J. Pharm. Pharmacol. 1995, 47: 120–123
Received October 14, 1993
Accepted September 7, 1994

Differential Effects of Modified β-Cyclodextrins on Pharmacological Activity and Bioavailability of 4-Biphenylacetic Acid in Rats after Oral Administration

G. PUGLISI, C. A. VENTURA, A. SPADARO, G. CAMPANA* AND S. SPAMPINATO*

*Istituto di Chimica Farmaceutica e Tossicologica, Università di Catania, V.le A. Doria 6, 95125 Catania, and *Dipartimento di Farmacologia, Università di Bologna, Via Irnerio 48, 40126 Bologna, Italy*

Abstract

Gastric tolerability, absorption and pharmacological activity of the non-steroidal anti-inflammatory drug 4-biphenylacetic acid (BPAA), as an inclusion complex with β-cyclodextrin (β-CyD) or chemically modified β-CyDs: 2,6-di-*O*-methyl-β-CyD (DM-β-CyD), 2,3,6-tri-*O*-methyl-β-CyD (TM-β-CyD) and 2-hydroxypropyl-β-CyD (HP-β-CyD), were investigated in the rat after oral administration.

BPAA absorption, determined from area under the plasma concentration-time curve (AUC), was increased by complexation with all β-CyDs in the following order: DM-β-CyD > TM-β-CyD > HP-β-CyD > β-CyD. The carrageenan paw oedema test demonstrated a significant increase in anti-inflammatory activity of BPAA and the ED50 values, compared with BPAA alone, were reduced to about a third for the BPAA-DM-β-CyD complex and halved for the others. BPAA complexed with DM-β-CyD, HP-β-CyD or β-CyD showed better gastric tolerability compared with uncomplexed drug, whereas the BPAA-TM-β-CyD complex produced marked gastric lesions similar in extent to BPAA alone. TM-β-CyD (500 mg kg^{-1}) and DM-β-CyD (1000 mg kg^{-1}) caused gastric erosions 21 h after oral administration.

The pharmacokinetic profiles of BPAA-β-CyD complexes have shown that DM-β-CyD is the most effective in enhancing the bioavailability of BPAA.

Inclusion of non-steroidal anti-inflammatory drugs (NSAIDs) with cyclodextrins (CyD) can improve their bioavailability (Nambu et al 1978a; Szejtli & Szente 1981) and tolerability (Nambu et al 1978b; Otero Espinar et al 1991). We reported (Puglisi et al 1991) that β-CyD increases the bioavailability, pharmacological activity and gastric tolerability of 4-biphenylacetic acid (BPAA), a useful NSAID (Tolman & Partridge 1975). In fact, this drug may cause gastric erosion and ulceration following oral administration as its low water solubility increases the contact time with the stomach wall (Sloboda & Osterberg 1976; Puglisi et al 1991). Unfortunately, β-CyD has a low water solubility (18 g L^{-1}) which limits the solubility of any complexes. Chemically-modified β-CyDs have therefore been investigated because they are more soluble in water and organic solvents, and also form inclusion complexes like β-CyD (Uekama 1985; Yoshida et al 1988). In a previous study we described the preparation, dissolution rate and solubility of the inclusion complexes between BPAA and β-CyD (Puglisi et al 1990) and of 2,6-di-*O*-methyl-β-CyD (DM-β-CyD), 2,3,6-tri-*O*-methyl-β-CyD (TM-β-CyD) and 2-hydroxypropyl-β-CyD (HP-β-CyD) and the diffusion rate of BPAA included in β-CyDs (this abbreviation is comprehensive of unmodified and modified β-CyD) across an artificial membrane. The diffusion rates of the BPAA-β-CyD complexes, particularly that of the BPAA-DM-β-CyD complex, were greater than that of the BPAA alone (Ventura et al 1994).

Correspondence: G. Puglisi, Istituto di Chimica Farmaceutica e Tossicologica, Università di Catania, V.le A. Doria 6, 95125 Catania, Italy.

In this study, we investigated the gastric tolerability, bioavailability and anti-inflammatory activity of BPAA, as an inclusion complex with chemically-modified β-CyDs, after oral administration in rats. The results obtained were compared with those of the free and β-CyD-complexed drug.

Materials and Methods

Chemicals

4-Biphenylacetic acid (BPAA) was obtained from Janssen (Belgium) and was recrystallized from ethanol. β-Cyclodextrin (β-CyD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD), with an 0·6 degree of average substitution, were kindly provided by SPAD (Italia SpA). Heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) and heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CyD) were supplied by Nikon Skoduhhin Kako Co. Ltd (Tokyo, Japan) and used without further purification. Indomethacin (Sigma, Italy) was used as an HPLC internal standard. All other chemicals and solvents were analytical reagent grade. De-ionized, double-distilled water was used.

Preparation of the solid complexes

The solid BPAA-β-CyD complexes in a 1 : 1 molar ratio were prepared by a freeze-drying method (Ventura et al 1994).

Animals and treatments

Male Sprague-Dawley rats, 120–150 g (Nossan, Correzzana, Italy), were starved 24 h before treatment while water was withheld 6 h before. BPAA or the corresponding

amount of BPAA-β-CyD complexes were administered orally as suspensions (BPAA, BPAA-β-CyD and BPAA-TM-β-CyD) or solutions (BPAA-DM-β-CyD and BPAA-HP-β-CyD) in distilled water (10 mL kg^{-1}). Control rats were treated with water alone.

Determination of blood BPAA concentration

Groups of three rats were anaesthetized using sodium pentobarbitone (30–40 mg kg^{-1}, i.p.) 15, 30, 60, 120, and 180 min after the oral administration of the compounds, and 8 mL blood was withdrawn through a catheter inserted into the jugular vein. Rats were killed immediately after (sodium pentobarbitone, 40 mg kg^{-1}, i.v.). One hundred microlitres of collected plasma was treated with 100 μL 1% $ZnSO_4 \cdot 7H_2O$ in methanol:water (70:30, v/v) containing 169·90 μg mL^{-1} indomethacin (internal standard), followed by vortexing for 1 min, to deproteinize the plasma. After centrifugation (6000 rev min^{-1} for 10 min at 4°C), the supernatant was filtered through a 0·20-μm Teflon membrane and 10 μL eluate was analysed by HPLC. Recovery was 98·5% (s.d. = 1·52). All plasma samples were analysed using an HPLC system, which consisted of a solvent pump (Varian Star 9010), a variable UV-vis detector (Varian Star 9050) on line with a Varian Star 9020 workstation. A reverse-phase column (Hypersil ODS; 5 μm of 150 mm × 4·6 mm i.d., obtained from Alltech) equipped with a direct-connect guard column (Hypersil ODS; 5 μm of 10 mm × 4·6 mm i.d., obtained from Alltech) was used in conjunction with the HPLC apparatus. The HPLC conditions were as follows: injection volume, 10 μL; flow rate, 1 mL min^{-1}; mobile phase, methanol: H_3PO_4 0·05 M (70:30, v/v); detection wavelength, 254 nm. Blanks were obtained on 100 μL serum from controls using the same procedure described above. The sensitivity of the assay was 9·5 ng for a signal/noise ratio of 3:1.

Carrageenan-induced paw oedema test

Paw oedema was induced in Sprague-Dawley rats by means of a subplantar injection into the right hindpaw of 0·05 mL 1% carrageenan suspension (Winter et al 1962). BPAA and BPAA-β-CyD complexes were administered orally 2 h after the irritant. The oedema was evaluated 2, 3 and 5 h after the carrageenan injection. Anti-inflammatory effects of the two assayed compounds were expressed as the percent inhibition of the control response and ED50 values were estimated by fitting a linear regression line to a plot of log dose vs percent inhibition.

Gastric ulceration assay

Rats were killed by an overdose of sodium pentobarbitone (100 mg kg^{-1}, i.p.); 21 h after the treatment, the stomachs were removed, inverted, washed in cold saline and the lesions on the gastric mucosa counted visually under 5× magnification. All lesions were counted regardless of size.

Results

BPAA-β-CyD complexes were investigated to evaluate the absorption rate of BPAA compared with that of uncomplexed drug. Fig. 1 shows the plasma concentrations of the drug following the oral administration of BPAA and BPAA-β-CyD complexes in rats.

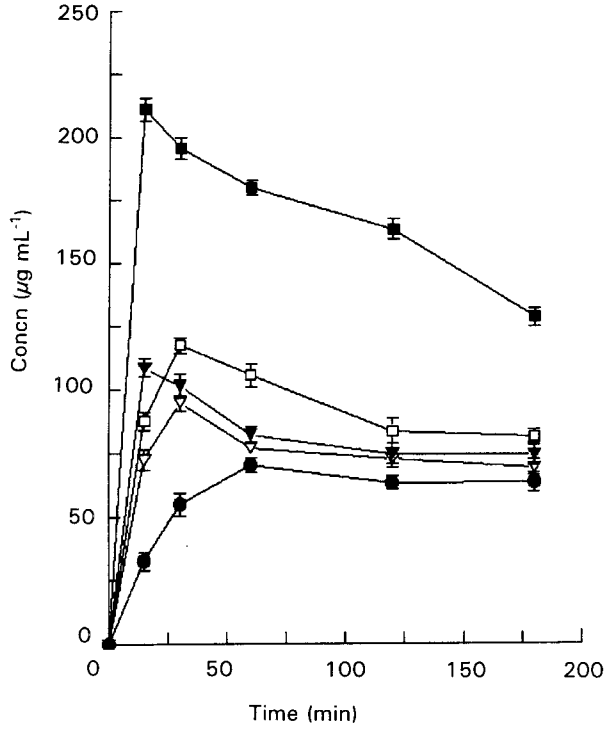

FIG. 1. 4-Biphenylacetic acid (BPAA) plasma levels in rats, following oral administration of 10 mg kg^{-1} BPAA. Values are the mean ± s.e.m. of three HPLC determinations. ● BPAA, ▽ BPAA-β-CyD, ▼ BPAA-HP-β-CyD, □ BPAA-TM-β-CyD, ■ BPAA-DM-β-CyD.

For uncomplexed BPAA, maximum plasma levels of 70·2 ± 1·1 μg mL^{-1} (s.e.m., n = 3) were reached 60 min after administration. An increase in absorption and an earlier appearance of the haematic peak was observed when BPAA was included with β-CyDs. Higher plasma levels, 211·03 ± 2.6 μg mL^{-1} (s.e.m., n = 3), were determined 15 min after the administration of the BPAA-DM-β-CyD complex. The area under the plasma concentration-time curve (AUC) of this complex (up to 3 h after treatment) was found to be approximately three times that of BPAA alone (Table 1). The administration of the other complexes led to increased bioavailability with respect to free drug, but to a lesser extent than that of the BPAA-DM-β-CyD complex. The $AUC_{0-3\,h}$ values were enhanced by 23, 34 and 55% for BPAA-β-CyD, BPAA-HP-β-CyD and BPAA-TM-β-CyD complexes, respectively, in comparison with uncomplexed BPAA.

Table 1. Pharmacokinetic parameters (± s.e.m., n = 3) of BPAA following oral administration of free BPAA and its inclusion complexes.

Compound	$AUC_{0-3\,h}$ (h μg mL^{-1})	C_{max} (μg mL^{-1})
BPAA	10736	70·2 ± 1·1
BPAA-β-CyD	13206	94·6 ± 1·5
BPAA-HP-β-CyD	14444	108·6 ± 2·5
BPAA-TM-β-CyD	16210	117·3 ± 2·0
BPAA-DM-β-CyD	30609	211·0 ± 2·6

Table 2. Inhibitory effects of BPAA-β-CyD complexes and BPAA alone on carrageenan hindpaw oedema in the rat. The compounds were administered orally 2 h after carrageenan and dose-response curves evaluated from the data 5 h after drug administration, were used to calculate the ED50 and 95% confidence limits. Values are the mean ± s.e.m. of six rats/group.

Compound	Dose (mg kg^{-1})	Increase in paw volume (mL) at time			ED50 (mg kg^{-1})
		2 h	3 h	5 h	
Control		0·63 ± 0·04	0·69 ± 0·04	0·61 ± 0·04	
BPAA	1	0·57 ± 0·04	0·66 ± 0·02	0·59 ± 0·08	
	3	0·50 ± 0·03	0·68 ± 0·04	0·36 ± 0·04	6·51 (13·91–3·05)
	10	0·52 ± 0·04	0·35 ± 0·06	0·22 ± 0·05	
	20	0·51 ± 0·05	0·28 ± 0·06	0·13 ± 0·04	
BPAA-β-CyD	6·4 (1)[a]	0·55 ± 0·03	0·51 ± 0·04	0·50 ± 0·03	
	19 (3)[a]	0·48 ± 0·06	0·55 ± 0·04	0·32 ± 0·03	3·11 (6·74–1·43)
	63·4 (10)[a]	0·47 ± 0·06	0·37 ± 0·05	0·12 ± 0·04	
	126·8 (20)[a]	0·53 ± 0·08	0·22 ± 0·06	0·04 ± 0·05**	
BPAA-HP-β-CyD	10·2 (1)[a]	0·55 ± 0·08	0·54 ± 0·07	0·48 ± 0·05	
	30·6 (3)[a]	0·51 ± 0·04	0·53 ± 0·03	0·30 ± 0·02	3·02 (7·40–1·23)
	102 (10)[a]	0·42 ± 0·06	0·31 ± 0·05	0·10 ± 0·04*	
	204 (20)[a]	0·52 ± 0·06	0·20 ± 0·06	0·04 ± 0·05**	
BPAA-DM-β-CyD	10·4 (1)[a]	0·56 ± 0·07	0·50 ± 0·03	0·42 ± 0·06	
	31·3 (3)[a]	0·55 ± 0·04	0·40 ± 0·04	0·25 ± 0·02*	2·02 (5·51–0·74)
	104 (10)[a]	0·53 ± 0·07	0·20 ± 0·04*	0·07 ± 0·04**	
	208 (20)[a]	0·52 ± 0·04	0·15 ± 0·05**	0·02 ± 0·01**	
BPAA-TM-β-CyD	10·1 (1)[a]	0·54 ± 0·09	0·55 ± 0·07	0·47 ± 0·04	
	30·3 (3)[a]	0·53 ± 0·08	0·50 ± 0·05	0·30 ± 0·03	3·09 (8·55–1·12)
	101 (10)[a]	0·44 ± 0·04	0·33 ± 0·08	0·15 ± 0·04	
	202 (20)[a]	0·46 ± 0·05	0·20 ± 0·05	0·07 ± 0·04**	

[a]Amount of active principle in the complex. *$P < 0·05$; **$P < 0·01$ vs BPAA-treated rats at the same dose (Newman–Keuls test).

BPAA and BPAA-β-CyD complexes were compared for anti-inflammatory activity in the rat paw oedema test (Table 2). The drugs were administered orally 2 h after carrageenan and significantly inhibited the intensity of oedema in a dose-dependent manner. The inclusion complexes displayed a higher activity than the drug alone. Dose-response curves, calculated from the data 5 h after drug treatment indicated the following order of activity: BPAA-DM-β-CyD > BPAA-HP-β-CyD ≥ BPAA-TM-β-CyD ≥ BPAA-β-CyD > BPAA (Table 2).

BPAA and BPAA-β-CyD complexes were compared for gastric tolerability by evaluating their tendency to cause gastric lesions. The inclusion complexes of BPAA with β-CyD, HP-β-CyD and DM-β-CyD displayed a significant reduction in gastric lesions compared with BPAA alone. On the contrary, the total number of gastric lesions in rats treated with BPAA-TM-β-CyD complex was similar to that detected in animals treated with BPAA alone (Table 3). Moreover, in rats treated with the TM-β-CyD alone (500 mg kg^{-1}, n = 6) and killed 21 h later, several small gastric erosions were observed (total lesions = 18); gastric erosions were also detected in rats treated with a higher dose of DM-β-CyD (1000 mg kg^{-1}; total lesions = 10; n = 6) while they were not observed in rats killed 6 h after treatment with the two methylated β-CyDs. No lesions were found in rats treated with β-CyD or HP-β-CyD (1000 mg kg^{-1}, n = 6).

Discussion

Pharmacokinetic profiles of BPAA-β-CyD complexes have shown a differential enhancement of bioavailability of the drug, with the BPAA-DM-β-CyD complex being the most effective. In parallel, the latter displayed the most potent anti-inflammatory activity in carrageenan-induced paw oedema in rats. These findings can be related to our previous in-vitro study on physicochemical properties and simulated gastric absorption of BPAA included in β-CyDs (Ventura et al 1994). Inclusion complexes of BPAA with DM-β-CyD or HP-β-CyD are rapidly dissolved in water and the solution does not precipitate in acidic conditions (pH < 2 (Ventura et al 1994)). Thus, the absorption of the free drug is primarily influenced by the stability constant of the complex (K_c) together with a second factor which plays a relevant role in-vivo—the interactions of β-CyDs with

Table 3. Gastric lesions in rats after oral administration of BPAA-β-CyD complexes and BPAA alone.

Compound	Dose (mg kg^{-1})	Number of animals with lesions	Total numbers of lesions
BPAA	25	3/6	10
BPAA	50	6/6	38
BPAA-β-CyD	158·5 (25)[a]	2/6	4*
BPAA-β-CyD	317·0 (50)[a]	6/6	25*
BPAA-HP-β-CyD	255·0 (25)[a]	2/6	5*
BPAA-HP-β-CyD	510·0 (50)[a]	6/6	26*
BPAA-DM-β-CyD	260·1 (25)[a]	2/6	4*
BPAA-DM-β-CyD	520·2 (50)[a]	6/6	16*
BPAA-TM-β-CyD	252·4 (25)[a]	6/6	9
BPAA-TM-β-CyD	504·8 (50)[a]	6/6	34

[a]Amount of active principle in the complex. *$P < 0·01$ vs BPAA at the same dose (Mann–Whitney U-test).

plasma membranes. Irie et al (1992), have reported that the DM-β-CyD is a more potent enhancer of nasal absorption of insulin than the parent β-CyD or HP-β-CyD and methylated β-CyDs have been shown to enhance cell permeability by solubilizing specific plasma membrane lipid components (Yoshida et al 1988; Ohtani et al 1989). Uekama & Otagiri (1987) have confirmed these findings for the gastrointestinal mucosa. DM-β-CyD may extract lipids from the gastrointestinal mucosa thereby facilitating BPAA absorption. This property may compensate for the higher K_c of the BPAA-DM-β-CyD complex (7394 M^{-1}) than that of BPAA-HP-β-CyD (4166 M^{-1}) or of BPAA-β-CyD (4519 M^{-1}) (see Ventura et al 1994) and could improve drug bioavailability. The BPAA-TM-β-CyD complex displays a low water solubility and was administered as a suspension in the rat; thus, the bioavailability of BPAA is influenced by the slow dissolution rate of the complex, which is only partially counterbalanced by its low K_c (1797 M^{-1} (see Ventura et al 1994)).

In agreement with our hypothesis, we found that the administration of higher doses of methylated β-CyDs alone may cause gastric erosions correlated with the residence time in the rat stomach. Cell damage induced by TM-β-CyD was more pronounced, possibly because this CyD is exhaustively methylated. Moreover, the BPAA-TM-β-CyD complex did not protect the stomach from the ulcerogenic effect of this NSAID, whereas, in agreement with our previous findings on the BPAA-β-CyD complex (Puglisi et al 1991), a marked reduction of the extent of lesion by BPAA-DM-β-CyD and BPAA-HP-β-CyD complexes, compared with uncomplexed BPAA, was observed. This could be a consequence of the low stability of the complex itself; in fact, given its slow rate of dissolution in-vivo, which is the limiting factor for BPAA absorption, a sustained release of free drug may contribute to the gastric damage observed 21 h after treatment.

Acknowledgements

The authors wish to thank Professor G. Mazzone for valuable discussions. This work was supported by a grant from Ministero dell'Univeritè e della Ricerca Scientifica e Tecnologica, Rome, Italy.

References

Irie, T., Wakamatsu, K., Arima, H., Aritomi, H., Uekama, K. (1992) Enhancing effects of cyclodextrins on nasal absorption of insulin in rats. Int. J. Pharm. 84: 129–139

Nambu, N., Shimoda, M., Takahashi, Y., Ueda, H., Nagai, T. (1978a) Bioavailability of powdered inclusion compounds of nonsteroidal antiinflammatory drugs with β-cyclodextrin in rabbits and dogs. Chem. Pharm. Bull. 26: 2952–2956

Nambu, N., Kikuchi, K., Kikuchi, T., Takahashi, Y., Ueda, H., Nagai, T. (1978b) Influence of inclusion of nonsteroidal antiinflammatory drugs with β-cyclodextrin on the irritation to stomach of rats upon oral administration. Chem. Pharm. Bull. 26: 3609–3612

Ohtani, Y., Irie, T., Uekama, K., Fukunaga, K., Pitha, J. (1989) Differential effects of α-, β- and γ-cyclodextrins on human erythrocytes. Eur. J. Biochem. 186: 17–22

Otero Espinar, F. J., Anguiano Igea, S., Blanco Mèndez, J., Vila Jato, J. L. (1991) Reduction in the ulcerogenicity of naproxen by complexation with β-cyclodextrin. Int. J. Pharm. 70: 35–41

Puglisi, G., Santagati, N. A., Pignatello, R., Ventura, C., Bottino, F. A., Mangiafico, S., Mazzone, G. (1990) Inclusion complexation of 4-biphenylacetic acid with β-cyclodextrin. Drug Dev. Ind. Pharm. 16: 395–413

Puglisi, G., Santagati, N. A., Ventura, C. A., Pignatello, R., Panico, A. M., Spampinato, S. (1991) Enhancement of 4-biphenylacetic acid bioavailability in rats by its β-cyclodextrin complex after oral administration. J. Pharm. Pharmacol. 43: 430–432

Sloboda, A. E., Osterberg, A. C. (1976) The pharmacology of fenbufen, 3-(4-biphenylylcarbonyl)propionic acid, and 4-biphenylacetic acid, interesting antiinflammatory-analgesic agents. Inflammation 1: 415–438

Szejtli, J., Szente, L. (1981) Interaction between indomethacin and β-cyclodextrin. Pharmazie 36: 694–698

Tolman, E. L., Partridge, R. (1975) Multiple sites of interaction between prostaglandins and non-steroidal antiinflammatory agents. Prostaglandins 9: 349–359

Uekama, K. (1985) Pharmaceutical applications of methylated cyclodextrins. Pharm. Int. 6: 61–65

Uekama, K., Otagiri, M. (1987) Cyclodextrins in drug carrier systems. CRC Crit. Rev. Ther. Drug Carrier Syst. 3: 1–40

Ventura, C. A., Puglisi, G., Giammona, G., Bottino, F. A. (1994) Comparative study on inclusion compounds of 4-biphenylacetic acid with β-cyclodextrin, hydroxypropylated-β-cyclodextrin and methylated-β-cyclodextrins. Drug Dev. Ind. Pharm. 20: 2245–2265

Winter, C. A., Risley, E. A., Nuss, G. V. (1962) Carrageenan-induced oedema in the hindpaw of the rat as an assay for antiinflammatory drugs. Proc. Soc. Exp. Biol. Med. 111: 544–547

Yoshida, A., Arima, H., Uekama, K., Pitha, J. (1988) Pharmaceutical evaluation of hydroxyalkyl ethers of β-cyclodextrins. Int. J. Pharm. 46: 217–222