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Comparative Study of Interactions between 4-Biphenylacetic Acid and Sulfamethizole, and between Fenbufen and Sulfamethizole in Rats

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The effects of fenbufen and 4-biphenylacetic acid (a pharmacologically active metabolite of fenbufen) on the plasma level of sulfamethizole were investigated in rats. The persistence of sulfamethizole in plasma was remarkably prolonged by coadministration of fenbufen and moderately prolonged by coadministration of 4-biphenylacetic acid.

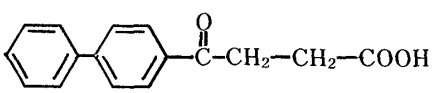
In the renal clearance experiments, the clearance ratio of sulfamethizole was greatly decreased after 4-biphenylacetic acid infusion, in accordance with the result for fenbufen reported previously. It is speculated that prolongation of the persistence of sulfamethizole in plasma by fenbufen or 4-biphenylacetic acid is mainly caused by competitive interactions between sulfamethizole and fenbufen or 4-biphenylacetic acid at the renal secretory level. The lesser effect of 4-biphenylacetic acid in prolonging the plasma level of sulfamethizole as compared with that of fenbufen might be attributed to rapid biotransformation of 4-biphenylacetic acid. In a protein binding experiment, fenbufen and 4-biphenylacetic acid were found to displace sulfamethizole bound to rat plasma protein, but the displacing activities were quite weak as compared with those of ibuprofen and bucolome.

Keywords——drug interaction; fenbufen; 4-biphenylacetic acid; sulfamethizole; proximal tubular secretion; renal excretion; protein binding; rat

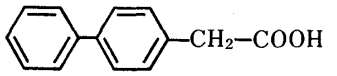
Introductions

Any biotransformation of a drug results in metabolites whose physicochemical properties differ from those of the original drug. Such a metabolite may display different pharmacokinetic behavior from the original drug. Therefore, clarification of the correlation between biotransformation and pharmacokinetic behavior is important for ensuring the safety of drug therapy. The anti-inflammatory propionic acid derivatives are currently widely used clinically. These drugs are frequently coadministered with chemotherapeutic drugs, and thus pharmacokinetic drug interactions in such combinations must be taken into account in establishing dosage schedules.

4-Biphenylacetic acid (4-BPA) is a major metabolite of fenbufen (FNB), which is one of the anti-inflammatory propionic acid derivatives.[1,2] 4-BPA has a similar spectrum of pharmacological activity to the original drug,[3] and its clinical application is under investigation. Up to now, pharmacokinetic drug interactions between 4-BPA and other drugs



fenbufen (FNB)



4-biphenylacetic acid (4-BPA)

Chart 1

have not been studied. In this study, we investigated comparatively the interaction between 4-BPA and sulfamethizole (SMZ) and the interaction between SMZ and FNB in rats.

Experimental

Materials——Fenbufen (FNB): Commercially available FNB was recrystallized from acetone, mp 185—187 °C. 4-Biphenylacetic acid (4-BPA): 4-BPA was purchased from Nippon Lederle Co., mp 147—148 °C. Sulfonamide: Sulfamethizole (SMZ) was recrystallized from EtOH, mp 207—208 °C. All other chemicals were of reagent grade and were used without further purification.

Plasma Level of Sulfamethizole in Rats——Male Wistar rats weighing 250—350 g were used in this study. They were anesthetized with sodium pentobarbital (30 mg/kg body weight), and were intubated to allow free respiration. After the intubation, the left femoral vein and right femoral artery were separately catheterized with polyethylene tubing (PE-50). SMZ at a dose of 30 mg/kg was administered to rats through the left femoral vein. In coadministration experiments, an anti-inflammatory agent at a dose of 10 mg/kg was also administered through the left femoral vein immediately after administration of SMZ. FNB and 4-BPA were used as suspensions in 0.5% Tween 80. At each sampling time, about 0.2 ml of blood was withdrawn through the right femoral artery, and the plasma was obtained by centrifugation.

Pharmacokinetic Analysis——Estimation of elimination half-life ($t_{1/2}$) was carried out by least-squares fitting of the plasma levels using a microcomputer (NEC PC-8801).

Protein Binding Experiment——The extent of binding of SMZ to rat plasma was determined by the equilibrium dialysis method, as described previously.[4] Percentage displacement of SMZ by FNB or 4-BPA was evaluated by the method of Anton.[5]

Renal Clearance Experiment——The retro-peritoneal approach procedure described by Sudo *et al.*[6] was employed for renal clearance studies in rats. After intubation, and catheterization of the left femoral vein and right femoral artery, the left ureter was catheterized with polyethylene tubing (PE-10) by the retro-peritoneal approach procedure. The rats were primed with SMZ (20 mg/body) and inulin (40 mg/body) through the left femoral vein, and a sustained infusion of SMZ (0.35 mg/min) and inulin (0.2 mg/min) in saline was continued throughout the whole experiment. For blockade of proximal tubular secretion of SMZ, FNB or 4-BPA (2 mg/body) was primed through the femoral vein after two or three control clearance periods, and a sustained infusion of the anti-inflammatory agents (0.35 mg/min) was continued until the experiments were performed. Drug clearance (C) in ml/min was calculated as $C = UV/P$, where U, P and V indicate urine and plasma concentrations of the drug in mg/ml, and urine flow rate in ml/min, respectively. To estimate the renal handling of the drug, clerance ratio (CR) has been conventionally used and was expressed as $CR = C/GFR$, where GFR represents the glomerular filtration rate in ml/min.

Analytical Method——For the determination of SMZ, a high-performance liquid chromatograph (Shimadzu LC-5A) equipped with an ultraviolet (UV) detector (245 nm, Shimadzu SPD-2A) was used with a stationary phase of Zorbax C_8 (5—6 μm particle diameter) packed in 25 cm × 4.6 mm i.d. stainless-steel tubing. The mobile phase was 0.2 M sodium phosphate (monobasic, pH = 5.6) mixed with acetonitrile at a volume ratio of 3/2, and the flow rate was maintained at 0.5 ml/min. Inulin was determined by a modification of the method described by Dische and Borenfreund.[7]

Results

Elimination Profile of SMZ with or without 4-BPA or FNB from Plasma

The plasma level of SMZ after coadministration with or without 4-BPA or FNB was studied in rats. In this study, when SMZ was coadministered with FNB or 4-BPA, we could not calculate the pharmacokinetic parameters of the distribution phase of plasma SMZ precisely, and only calculation of the elimination half-life ($t_{1/2}$) of plasma SMZ was carried out from the slope of the linear portion of the elimination phase of plasma SMZ. The partial time course of plasma concentration of SMZ after intravenous administration of SMZ with or without 4-BPA or FNB is shown in Fig. 1. The elimination half-life ($t_{1/2}$) of SMZ is also shown in Table I. When SMZ was coadministered with FNB, its elimination half-life ($t_{1/2}$) was 13.4 ± 2.02 h and this value is approximately 4 times longer than the control value (3.38 ± 0.206 h), as shown in Table I. On the other hand, when SMZ was coadministered with 4-BPA, its elimination half-life ($t_{1/2}$) was 5.57 ± 1.64 h, which is only moderately increased as compared with the control value (Table I).

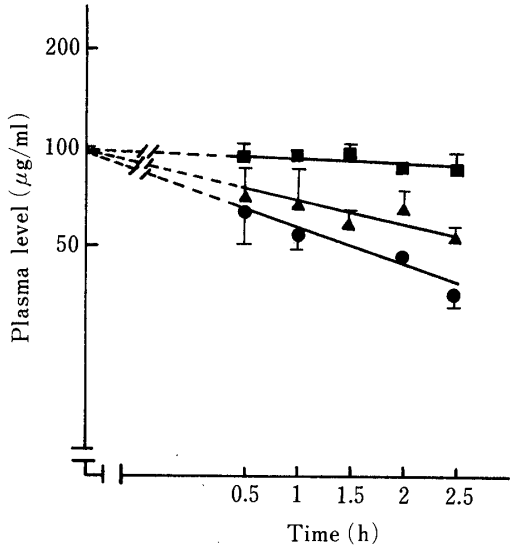


Fig. 1. Effects of FNB and 4-BPA on the Plasma Level of SMZ in Rats

●, SMZ alone ($n=3$); ■, SMZ with FNB ($n=4$); ▲, SMZ with 4-BPA ($n=3$). The S.D. is indicated by a bar (where more than one rat was used).

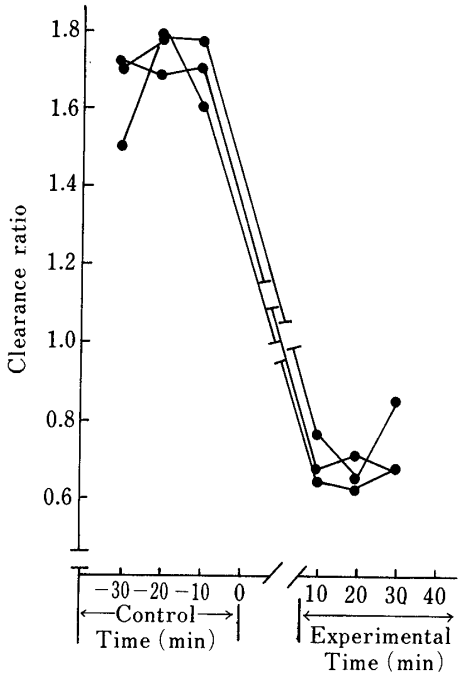


Fig. 2. Clearance Ratio of SMZ before and after Blockade of Proximal Tubular Secretion by 4-BPA in Rats

TABLE I. Values of Elimination Half-life for SMZ with or without Coadministration of FNB or 4-BPA in Rats

Drug	$t_{1/2}$ (h^{-1})
SMZ ($n=3$)	3.38±0.206
SMZ+FNB ($n=4$)	13.4 ±2.02
SMZ+4-BPA ($n=3$)	5.57±1.64

Each value is the mean±S.D.

Interaction between SMZ and 4-BPA at the Renal Level

For renal clearance experiments, three rats were used to determine whether the renal excretion of SMZ could be altered by 4-BPA infusion. The result is shown in Fig. 2. The data obtained in a typical renal clearance experiment are listed in Table II.

As shown in Fig. 2, a marked decline in the clearance ratio of SMZ after 4-BPA infusion was observed.

Effects of 4-BPA and FNB on Plasma Protein Binding of SMZ

FNB and 4-BPA were examined for ability to displace SMZ from the binding plasma protein. As shown in Table III, the displacements of bound SMZ by FNB amounted to 8.26% and 24.8% at equal concentrations of the two drugs of 50 and 100 μg/ml, respectively. The displacements of bound SMZ by 4-BPA were almost the same as in the case of FNB,

TABLE II. The Effect of 4-BPA on Renal Clearance of SMZ in a Rat[a]

	Time (min)	V[c] (ml/min)	GFR[d] (ml/min)	SMZ U[e] (mg/ml)	SMZ P[f] (mg/ml)	SMZ C[g] (ml/min)	SMZ CR[h]
Control	30—20	0.56	14.3	6.14	0.139	24.7	1.73
	20—10	0.58	13.2	4.05	0.105	22.4	1.70
	10— 0	0.86	14.8	3.63	0.123	25.4	1.72
Exptl.[b]	30—40	1.64	14.3	0.766	0.128	9.81	0.686
	40—50	1.62	13.1	0.627	0.108	9.41	0.718
	50—60	1.88	13.9	0.541	0.109	9.33	0.671

a) Rat: 250 g. *b*) Experimental. *c*) Urine flow rate. *d*) Glomerular filtration rate. *e*) Urine concentration. *f*) Plasma concentration. *g*) Drug clearance. *h*) Clearance ratio.

TABLE III. Interference by FNB or 4-BPA with the Binding of SMZ to Rat Plasma Protein

SMZ concentration (μg/ml)	% bound in rat plasma	Displacing activity *in vitro*[a]	
		FNB[b]	4-BPA[b]
50	81.2±2.62	8.26±2.07	9.91±2.91
100	65.7±5.28	24.8 ±8.46	19.0 ±5.32

a) Displacing activity is defined as $DAc = 100 - (a/b \cdot 100)$, where DAc = displacing activity *in vitro*, a = %SMZ bound in the presence of the anti-inflammatory agent, and b = %SMZ bound in the absence of the drug. *b*) FNB or 4-BPA was used at the same concentration as SMZ.

amounting to 9.91% and 19.0% at concentrations of both drugs of 50 and 100 μg/ml, respectively.

Discussion

4-BPA is one of the main metabolites of FNB in several laboratory animals and man.[1,2] Among the main metabolites of FNB, only 4-BPA has been found to have anti-inflammatory activity.[3] It is probable that 4-BPA is the agent responsible for the anti-inflammatory activity of FNB. Therefore FNB is a kind of prodrug which is partially converted into 4-BPA *in vivo*.

To estimate the alteration in pharmacokinetic behavior of a drug upon coadministration of another drug, several factors concerning drug interactions must be considered. One such factor is alteration of the renal tubular processes: tubular secretion of one drug may be decreased by competition of the other drug for the same transport system. There are several examples of drug interactions resulting from interference with renal tubular processes.[8-12]

As shown in Table I and Fig. 1, the plasma elimination half-life of SMZ ($t_{1/2}$) was remarkably prolonged by FNB. Previously we reported that FNB competitively blocks the renal tubular secretion of SMZ.[12] The present result on the prolonging effect of FNB on plasma SMZ indicates that the effect of FNB on plasma SMZ might be attributed to inhibition of renal tubular secretion of SMZ by FNB. On the other hand, in spite of the remarkable inhibitory effect of 4-BPA on renal handling of SMZ, as shown in Fig. 2, the prolonging effect of 4-BPA on plasma SMZ was considerably less than that of FNB. It has been reported that rapid 4′-hydroxylation of 4-BPA occurs in rats, but not in some other

species.[1] Thus, 4-BPA administered by bolus intravenous administration might undergo rapid biotransformation, consequently decreasing the prolonging effect on plasma SMZ.

It is well known that FNB and 4-BPA bind to plasma protein to considerable extents.[1,2] Thus, the activities of FNB and 4-BPA for displacing SMZ from bound protein were examined. For comparison with the data obtained on other non-steroidal anti-inflammatory drugs, equal concentrations of FNB or 4-BPA and SMZ were used in the *in vitro* experiment, although only one-third as much FNB or 4-BPA relative to SMZ was used in the *in vivo* experiment. As shown in Table III, the activities of both FNB and 4-BPA for displacing protein-bound SMZ are weak as compared with those of bucolome and ibuprofen reported previously.[11] In view of this result, the influence of displacing activity of FNB or 4-BPA on the elimination profile of plasma SMZ may be negligible *in vivo* in rats.

References

1) F. S. Chiccarelli, H. J. Einser and G. E. Van Lear, *Arzneim-Forsch.*, **30**, 707 (1980).
2) F. S. Chiccarelli, H. J. Einser and G. E. Van Lear, *Arzneim-Forsch.*, **30**, 728 (1980).
3) A. E. Sloboda, E. L. Tolman, A. C. Osterberg and J. Panagides, *Arzneim-Forsch.*, **30**, 716 (1980).
4) T. Arita, R. Hori, M. Takada and M. Misawa, *Chem. Pharm. Bull.*, **19**, 930 (1971).
5) A. H. Anton, *J. Pharmacol. Exp. Ther.*, **134**, 291 (1961).
6) J. Sudo, A. Ishihara and T. Tanabe, *Chem. Pharm. Bull.*, **31**, 4524 (1983).
7) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).
8) M. Takada, S. Akuzu, A. Misawa, R. Hori and T. Arita, *Chem. Pharm. Bull.*, **22**, 542 (1974).
9) M. Takada, A. Misawa, K. Fujimoto, R. Hori and T. Arita, *Chem. Pharm. Bull.*, **22**, 551 (1974).
10) K. Chiba, M. Sakamoto, M. Ito, N. Yagi, H. Sekikawa, S. Miyazaki and M. Takada, *Chem. Pharm. Bull.*, **30**, 3362 (1982).
11) K. Chiba, M. Yoshida, A. Ishihara, J. Sudo, T. Tanabe, S. Miyazaki and M. Takada, *Chem. Pharm. Bull.*, **33**, 3415 (1985).
12) K. Chiba, S. Nishimura, S. Kikuchi, N. Higuchi, S. Miyazaki and M. Takada, *Chem. Pharm. Bull.*, **33**, 5100 (1985).