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Oral bioavailability of naproxen-β-cyclodextrin inclusion compound

F.J. Otero-Espinar, S. Anguiano-Igea, N. García-González, J.L. Vila-Jato and J. Blanco-Méndez

Departamento Farmacología, Farmacia y Tecnología Farmacéutica, Laboratorio de Farmacia Galénica, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela (Spain)

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Summary

An inclusion complex between naproxen and β-cyclodextrin was prepared by the freeze-drying method. Dissolution profiles in a pH 1.2 medium (gastric juice) of naproxen, freeze-dried naproxen, a physical mixture and 1:1 and 1:3 inclusion complexes demonstrated a faster dissolution rate of the inclusion complex. After 5 min the amount of drug dissolved was around 5% for the drug, 12% for freeze-dried naproxen, 11% for the physical mixture and 61 and 99% for the 1:1 and 1:3 inclusion complexes, respectively. The bioavailability of naproxen administered as its 1:1 inclusion complex with β-cyclodextrin has been evaluated using a new HPLC method for determining the drug and its glucuronide conjugate in urine. Renal excretion levels after oral administrations of naproxen, freeze-dried naproxen and the 1:1 inclusion complex with β-cyclodextrin, measured by this method, show no statistical differences, indicating that the three formulations assayed are bioequivalent.

Introduction

Cyclodextrins (CDs) are well-known for their ability to form inclusion complexes with lipophilic drug molecules. Inclusion complexes of drugs may offer a number of advantages over the pure compounds, such as enhancement of dissolution rate (Corrigan and Stanley, 1982; Nozawa and Yamamoto, 1989), drug stability (Uekama et al., 1983a, 1985; Vikmon et al., 1986) and reduction in the side effects (Nambu et al., 1978a; Uekama et al., 1982; Otero-Espinar et al., 1991). Many reports have been published on improved drug bioavailability as a result of complex formation with cyclodextrin in laboratory animals (Nambu et al., 1978b; Uekama et al., 1983b; Vila-Jato et al., 1988; Stracciari et al., 1989), however, few studies have appeared concerning the oral administration of the drug-cyclodextrin complexes in humans (Uekama et al., 1983c; Vila-Jato et al., 1986; Bootsma et al., 1989).

Naproxen (NAP) is a non-steroid anti-inflammatory of the group of aryl acetate derivatives. The solubility and dissolution rate of naproxen in acid media are poor. It was reported previously that the freeze-drying method was the

Correspondence: F.J. Otero-Espinar, Dpto Farmacología, Farmacia y Tecnología Farmacéutica, Laboratorio de Farmacia Galénica, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain.

most suitable for obtaining an inclusion complex of naproxen with β-cyclodextrin; this was confirmed by various physico-chemical methods (Blanco et al., 1991). The inclusion complex obtained had a 1:1 stoichiometry. The improvement in solubility and good stability characteristics of the complex in acid medium ($k_c = 1378$ M^{-1}, pH 1 and 25 °C) have been shown to lead to a significant reduction in the adverse collateral effects in the gastric mucosa that are associated with oral administration of this drug. In a previous study in rats (Otero-Espinar et al., 1991), the inclusion complex was found to be the cause of a considerable reduction in the ulcerogenic potential of naproxen.

In this communication, the formation of the naproxen-β-cyclodextrin complex has been studied inorder to determine whether it is related to some modification of the drug's oral bioavailability in humans.

Experimental

Materials

Naproxen (Sigma^R), β-cyclodextrin (Chinoin^R), HPLC-grade acetonitrile UV (Scharlau^R), phosphoric acid (Merck^R); Milli-Q water for HPLC, phosphate buffer pH 7 and β-glucuronidase from *Escherichia coli* type VII-A (Sigma^R) were obtained from the indicated sources.

Preparation of the inclusion complex

Corresponding quantities of naproxen and β-cyclodextrin were weighed out and dissolved in deionized water. Small quantities of ammonium hydroxide (0.5 ml of 35% ammonium hydroxide/l H_2O) were added to assist the dissolution of naproxen. Once transparent, the solution was stirred for 24 h and freeze-dried (Telstar Freeze-dryer).

The drug was taken through the same process in the absence of β-cyclodextrin.

Dissolution studies

Drug dissolution tests were performed in a USP XXII Ed. Type 2 dissolution apparatus. The dissolution medium consisted of 900 ml of artificial gastric juice pH 1.2 (USP XXII Ed.), stirred at 75 rpm and maintained at 37 ± 0.5 °C. An amount of each sample equivalent to 50 mg of naproxen was added to the medium. At appropriate intervals, 5-ml aliquots were withdrawn and filtered. A correction was applied for the cumulative dilution caused by the replacement of the sample by an equal volume of fresh medium. The concentration of naproxen dissolved was determined spectrophotometrically at 272 nm ($E_1^1 = 218$).

For comparison of the dissolution profiles, the percentage of naproxen dissolved at 5 min (D_5), 30 min (D_{30}) and the dissolution efficiency at 120 min D.E. (Khan and Rhodes, 1975) were used:

$$\text{D.E.} = \frac{\int_0^t M \, dt}{M_t t}$$

where M_t is the percentage dissolved and t denotes the time.

In vitro study design

Bioavailability was evaluated by a method based on determining the naproxen excreted in urine; the excreted, unaltered drug and its glucuronide derivative (after hydrolysis with β-glucuronidase) are determined.

Twelve healthy and informed volunteers of both sexes, aged between 20 and 30 years without any history of renal pathologies participated in the study. They were divided into three equal groups at random, according to a 3 × 3 Latin square design.

Formulations were administered in gelatin capsules containing 200 mg of naproxen, freeze-dried naproxen and a quantity of complex equivalent to 200 mg of drug. The volunteers took these formulations immediately following a light breakfast. Consecutive administrations were separated by 7 days.

Bioanalysis

The chromatographic method proposed by Owen et al. (1987) for determining non-steroid anti-inflammatories in plasma has been modified

to determine naproxen and its glucuronide excreted in urine. The conjugate derivative is broken down prior to determination using β-glucuronidase. Finally, the unaltered naproxen and the treated glucuronide excreted are assayed together.

The volumes of urine excreted are measured and 0.5 ml is placed in a screw-top tube, to which is added 250 μl of a solution of β-glucuronidase, containing 100 units of enzyme per ml. The solution is made up to 2 ml with pH 7 phosphate buffer and stirred. These solutions are maintained for at least 12 h at 37 °C in an incubation chamber. The incubated samples are then filtered through 0.45 μm nylon membranes (Millipore).

The samples were introduced by a manual injector (Rheodyne) with a 100 μl loop into the chromatographic system (Hewlett Packard 1081A liquid chromatograph) with a fixed-wavelength (254 nm) UV detector and a Hewlett Packard 3380A intergrator. An acetonitrile : phosphoric acid (0.03% phosphoric acid pH 3.5 ± 0.2) mixture (40 : 60 v/v) was used as the mobile phase, filtered through 0.45 μm nylon membranes (Millipore) and degassed ultrasonically. The stationary phase was a C_{18}-μBondapak column (300 × 3.9 mm, particle size 10 μm), preceded by a Waters precolumn containing particles of C_{18}-μBondapak. The samples were diluted in mobile phase for injection and all the chromatograms were obtained at room temperature.

The calibration curve was plotted for known quantities of drug in urine, the range of concentrations employed being 5–100 μg/ml. The standard curves were determined as the area under the peaks against the concentration of drug. For the interval used, the relation between the area and the concentration was linear and could be fitted by the following equation using linear regression:

$$[\text{NAP}]\ (\mu\text{g/ml}) = -0.2203 + 8.2259 \times 10^{-6}$$

$$\text{area}\ (r = 0.9955)$$

Under the chromatographic conditions described, the drug is eluted with a retention time of around 8 min. For all the samples examined, drug chromatograms did not show any interference with the peaks corresponding to urine (Fig. 1).

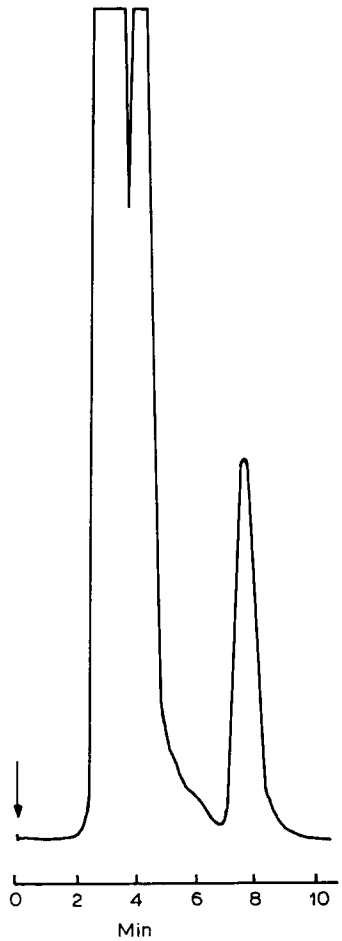

Fig. 1. HPLC profile of urine from a healthy volunteer following oral administration of 200 mg of naproxen.

Evaluation of bioavailability: parameters

For the evaluation and characterization of the excretion curves, the three statistical moments proposed by Yamaoka et al. (1978) for curves of plasma levels, and as modified by Vila-Jato et al. (1980) for urine excretion curves were used. These moments are: (1) total amount of drug excreted (Xu_{48h}), or zero-order moment; (2) mean residence time (MRT), or first-order moment with respect to the origin; and (3) variance of the

mean residence time (VRT), or second-order moment with respect to the origin.

Xu_{48h} was determined directly from the urine excretion data. The first- and second-order moments were calculated from:

$$MRT = \frac{\int_0^t (dXu/dt)\, dt}{Xu_{48h}}$$

$$VRT = \frac{\int_0^t (t - MRT)^2 (dXu/dt)\, dt}{Xu_{48h}}$$

where dXu/dt is the excretion rate, and t is the time.

Statistical analysis

A two-way multivariant analysis of variance (MANOVA) (Vila-Jato et al., 1980), grouping by formulations and subjects was used. To verify the null hypothesis:

$$\boldsymbol{H}_0: \boldsymbol{C}\phi\boldsymbol{M} = 0$$

where $\boldsymbol{C}$ is the matrix of the contrasts for the different parameters, ϕ is the parameter matrix and $\boldsymbol{M}$ is the identity matrix, the greatest eigenvalue of the matrix $\boldsymbol{HE}^{-1}$ ($\boldsymbol{C}_s$) was calculated, where $\boldsymbol{H}$ is the matrix of the sum of squares and products of the treatments term and $\boldsymbol{E}$ is the matrix of the 'error'. The $\boldsymbol{C}_s/(\boldsymbol{C}_s + 1)$ statistic must be looked up in the Heck charts for the distribution parameters $s = \min[(k-1), p]$, $m = [|k - 1 - p| - 1]/2$, $n = [k(b-1) - b - p]/2$, where b is the number of subjects, p the number of parameters and k the number of formulations.

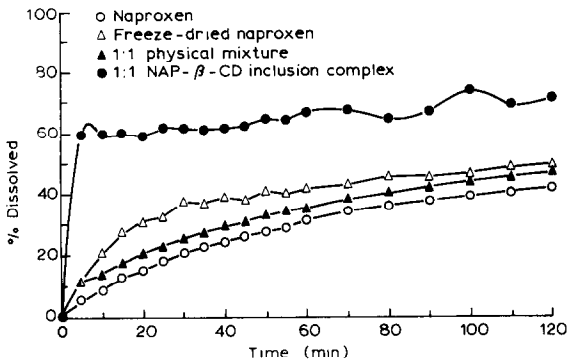

Fig. 2. Percentages of naproxen dissolved (mean of six assays), determined at pH 1.2 and 37 °C.

Results and Discussion

Dissolution studies

The dissolution profiles of drug, freeze-dried drug, physical mixture and 1 : 1 inclusion complex are shown in Fig. 2. It is evident that the complex demonstrates a faster dissolution rate than the free drug. In order to ascertain whether the differences found were statistically significant, the parameters D_5, D_{30} and D.E. were compared using an ANOVA (Table 1). Application of the least significant difference test (LSD) showed an improvement in the dissolution characteristics of naproxen in the inclusion complex. However, the 1 : 1 inclusion complex does not provide complete dissolution. In aqueous solution, the dissolution and dissociation equilibrium simultaneously de-

TABLE 1

Results of one-way analysis of variance and least significant difference test for D_5, D_{30} and dissolution efficiency (D.E.) at 120 min

Parameter	F	α	LSD	Mean value of the tested parameters for each formulation				
				NAP	Physical mixture	Freeze-dried NAP	1 : 1 inclusion compound	1 : 3 inclusion compound
D_5	1000.0	< 0.01	3.76	5.49	11.53	12.22	60.80	99.03
D_{30}	944.79	< 0.01	4.95	20.92	25.31	37.43	63.58	99.78
D.E.	144.33	< 0.01	0.0058	0.2856	0.3274	0.3855	0.6426	–

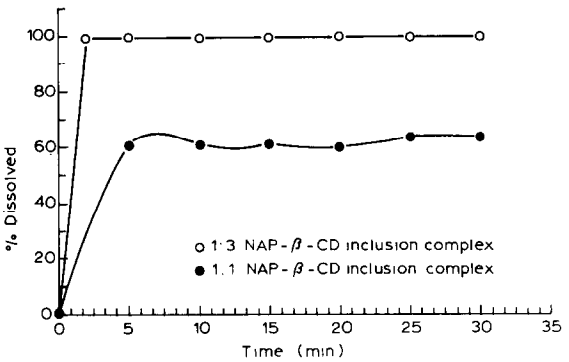

Fig. 3. Dissolution profiles of naproxen from NAP/β-CD systems in gastric juice (pH 1.2) at 37 °C.

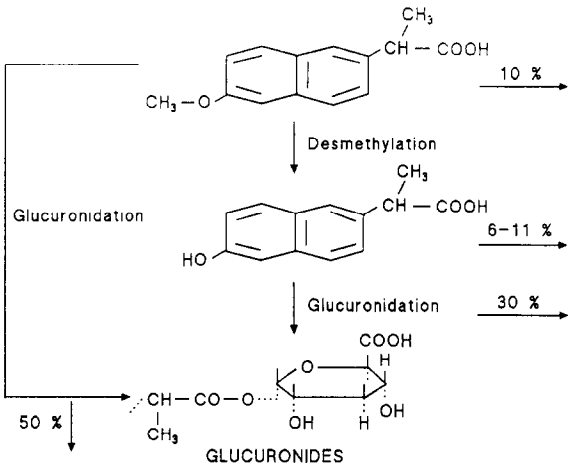

Fig. 4. Biotransformation and urinary excretion scheme of naproxen.

termine the amount of dissolved drug in either complexed or free form. The degree of dissociation of the complex depends on its stability constant and its concentration conditions in the solution. Under our conditions (900 ml of gastric juice at 37 °C) dissolving the solid complex, the high dilution results in the instantaneous dissociation of the complex. When the concentration of free drug, released by dissociation, surpasses its saturation concentration, the excess will precipitate as a solid uncomplexed microcrystalline drug. If excess cyclodextrin is present together with the inclusion complex, the dissociation equilibrium can be shifted towards a higher molar ratio of complexed drug in the dissolution medium, and thereby give rise to an increase in the amount of drug dissolved. Fig. 3 shows the dissolution profiles for 1:1 and 1:3 (drug:CD) naproxen-β-cyclodextrin inclusion complexes. The dissolution profiles confirm the previous findings that the addition of excess cyclodextrin with the complex significantly enhanced the amount of dissolved drug.

In vivo studies

Almost the entirety of naproxen is eliminated in urine (94% of the dosage) with a mean elimination time of 14 h (10–20 h); 10% is eliminated unaltered, 5–11% as 6-*O*-desmethylnaproxen and 50 and 30% as conjugated naproxen glucuronide and 6-*O*-desmethylnaproxen glucuronide, respectively (Runkel et al., 1976) (Fig. 4). Hence, the rapid and complete renal clearance of this drug is the result of the formation of soluble conjugates in water. Runkel et al. (1972) also found that, after i.v. bolus administration of the drug, the excretion rate is proportional to the plasma concentrations, therefore determining naproxen excreted renally would appear to represent an accurate means for measurement of its bioavailability.

Figs 5 and 6 show the mean urinary excretion rate profiles for naproxen and the accumulative excretion curves, respectively. The profiles obtained show no variations among the three formulations assayed; the rate and quantity excreted are similar in the three cases.

The curves were used to calculate the three statistical moments chosen to define the excretion profiles of naproxen: Xu_{48h}, MRT and VRT

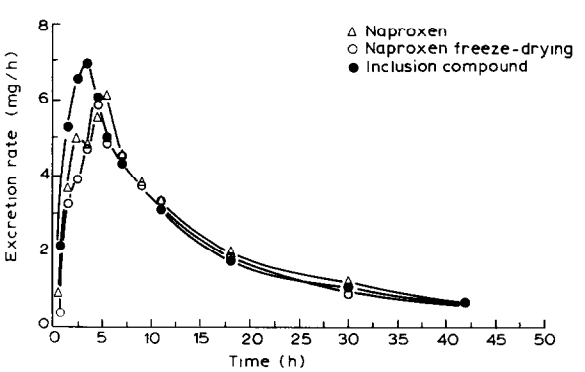

Fig. 5. Mean curves of urinary excretion rate.

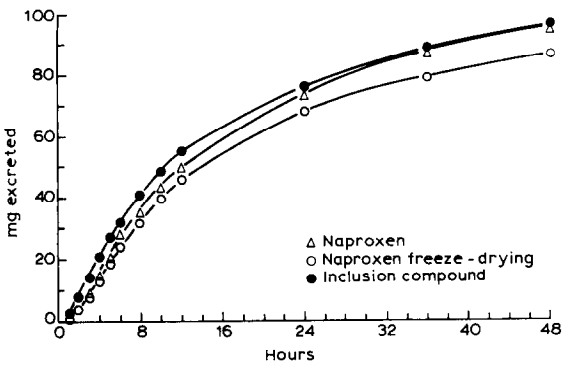

Fig. 6. Cumulative amount of naproxen excreted from human volunteers.

(Table 2). MANOVA confirmed the null hypothesis (H_0); the results are listed in Table 3.

The results obtained demonstrate that the three formulations are bioequivalent under the conditions imposed in the assay. Among the formulations there is no difference in the total amount of naproxen absorbed, or in the rate of absorption, despite the large differences among them in dissolution behaviour.

The lack of effect of increasing solubility and dissolution rate on drug absorption may be because solubility is not the limiting step in the absorption of naproxen. Similar findings were reported by Szejtli (1988) for the indomethacin–β-cyclodextrin (1 : 1) complex.

The absorption of orally administered naproxen begins in the stomach but occurs mainly in the intestine, and is dose-independent up to 500 mg.

TABLE 3

Results of MANOVA [a]

Source	Matrix	Sums of squares and products		
Formulations	***H***	697.4502		
		−49.9463	9.7458	
		−226.4589	−3.6087	137.2476
Subjects	***S***	5203.3870		
		259.0765	99.1036	
		424.1195	1061.2253	16401.6887
Error	***E***	3559.8650		
		215.7052	114.9910	
		2397.5648	1156.5767	14487.4612
Total	***T***	9540.7032		
		424.0355	223.8405	
		2595.2254	2214.1934	31026.3975

[a] The greatest eigenvalue of HE^{-1} is $C_s = 0.4$ and $C_s/(1+C_s) = 0.5742$; the parameters for its distribution are $s = 2$, $m = 0$ and $n = 9$. Null hypothesis accepted at $\alpha = 0.05$ level.

TABLE 2

Calculated values of statistical moments for each subject and formulation

Subject	Naproxen			Freeze-dried naproxen			Inclusion complex		
	Xu_{48h}	MRT	VRT	Xu_{48h}	MRT	VRT	Xu_{48h}	MRT	VRT
1	141.81	18.35	169.98	95.78	15.51	126.10	141.60	17.12	190.99
2	110.46	16.43	170.89	71.74	16.33	178.73	102.21	16.80	181.38
3	107.22	20.94	173.93	88.14	15.98	163.03	95.26	16.80	161.01
4	92.91	13.81	133.51	99.82	12.95	177.33	87.95	11.11	100.58
5	77.17	12.96	124.28	88.11	20.64	203.79	85.13	13.57	156.34
6	75.70	11.20	106.06	93.18	16.42	170.68	92.67	11.65	128.10
7	86.08	13.05	131.14	77.01	14.78	152.10	96.89	11.42	163.04
8	92.95	14.11	150.60	84.42	15.30	149.05	106.61	14.09	155.15
9	88.11	20.64	230.79	84.14	12.74	152.39	81.72	15.63	181.97
10	80.25	13.77	139.89	63.14	14.76	137.11	85.63	11.37	174.08
11	84.61	14.37	144.21	84.61	14.37	144.21	84.06	11.37	104.65
12	104.94	13.49	104.59	82.65	15.26	140.71	82.65	15.26	140.71
Mean	95.18	15.26	148.32	85.85	15.42	151.48	95.20	14.24	150.08
STD	17.86	3.00	34.78	12.79	1.93	25.29	15.94	2.26	27.38

Xu_{48h} in mg, MRT in h, and VRT in h^2.

The increase in solubility at acid pH achieved by the formation of the complex with β-cyclodextrin will influence absorption in the stomach, but not in the small intestine, since in this region the drug is ionized, and hence its solubility and dissolution rate will be high. From these considerations, the gastric emptying time is a determining factor in the initial moments of absorption. However, the rate at which solids pass to the intestine depends on intrinsic factors of the stomach, whether or not food is present and on the characteristics of the dosage form (Davis, 1986; Davis et al., 1990): multiparticulate forms, such as those used in this work, as against monolithic or large particles, will not be retained by the pylorus, and can pass to the intestine, although other factors, such as the presence of food in the stomach, can delay this somewhat.

Given that the permanence time of the drug and the complex in the stomach is short and variable, a partial explanation for the lack of differences in the absorption rate and the large variability found among subjects is therefore evident.

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