\alpha/k(k-1)} \sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

where the Z are tabulated values for different probability levels and different comparison numbers ($k(k-1)/2$).

In our study we determined the critical value of Z to be 2.394: the critical difference is then equal to 8.46.

Taking the differences between the average rankings:

$$|\bar{R}_{\mathrm{NAP}} - \bar{R}_{\mathrm{COMP}}| = 8.5 > 8.46$$

$$|\bar{R}_{\mathrm{NAP}} - \bar{R}_{\mathrm{CONTROL}}| = 14.75 > 8.46$$

$$|\bar{R}_{\mathrm{COMP}} - \bar{R}_{\mathrm{CONTROL}}| = 6.25 < 8.46$$

Hence, the degree of ulceration was significantly greater for the group treated with naproxen (NAP), whereas no differences are indicated between the animals treated with the inclusion complex (COMP) and the control.

To establish whether a possible relation exists between loss of body weight and the formulation administered, the weight loss was calculated for each animal as a percentage of body weight, i.e.:

$$\text{decrease of body weight } (\%) = \frac{W_{n-1} - W_n}{W_{n-1}} \times 100$$

where W_{n-1} is the weight of the rat the day before the n-th administration and W_n is the weight of the rat the day of the n-th administration.

The animals treated with the inclusion complex and those used as a control show a weight loss that is low throughout the treatment. In comparison, the administration of naproxen produces an increase in weight loss by the last day of the study (Fig. 6).

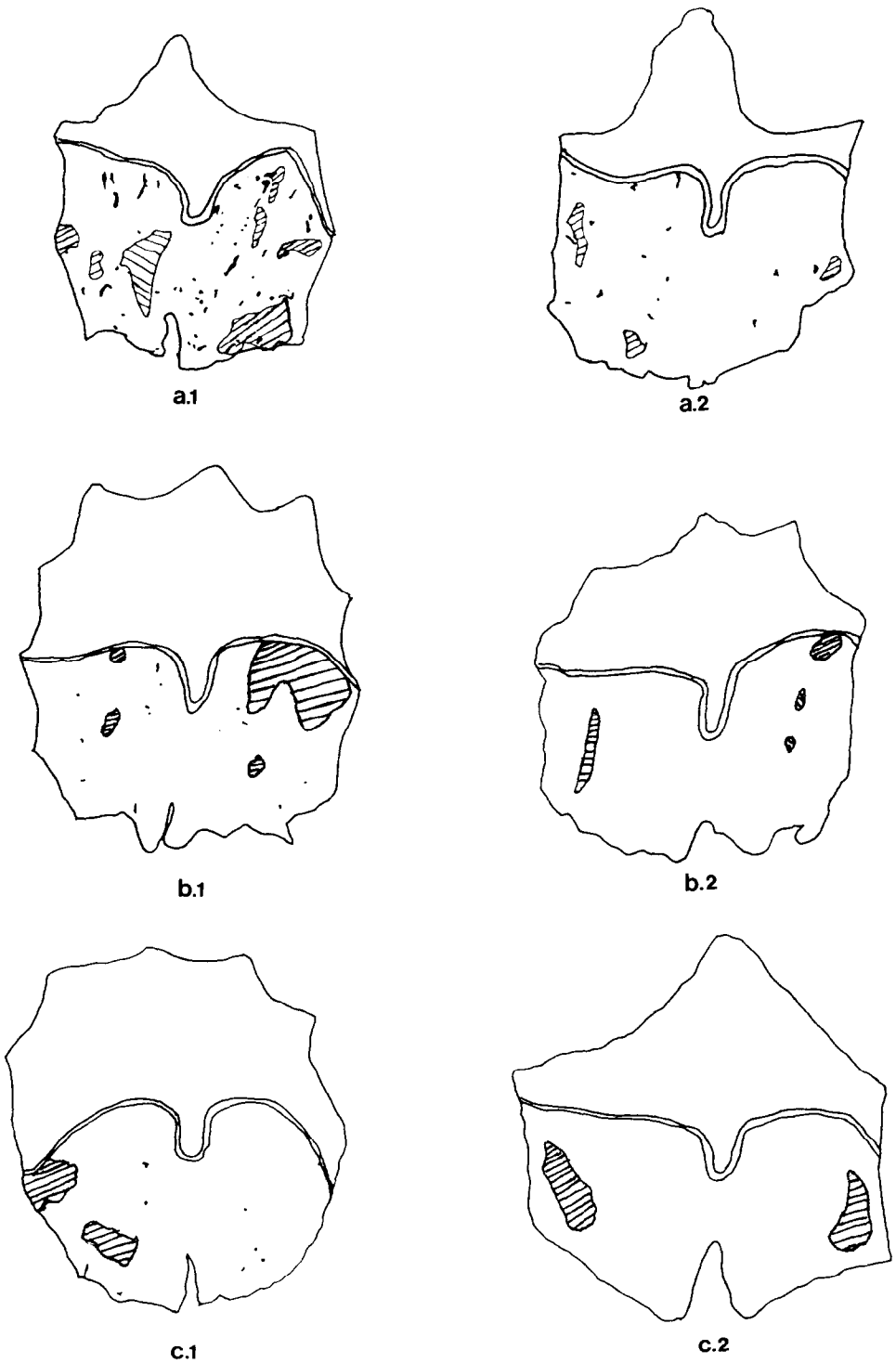

Fig. 5. Schematic representation of the inside of the rats' stomachs showing the limiting cases of damage produced by each formulation: (a) naproxen, (b) inclusion complex, (c) control. (1) Damage of upper valves. (2) Damage of lower valves. (■) Ulcers, (▨) Lessening of the consistency of the gastric tissue.

TABLE 1

Ulcerogenic index assigned by each observer to each rat ($\overline{UI}$: average ulcerogenic index)

Rat	Observer					$\overline{UI}$
	A	B	C	D	E	
1	115	147	90	147	102	120.2
2	97	131	92	92	111	104.6
3	55	57	55	49	59	55.0
4	38	24	28	24	50	32.8
5	118	168	224	168	184	172.4
6	37	54	54	51	49	49.0
7	57	73	57	49	48	56.5
8	30	34	29	24	36	30.6
1	32	38	41	46	56	42.6
2	19	27	34	29	14	24.6
3	9	4	4	4	4	5.0
4	4	4	4	4	4	4.0
5	14	9	4	4	9	8.0
6	15	22	28	28	28	24.2
7	29	24	30	16	26	25.0
8	17	24	20	26	21	21.6
1	4	4	9	10	4	6.2
2	14	19	12	12	11	13.6
3	4	4	9	4	4	5.0
4	4	4	4	4	4	4.0
5	4	4	4	4	4	4.0
6	4	4	0	4	4	3.2
7	4	4	0	4	4	3.2
8	4	4	4	4	4	4.0

TABLE 2

Values of the average ulcerogenic index and their corresponding ranks, obtained for each rat in the different groups

Rats	Naproxen		Complex		Control	
	$\overline{UI}$	Ranks	$\overline{UI}$	Ranks	$\overline{UI}$	Ranks
1	120.2	23	42.6	18	6.2	9
2	104.6	22	24.6	14	13.6	11
3	55.0	20	5.0	7.5	5.0	7.5
4	32.8	17	4.0	4.5	4.0	4.5
5	172.4	24	8.0	10	4.0	4.5
6	49.0	19	24.2	13	3.2	1.5
7	56.5	21	25.0	15	3.2	1.5
8	30.6	16	21.6	12	4.0	4.5
Global $\overline{UI}$	78.75		19.29		5.67	
ΣR		162		64		44
$\overline{R}$		20.25		11.75		5.5

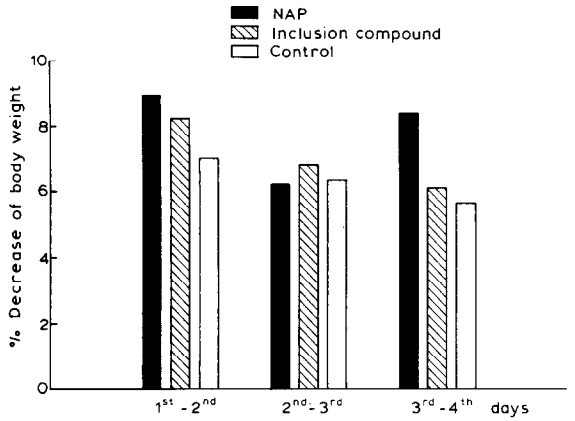

Fig. 6. Weight loss, expressed as a percentage of body weight, suffered by the animals of each group over the course of the treatment.

The ANOVA (Table 3) made clear statistically significant differences on the last day of the experiment among the three formulations used. Application of the least significant difference test (LSD) showed the differences arose in the group treated with naproxen. Interestingly, in some rats of this group at the end of the study we observed the presence of diarrhoea and blood in the stomach, which could have been produced by haemorrhaging ulcers caused by the presence of uncomplexed naproxen.

The decrease observed in the local toxicity of naproxen at the level of the gastrointestinal tract is in keeping with both the solubility increase, as shown by the solubility diagram, of this active principle in an acid medium after its inclusion in β-cyclodextrin, and the stability of the complex in the gastric medium, the drug being held in the cavity of the host so that it does not come into direct contact with the mucus.

TABLE 3

Analysis of variance (ANOVA) and least significant difference test (LSD) for the mean percentage of decreasing body weight

Source	Sum of squares	D.F.	Mean square	F	α
Formulation	35.086	2	17.543	3.49	< 0.05
Error	79.013	21	3.763		
Total	114.098	23			

LSD = 2.22

Third-Fourth day

$|\bar{X}_{NAP} - \bar{X}_{COMP}| = 2.28 > LSD$

$|\bar{X}_{NAP} - \bar{X}_{CONTROL}| = 2.75 > LSD$

$|\bar{X}_{COMP} - \bar{X}_{CONTROL}| = 0.47 < LSD$

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