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Effect of Bile Salt on the Pharmacokinetics of Bretylium in the Rat. (I)

— Increased Lipophilicity of Bretylium by Ion-Pair Complexation with Taurodeoxycholate —

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Abstract—Bretylium tosylate is a quaternary ammonium compound used for the treatment of ventricular fibrillation in humans. It is advantageous to other cationic compound in the study of biliary excretion in that negligible amount is bound to plasma protein and metabolite is not likely to be formed. Some researchers reported that the formation of ion-pair complex caused to increase the lipophilicity of cationic compound. The partition of bretylium between water and organic phase was increased with the addition of sodium taurodeoxycholate. Also sensitive gas chromatographical assay procedure using flame ionization detector was studied. This procedure can detect as low as 0.1 mg/ml using 0.1 ml biological sample, but contamination by previous injection is the major problem of this method.

Keywords—Bretylium tosylate, Taurodeoxycholate, Ion-pair complex Apparent partition coefficient, Lipophilicity, Electron capture detector, Flame ionization detector, Capillary column gas chromatography, Splitless method, Sensitive determination of bretylium.

Bretylium [(o-bromobenzyl) ethyl (dimethylamine)] is a quaternary ammonium compound used as the tosylate salt for the treatment of ventricular fibrillation in humans. Relatively high concentrations of bretylium initially release norepinephrine from adrenergic nerve endings.[1,2] Subsequently and lower concentrations, the drug inhibits the release by the nerve action potentional of norepinephrine and thereby potentiates the action of these agonists on adrenergic recepters. Bretylium also increases the action potential of duration (APD) and prolongs the effective refractory period (ERP) of isolated Purkinje fibers and ventricular muscle fiber without altering ERP/APD.[1]

Although bretylium is strongly basic and the 70% of the administered dose excreted in urine,[3] biliary excretion should not be neglected or underestimated as it is the second major eliminating route. Furthermore, bretylium tosylate is advantageous to other organic cations in the study of the biliary excretion of cationic drugs in that negligible amount is bound to plasma protein and metabolite is not likely to be formed in the body.[1]

According to Shim,[3] the formation of ion-pair with anion markedly increased the lipophilicity of cationic compound like methylene blue, isopropamide iodide, tetrabutyl ammonium. Also Shim et al.[4] reported that sodium taurodeoxycholate (TDC) infusion into a rat caused to increase the elimination and distribution of methylene blue (MB). Increased lipophilicity through ion-pair formation between MB and TDC seemed to be the possible caused of it. Likewise, the possible increase of the lipophilicity of bretylium caused by the formation of ion-pair complex with TDC may influence the pharmacokinetics of bretylium.

In order to study the effect of possible ion-pair formation on the pharmacokinetics of bretylium, the possibility of the formation of bretylium-TDC ion-pair complex and partitioning of bretylium between water and organic solvent in the presence of TDC were studied.

Also, sensitive assay procedure of bretylium in biological fluid was examined gas chromatographically using flame ionization detector (FID).

EXPERIMENTAL METHOD

Materials

Bretylium tosylate was kindly supplied by the Wellcome Foundation Ltd (England). Sodium taurodeoxycholate (Sigma) was used as anionic substance.

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Thiophenol (TCI) was used as sodium salt in the derivatization of bretylium. Solvents and other reagents were commercially available ones and used without further purification.

Partition of bretylium tosylate between n-octanol phosphate buffer

Partition experiment was performed according to Shim et al. The phosphate buffer soln' (pH 7.4) of bretylium (1×10^{-5} M) and TDC (1×10^{-5} — 5×10^{-5} M) was mixed vigorously with the aliquot of n-octanol. n-Octanol and phosphate buffer were presaturated mutually before the experiment. After separation of organic phase from water phase by centrifugation, concentration of bretylium in water phase was determined by the GLC assay procedure.

Sample preparation for GLC in partition study

Bretylium in water phase was extracted by the procedure according to Lai et al.[6] The residue was derivatized with sodium benzene thiolate prepared according to Lai et al[6] in 100-120°C sand bath.[7] The medium was evaporated using nitrogen stream. 20 μl of n-hexane was added and 2 μl of hexane was injected to GLC. GLC condition is described in the section GLC assay condition.

Sample preparation for GLC in biological fluid

Extraction of bretylium in plasma or bile was performed according to Lai et al. 0.1 ml of biological sample was added to 0.1 ml of the internal standard soln' containing 500 ng of UM-360 [(o-iodobenzyl) trimethylamine], 0.5 ml of 0.1 N sodium chloride solution and 1 ml of acetonitrile. The sample was vortexed for 10 sec and centrifuged at 4000 rpm for 5 min. The supernate was transferred into a tube to which 0.2 ml of 1 N NaOH was added. The mixture was extracted with 4 ml of methylene chloride by gentle shaking for 30 min and was centrifuged at 4000 rpm for 5 min. 3.5 ml of the organic phase was evaporated to dryness with nitrogen stream in a new conical tube. Sodium benzene thiolate (1 mg/ml, in ethyl acetate), 100 ul, was added and tube was kept tightly. The sample were refluxed in a sand bath (100-200°C) for one hour. The ethyl acetate was removed under a stream of nitrogen gas and the residue was dissolved in 20 μl of hexane and 2 μl of hexane was injected to GLC.

GLC assay condition

For the assay of bretylium in partition study, gas chromatograph equipped with glass column packed with 3% SE-30 on Chromosorb W (100-200 mesh) and flame ionization detector was used. The injection port, column and detector temperature were 250°C, 170°C and 250°C respectively. Nitrogen gas was used as the

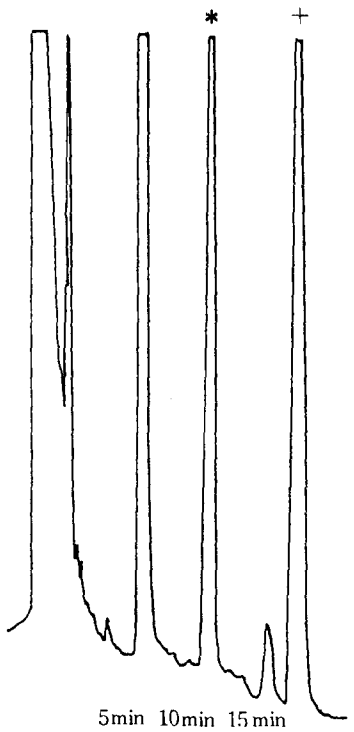

Fig. 1. Typical chromatogram of bretylium derivative. Injector and column temperature was 250°C and 170°C, respectively. (Key; * = bretylium deravative, + = UM-360 derivative)

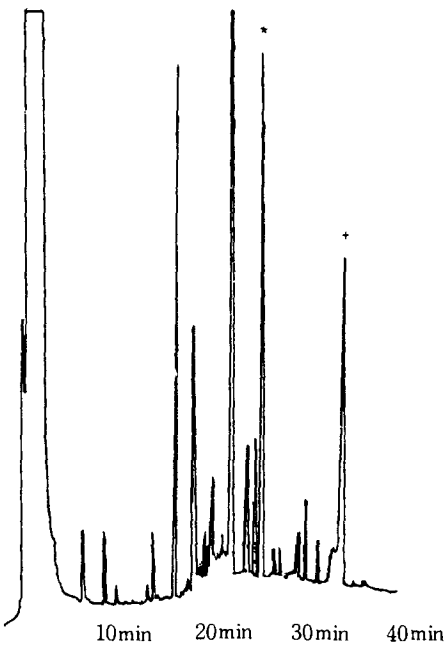

Fig. 2. Typical chromatogram in capillary column assay. (Key; * = bretylium derivative, + = UM-360 derivative)

carrier gas with a flow rate of 50 ml/min. The typical chromatogram is shown in Fig. 1. Column temperature should be kept under 170 degree unless the peak of bretylium derivative was separated incompletely.

For the quantitative assay of bretylium in biological fluid, gas chromatograph equipped with fused-silica capillary column (CBP-1, 0.20mm ID × 50m) and flame ionization detector was used. Not only the sensitivity and resolution can be improved when compared to packed column assay, but also injection volume can be reduced less than 1 μl. The splitless method (1.8 min of purging wait time) was used as it introduces concentrated sample to the column. The injection port and detector temperature was 280°C. The column temperature was raised by the rate 10°C/min (65-200°C) or 5°C/min (200-250°C) (Fig. 2).

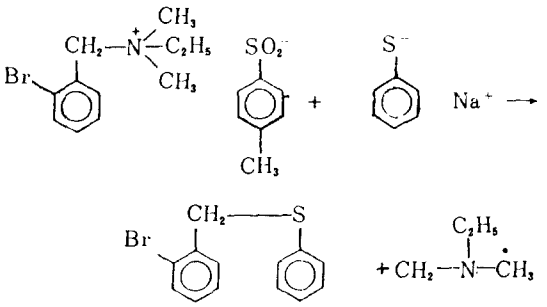

Fig. 3. Reaction mechanism of bretylium tosylate and sodium benzene thiolate.

RESULTS AND DISCUSSION

The reaction mechanism of bretylium with sodium benzene thiolate for GLC analysis—According to Lai et al. the reaction of the quaternary ammonium compound with sodium benzene thiolate yields a volatile derivative, o-bromobenzylphenlythioether. Although the benzenethiolate anion can attack at any of the four amine substituents by an SN2 process, attack at the o-bromobezyl group seems to be preferred due to resonance stabilization of the reaction transition state (Fig. 3). TLC data was also in agreement with this assumption (Fig. 4).

The calibration curve of bretylium in buffer phase—The calibration curve of bretylium in buffer phase was shown in Fig. 5. It showed reliable linearity in the region studied.

Partition experiment—The result of partition study was shown in Fig. 5. This study shows that the partition of bretylium into organic phase was increased in the presence of TDC. It implicates that the polarity of bretylium is reduced by ion-pair formation with TDC. The increased lipophilicity of bretylium-TDC complex may affect the lipoidal membrane transport of bretylium.

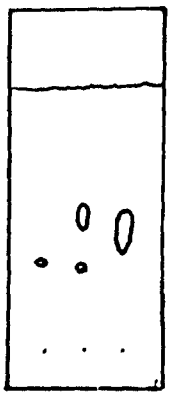

Fig. 4. TLC anaylsis of bretylium, o-bromobenzylthio ether and sodium benzene thiolate. The R_f value was 0.33 for bretylium and 0.44 for sodium benzene thiolate.

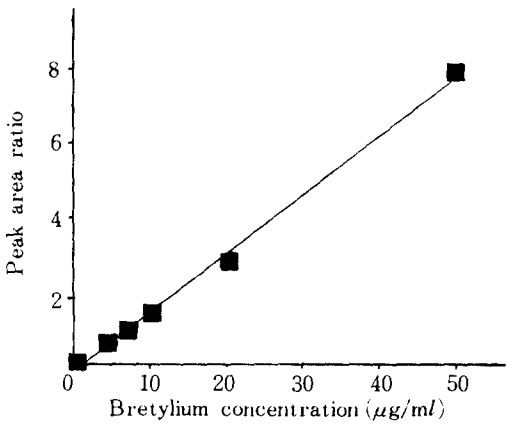

Fig. 5. The calibration curve of bretylium in partition study. Correlation coefficient was 0.9984.

There are two major categories in the determination of bretylium in biological fluids using chromatographical methods. The more sensitive one, GLC-electron capture detector (ECD) method,[6,7] is hard to apply and the other, HPLC method,[8] is lack of sensitivity. GLC-ECD method is difficult in routine analysis. Especially, the choice of the carrier gas for the detector is the most difficult one. Widely used nitrogen gas must be nano-grade-quality and is expensive. Furthermore ECD is so sensitive that the peak response alters occasionally. This variation makes the routine application even more difficult. On the other hand, the HPLC method is not suitable for the assay of bretylium in biological fluid as it lacks sensitivity.

Compared to ECD, FID[8] can be readily applicable with the variety choice of carrier gases and with sensitivity in GLC. In this study, however, previous injection of plasma sample affected the number of the peaks

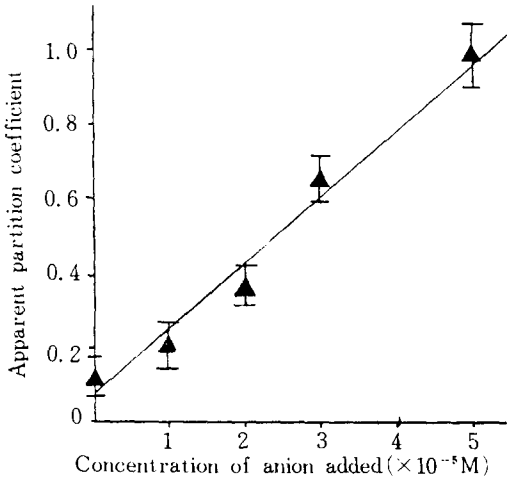

Fig. 6. The plot of apparent partition coefficient and concentration of anion added. It was shown that apparent partition coefficient was increased with the addition of anionic substance.

of consecutive sample. It seemed to be due to the contamination by previous injection. Presented data failed to show the linearity to assay the bretylium in biological fluid, but plasma concentration of bretylium could be detected as low as 0.1 μg/ml using 0.1 ml sample. The plasma concentration of bretylium was about 0.1 μg/ml at 24 hr after the intravenous administration of the adult dose (10 mg/kg) of bretylium.[10] More intensive study would be necessary for the development of readily applicable and sensitive GLC-FID assay for bretylium in biological fluids.

ACKNOWLEDGEMENTS

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