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COMMUNICATIONS

Enhancement of 4-biphenylacetic acid bioavailability in rats by its β-cyclodextrin complex after oral administration

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Abstract—4-Biphenylacetic acid, a potent non-steroidal anti-inflammatory agent forms a solid inclusion complex with β-cyclodextrin in a 1 : 1 molar ratio, which exhibits better solubility and dissolution characteristics than the uncomplexed drug. Following oral administration of the complex to rats, quicker and higher drug plasma concentrations can be achieved than with the drug alone. Parallel studies, using the carrageenan paw oedema test, demonstrate a greater anti-inflammatory activity of the complex (ED50 of 2·9 mg kg^{-1} for the complex and of 6·2 mg kg^{-1} for the free drug). The complex displayed a better gastric tolerability in the rat than drug alone.

Positive effects on the bioavailability and pharmacological activity of various classes of drugs by their encapsulation into cyclodextrins have been observed (Uekama et al 1979; Koizumi et al 1980; Szejtli et al 1980, 1982). The complexation of non-steroidal anti-inflammatory drugs (NSAID) with cyclodextrins improved their biological activity and tolerability in-vivo (Nambu et al 1978a; Szejtli et al 1979; Szejtli & Szente 1981; Chow & Karara 1986).

In a previous paper (Puglisi et al 1990) we described the preparation, in-vitro dissolution rate and solubility of an inclusion complex between β-cyclodextrin (β-CyD) and 4-biphenylacetic acid (BPAA), a NSAID particularly active as an antiphlogistic (Tolman & Partridge 1975; Tolman & Sloboda 1976; Tolman et al 1976).

In this study the complex was administered orally to rats, to evaluate the influence of complexation with β-CyD on bioavailability and on pharmacodynamic activity of BPAA.

It has been claimed that NSAID-cyclodextrin complexes display less irritant effects on gastric mucosa after oral administration (Nambu et al 1978b; Szejtli et al 1979). β-CyD may promote the absorption of the drug, lowering its effective contact time with the stomach walls and, consequently, the risk of ulcerous lesions (Szejtli & Szente 1981). Therefore, pharmacological analysis was extended to evaluate any possible reduction of gastric lesions caused by BPAA.

Materials and methods

Chemicals. 4-Biphenylacetic acid (BPAA) was obtained from Janssen (Belgium) (analytical grade) and was recrystallized from ethanol. Its purity, checked by HPLC, was found to be 99·6%. β-Cyclodextrin (β-CyD) from Fluka Chemical Co. (Buchs, Switzerland) was used after recrystallization from water and drying on P_2O_5 in-vacuo. Piperonylic acid (Aldrich Chimica, Milan, Italy; purity 99%) was used as an HPLC internal standard. All other chemicals were of analytical grade and double distilled water was used.

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Preparation of the solid complex. The solid BPAA-β-CyD complex in a 1:1 molar ratio was prepared by a homogeneous coprecipitation method (Puglisi et al 1990).

Animals and treatments. Male Sprague-Dawley rats, 120–150 g (Nossan, Correzzana, Italy), were fasted 24 h before treatment while water was withheld 6 h before. BPAA or the corresponding amount of β-CyD-BPAA 1:1 complex was suspended in distilled water (10 mL kg^{-1}) and immediately administered orally. Control rats were treated with water alone.

Carrageenan-induced paw oedema test. Paw oedema was induced in Sprague-Dawley rats by a subplantar injection of 0·05 mL of a 1% carrageenan suspension into the right hindpaw (Winter et al 1962). Both BPAA and BPAA-β-CyD were given orally 2 h after the irritant and 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 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 by visual examination under 5 × magnification. All lesions were counted regardless of size.

Determination of blood BPAA concentration. Groups of 3 rats were anaesthetized by sodium pentobarbitone (30–40 mg kg^{-1}, i.p.) 30, 60, 90, 120, 180, and 210 min after the oral administration of the two compounds and 8 mL of blood withdrawn through a catheter inserted into the jugular vein. Rats were killed immediately (sodium pentobarbitone, 40 mg kg^{-1}, i.v.).

One millilitre of the collected samples and 250 μg of internal standard were added to an extraction tube, with 10 mL methanol, and vortexed vigorously for 15 min. The mixture was centrifuged at 4°C to precipitate protein and the methanolic solution transferred to a fresh tube. After evaporation of the methanol under a nitrogen stream, the residue was reconstituted in 500 μL of the mobile phase and a 10 μL aliquot was injected into the chromatograph.

Sample analysis was carried out by HPLC on a Varian 5000 liquid chromatograph equipped with a UV-100 variable-wavelength detector and a Varian 4270 Integrator. A reversed phase C_{18} column (Hypersil ODS 5 μm, Policonsult Scientifica S.r.l., Italy) with a 10 μL loop was used. The mobile phase consisted of (A) acetate pH 4·6 buffer (B) methanol, (55:45 v/v); flow rate was 1·5 mL min^{-1}. The detector was set at 254 nm and 0·05 aufs. Fig. 1 shows a representative chromatogram of BPAA and internal standard.

Blanks were obtained by adding to 1 mL of serum, from

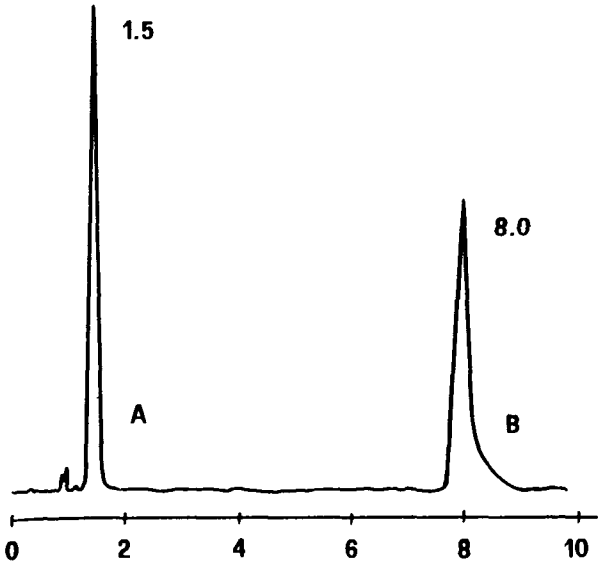

FIG. 1. A typical chromatogram of a methanol extract of plasma containing 100 μg mL^{-1} of internal standard (A) and 60 μg mL^{-1} of BPAA (B).

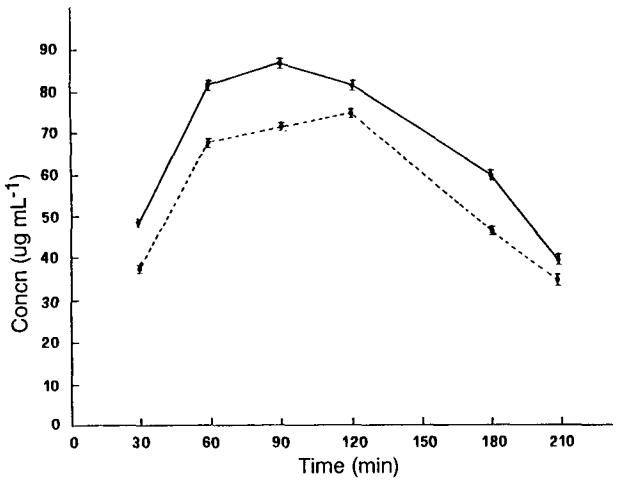

FIG. 2. 4-Biphenylacetic acid serum levels in rats, following oral administration of 10 mg of BPAA (●- -●) or an equivalent amount of β-CyD complex (●—●). Values are the mean ±s.e., of three HPLC determinations.

untreated animals, 250 μg of the internal standard and 80 μg of BPAA. After the same extraction procedure described above, 10 μL of each sample was injected into the chromatograph. The assay was repeated on six samples and the average recovery was found to be 91%.

The same aliquots were used to detect the precision of the method; it was found to be reproducible, with a relative standard deviation of 1·80%.

The linearity of the assay was also determined; five standards containing concentrations of BPAA in the range 10–150 μg mL^{-1} were analysed. The calibration curve obtained showed a correlation coefficient of 0·9999 and the equation for the linear regression line was $y = 3{\cdot}618x + 0{\cdot}06105$, where y was the known concentration and x was the area ($\times 10^3$) under the peak.

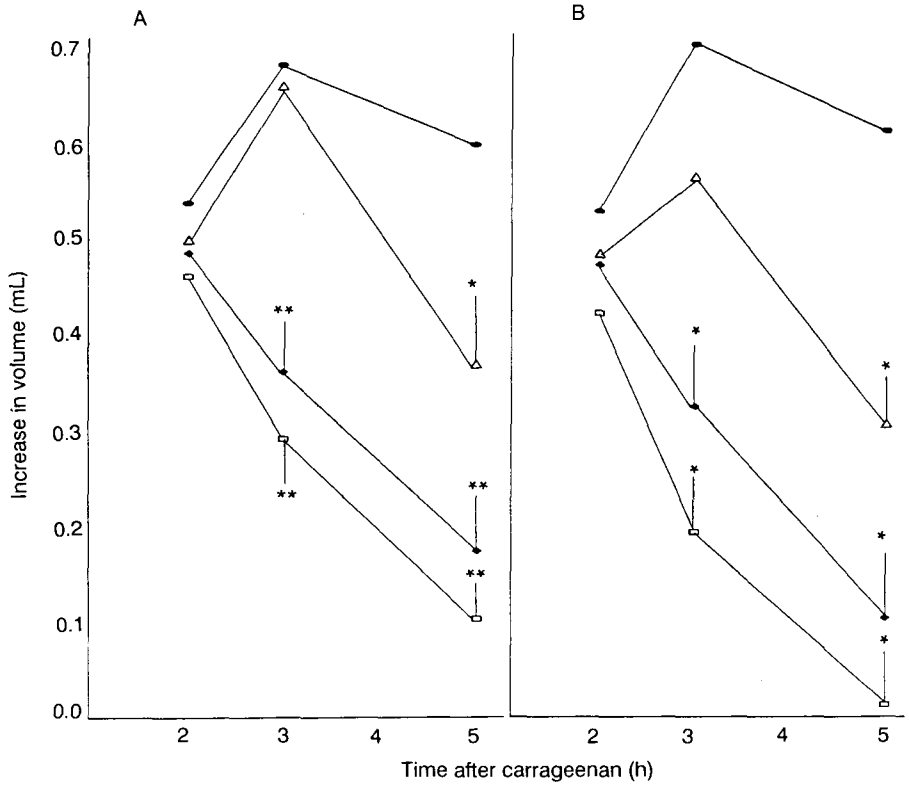

FIG. 3. Inhibitory effects of BPAA (panel A) and BPAA-β-CyD complex (panel B) on carrageenan oedema in rats. Three doses of BPAA or of the complex containing an equivalent amount of drug were administered orally 2 h after carrageenan. (●-●) control; (△-△) 3 mg kg^{-1}; (◆-◆) 10 mg kg^{-1}; (□-□) 20 mg kg^{-1}. Values are the mean ±s.e. of 6 animals/group. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ vs control group.

Results

To ascertain whether the β-CyD complex acts as a sustained-release drug carrier in-vivo, the free drug (10 mg kg^{-1}) or an equivalent dose of the complex was administered orally to rats. Fig. 2 shows the plasma concentrations of BPAA following oral treatment. The plasma peak of BPAA from the complex appears at a shorter time (90 min) compared with that from the free drug (120 min), and there was significant increase in the maximal concentrations. The area under the curve (AUC) value for BPAA was 10958·86, whereas for the complex it was 13105·62, corresponding to an enhancement of the bioavailability of 19·6% for the complex.

BPAA and BPAA-β-CyD complex were compared for oral efficacy of anti-inflammatory action in the rat paw oedema test (Fig. 3). Drugs administered 2 h after carrageenan significantly inhibited the intensity of oedema in a dose-dependent manner. The inclusion complex showed a higher activity than the uncomplexed compound.

Dose-response curves, calculated from the data 5 h after drug administration (see Fig. 3), indicated that the ED50 of BPAA-β-CyD is 2·9 mg kg^{-1} (as BPAA dose), approximately one half that of the uncomplexed drug (ED50 = 6·2 mg kg^{-1}). At the same BPAA dose (20 mg kg^{-1}), β-CyD complex gives a practically total inhibition of oedema.

The greater activity of BPAA-β-CyD complex in an acute inflammation model was associated with a significant reduction of gastric lesions in the same species (Table 1), as a consequence of the capacity of β-CyD in enhancing gastrointestinal absorption of the drug, thus preventing its irritant local effects on the mucosa.

Table 1. Gastric lesions in rats after oral administration of BPPA-β-CyD and BPAA alone.

Compound	Dose (mg kg^{-1})	No. of animals with lesions	Total number of lesions
BPAA-β-CyD	158·5 (25)[a]	2/6	6*
BPAA-β-CyD	317·0 (50)[a]	6/6	31*
BPAA	25·0	3/6	12
BPAA	50·0	6/6	44

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

Discussion

Present findings demonstrate an enhancement of bioavailability of BPAA following oral administration of its β-CyD complex in the rat, since more rapid and higher plasma levels of the drug were indicated.

Results of pharmacodynamic activity indicate the increased water solubility of BPAA on β-cyclodextrin complexation clearly enhances its anti-inflammatory effects. The administration of the complex permits a reduction in the efficacious dose of the drug and a greater anti-inflammatory action. These findings are in good agreement with physicochemical results (Puglisi et al 1990), demonstrating an increasing dissolution rate and a greater solubility in water of BPAA by its complexation with β-CyD. Although further specific studies would be necessary, the parallel increase in both serum levels and in-vivo activity could indicate that encapsulation of this drug modifies the absorption phase, with possible effects on other pharmacokinetic parameters.

Finally, the enhanced gastric tolerability shown by this complex confirms the validity of β-cyclodextrin as a carrier for anti-inflammatory drugs.

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