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Influence of Ion-pair Formation on the Pharmacokinetic Properties of Drugs. Pharmacokinetic Interactions of Bretylium and Hexylsalicylic Acid in Rabbits

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Abstract—The simultaneous i.v. administration of equimolar doses of bretylium and hexylsalicylic acid results in an increase in plasma area under the curve value of both substances in comparison with their separate administration. The higher plasma levels of both compounds were associated with a reduced renal excretion and an increased biliary elimination. However, the increase in biliary excretion did not compensate for the reduced elimination of bretylium and hexylsalicylic acid via the kidney. The results presented in this paper give further evidence that ion-pairing in-vivo may result in altered pharmacokinetics of drugs particularly due to changes in biliary or renal excretion.

Ion-pair formation seems to be a rational way to increase lipophilicity as well as the transport rate of suitable drugs across lipid membranes (Neubert 1989; Neubert & Fischer 1991). The mechanism of ion-pair transport has been intensively studied in-vitro (Neubert 1989; Hadgraft et al 1985; Neubert et al 1984). Furthermore, ion-pair formation can be considered as a possible mechanism of pharmacokinetic drug interaction. In this study the interactions of a hydrophilic and a lipophilic counterion were investigated in rabbits using bretylium and hexylsalicylic acid as model substances. In a previous article it was shown that hexylsalicylic acid is able to increase markedly the transport of bretylium across lipid membranes (Neubert et al 1987), and the lipophilicity of both substances was enhanced in the presence of the other.

Bretylium and hexylsalicylic acid were administered i.v. either separately or in combination at equimolar doses. In contrast to the previous articles of this series, both the amount of bretylium and hexylsalicylic acid excreted into urine and into bile were quantitatively measured using a rabbit model which allows parallel sampling of plasma, bile and urine in the same animal (Amlacher et al 1990).

Materials and Methods

Bretylium tosylate was provided by Arzneimittelwerk Dresden GmbH (Dresden, Germany). Hexylsalicylic acid was synthesized as described previously (Neubert et al 1987). Pentobarbitone sodium was supplied by Spofa (CSFR). All other reagents were of analytical grade.

Partition coefficient

The n-octanol/buffer system was used to determine the partition coefficients of bretylium and hexylsalicylic acid. Five mL of a solution of each compound (0·5 mM) in phosphate buffer pH 7·2 and 5 mL of n-octanol were added to a glass test tube. The covered tubes were equilibrated for 24 h using a Thys 2 shaker at 20°C. The aqueous phase was assayed for drug content.

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Animals

Rabbits of either sex, 3–5 kg, were fasted for 24 h. For experimental preparation the rabbits were narcotized with pentobarbitone sodium (50 mg kg^{-1}, i.v.). About 50% of this dose was given as a bolus and the remainder was dosed as required. The animals were fixed onto an operating table which was kept at constant temperature (38°C). Polyethylene tubings of different diameters were inserted into the arteria carotis, ductus choledochus and both ureters. In order to prevent clotting and to stabilize the blood circulation an infusion of 36 mL h^{-1} of heparinized physiological saline was administered using an infusion pump (Diel, Germany).

Drug administration and sampling

The rabbits received bretylium or hexylsalicylic acid or both (hexylsalicylic acid immediately before bretylium) as an i.v. bolus injection into a marginal ear vein. Bretylium was given at a dose of 24·2 μmol kg^{-1} (10 mg kg^{-1}) in 2·0 mL Soerensen phosphate buffer pH 7·2. Hexylsalicylic acid was administered at a dose of 24·2 μmol kg^{-1} (5·4 mg kg^{-1}) in 5·0 mL kg^{-1} ethanol/Soerensen phosphate buffer pH 7·2 (1:5). Blood, bile and urine were sampled via the cannulas at intervals as indicated.

Analytical assays

Bretylium and hexylsalicylic acid were assayed in the bile, urine and plasma as described previously (Neubert et al 1989). In order to determine total hexylsalicylic acid the metabolic conjugates were enzymatically hydrolysed as described by Neubert et al (1989).

Calculation of the pharmacokinetic parameters

The pharmacokinetic parameters were calculated according to a model-independent method (Weiss 1982). The area under the curve (AUC) was calculated to infinity.

Table 1. Partition coefficients of bretylium and hexylsalicylic acid alone and together in the system n-octanol/buffer pH 7·2.

	Partition coefficient	
	Bretylium	Hexylsalicylic acid
Bretylium	<0·01	
Hexylsalicylic acid		15
Bretylium + hexylsalicylic acid	1·55	68

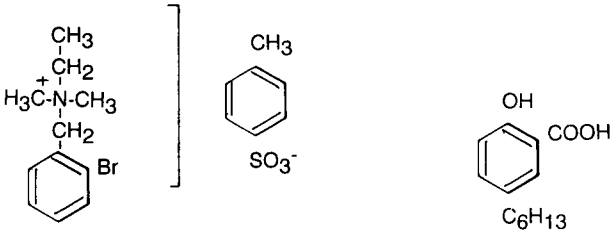

FIG. 1. Structures of the compounds studied.

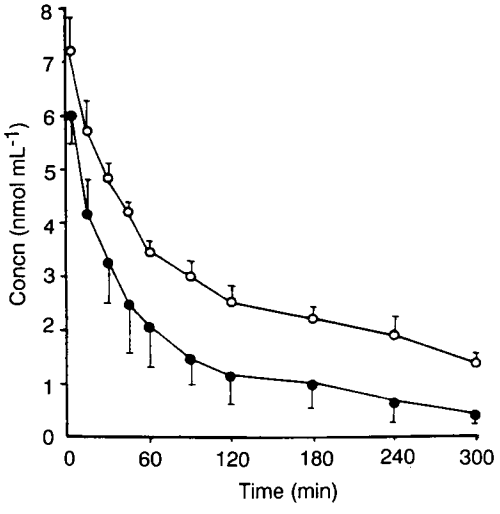

FIG. 2. Plasma concentration-time profiles of bretylium after i.v. administration alone (24·2 μmol kg^{-1}) (●) or simultaneously with hexylsalicylic acid (24·2 μmol kg^{-1}) (○). Vertical lines indicate s.d. (n = 4).

Results

Plasma concentration-time profiles

The levels of bretylium in the plasma of rabbits after i.v. administration of bretylium alone (24·2 μmol kg^{-1}) or simultaneously with an equimolar dose of hexylsalicylic acid

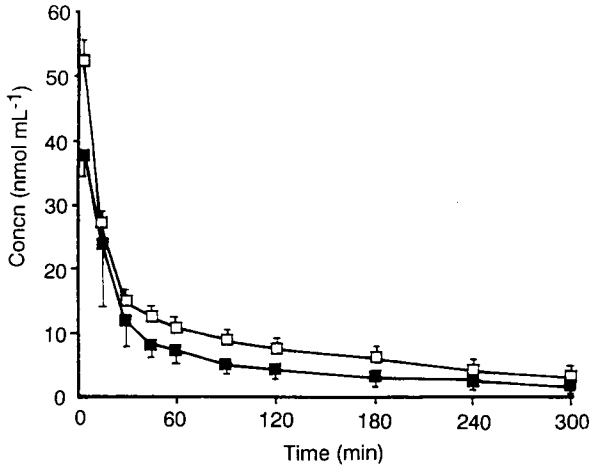

FIG. 3. Plasma concentration-time profiles of hexylsalicylic acid after i.v. administration alone (24·2 μmol kg^{-1}) (■) or simultaneously with bretylium (24·2 μmol kg^{-1}) (□). Vertical lines indicate s.d. (n = 4).

are depicted in Fig. 2. Intravenous administration of bretylium resulted in rapidly decreasing plasma levels of this compound. After injection with hexylsalicylic acid, the plasma levels of bretylium were found to be elevated in comparison with single drug administration. The pharmacokinetic parameters are given in Table 2. The simultaneous administration of the compounds resulted in a significant doubling of the AUC as well as a significant increase in mean residence time (MRT) of bretylium. In contrast, the steady state distribution volume (V_{dss}) was not influenced. After combination with bretylium the plasma concentration-time profiles of hexylsalicylic acid were found to be increased in comparison with the single administration of hexylsalicylic acid (Fig. 3). An insignificant increase in MRT of hexylsalicylic acid was found when both compounds were given simultaneously, and a significant change in V_{dss} could not be demonstrated.

Biliary excretion

After combination with hexylsalicylic acid the biliary excretion of bretylium was increased up to 140%, and that of hexylsalicylic acid up to about 400% (Figs 4, 5).

Renal excretion

Cumulative excretion data of bretylium and hexylsalicylic acid after their single and simultaneous administration are summarized in Table 4. The renal excretion of both substances was found to be markedly reduced after simultaneous administration.

Table 2. Pharmacokinetic parameters of bretylium (24·2 μmol kg^{-1}) after separate or simultaneous i.v. administration with hexylsalicylic acid (24·2 μmol kg^{-1}).

Drug	AUC (nmol h mL^{-1})	MRT (h)	Vd_{ss} (mL kg^{-1})	CL_r (mL h^{-1} kg^{-1})
Bretylium	7·2 ± 4·0	1·5 ± 0·3	5040 ± 2400	1014 ± 270
Bretylium + hexylsalicylic acid	13·6* ± 1·5	1·9* ± 0·1	3380 ± 1080	243* ± 95

Mean ± s.d., n = 4, * $P < 0·05$ compared with bretylium alone.

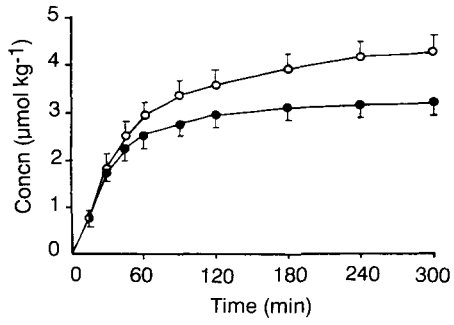

FIG. 4. Cumulative biliary excretion of bretylium after i.v. administration alone (24·2 μmol kg^{-1}) (●) or simultaneously with hexylsalicylic acid (24·2 μmol kg^{-1}) (○). Vertical lines indicate s.d. (n = 4).

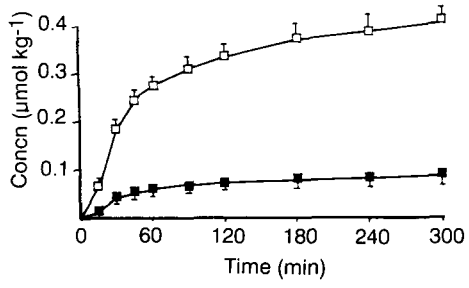

FIG. 5. Cumulative biliary excretion of hexylsalicylic acid after i.v. administration alone (24·2 μmol kg^{-1}) (■) or simultaneously with bretylium (24·2 μmol kg^{-1}) (□). Vertical lines indicate s.d. (n = 4).

Discussion

The ability to form ion-pairs (Lippold 1973) and the mechanism of ion-pair transport has been extensively studied in-vitro (Neubert et al 1984; Hadgraft et al 1985; Neubert et al 1985; Green & Hadgraft 1987; Neubert 1989; Neubert & Fischer 1991). For the model compounds used in this study it was found in-vitro that the physicochemical properties (e.g. lipophilicity, Table 1) of the highly hydrophilic drug bretylium can be markedly changed by lipophilic counterions such as hexylsalicylic acid (Neubert et al 1987; Neubert & Fischer 1991).

Earlier studies of ion-pair transport in-vivo were focused on ion-pair absorption from the gastrointestinal tract (Gasco et al 1984; Langguth & Mutschler 1987; Neubert et al 1988a, b; Trotta et al 1988; Neubert 1989), the peritoneal cavity (Neubert 1989) or through the skin (Langguth & Mutschler 1987). Furthermore, there is evidence (Neubert 1989; Härtl et al 1990) that the movement of ionizable drugs can be facilitated by ion-pair transport throughout the entire body.

Table 4. Cumulative urinary excretion of bretylium and hexylsalicylic acid after separate or simultaneous administration. Both drugs administered at 24·2 μmol kg^{-1}.

	Cumulative amount excreted	
Drug	Bretylium	Hexylsalicylic acid
Bretylium	7·08 ± 1·1	
Bretylium + hexylsalicylic acid	3·21 ± 0·8*	0·23 ± 0·07
Hexylsalicylic acid		0·47 ± 0·018*

Means ± s.d., n = 4, * $P <$ compared with the experiment with the single drug.

However, the preferred administration of one of the respective counter-ions in molar excess may be considered as a potential disadvantage in the studies cited above. Furthermore, most data were related to the plasma compartment.

In this study the experimental conditions for the investigation of pharmacokinetic drug interactions due to ion-pair transport have been optimized taking earlier studies into account (Neubert et al 1989; Härtl et al 1990). Firstly, a rabbit model was used which allows parallel intra-individual sampling of blood, bile and urine. Thus, in addition to evaluation of plasma concentration-time profiles both main excretion routes were taken into consideration. Secondly, the potential interaction of the hydrophilic bretylium and the lipophilic hexylsalicylic acid was studied under conditions of equimolar dosing of both substances as with earlier in-vitro studies (Neubert et al 1987; Neubert & Fischer 1991). The results presented in this paper are in agreement with earlier findings (Neubert et al 1988a, b; Neubert 1989; Härtl et al 1990) demonstrating that drugs which are only transported as an ion-pair in-vitro (Neubert et al 1987) may cause altered concentration-time profiles in-vivo after simultaneous administration. However, in rats (Neubert et al 1989) and in dogs (Härtl et al 1990) drug interactions between bretylium and hexylsalicylic acid changed the pharmacokinetic parameters of hexylsalicylic acid more than those of bretylium. As shown in this study for rabbits, the simultaneous equimolar administration of bretylium and hexylsalicylic acid resulted in a marked elevation of AUC and a slight increase in MRT both for hexylsalicylic acid and for bretylium. Furthermore, it was shown in this study that the renal elimination of both drugs was significantly reduced. The reduced renal excretion of both substances, together with the increased AUC demonstrate a marked decrease in renal clearance of both substances (Tables 2, 3). An increase in renal reabsorption caused by ion-pairing of bretylium and hexylsalicylic acid may be considered as one reason for the

Table 3. Pharmacokinetic parameters of hexylsalicylic acid (24·2 μmol kg^{-1}) after separate or simultaneous i.v. administration with bretylium (24·2 μmol kg^{-1}).

Drug	AUC (nmol h mL^{-1})	MRT (h)	Vd_{ss} (mL kg^{-1})	CL_r (mL h^{-1} kg^{-1})
Hexylsalicylic acid	29·8 ± 4·0	1·4 ± 0·4	1100 ± 470	16·4 ± 2·3
Hexylsalicylic acid + bretylium	49·9* ± 5·8	1·6 ± 0·1	780 ± 290	5·03* ± 0·85

Means ± s.d., n = 4, * $P < 0·05$ compared with hexylsalicylic acid alone.

elevated plasma levels of these substances after simultaneous administration. As discussed earlier (Neubert et al 1989; Härtl et al 1990), an increased intestinal reabsorption of both drugs could also be considered as a second principle reason for their elevated plasma levels. However, under the experimental conditions used in this study the common bile duct was interrupted for bile sampling and therefore the elevated plasma concentration-time profiles of bretylium and hexylsalicylic acid could only be due to the changes in tubular reabsorption. Nevertheless, the elevated biliary excretion of bretylium and hexylsalicylic acid after their simultaneous administration has to be considered as a prerequisite for an elevated intestinal reabsorption of both compounds.

Hexylsalicylic acid is extensively metabolized in the liver, and only the sulphuric and glucuronic acid conjugates are detected (Neubert et al 1989). Therefore, a lower amount of hexylsalicylic acid was measured in the bile than was probably there. As discussed in detail (Neubert 1989), ion-pair transport of bretylium and hexylsalicylic acid may be considered as a principle mechanism for an increased transport of these compounds into the liver cell and, thus, as a prerequisite for an elevated biliary excretion.

On the other hand, bretylium is excreted unchanged (Garrett et al 1982). Therefore, an influence of the co-administered counter-ion on the metabolic fate of this drug can be excluded. There are no references in the literature that compounds such as bretylium influence the metabolic fate of drugs; however, the influence of bretylium on the metabolic fate of hexylsalicylic acid needs further study.

It is demonstrated in this paper that pharmacokinetic interactions of drugs due to ion-pair transport are not limited to drug absorption but also include the pharmacokinetic process of drug elimination. The rabbit model used in this study was found to be very useful for parallel investigation of biliary and renal excretion in comparison with plasma concentration-time profiles of drugs after separate or simultaneous administration.

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