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Effect of Ion-Pair Complexation with Bile Acids on the Biliary Excretion and Systemic Distribution of Organic Cationic Drugs

Chang-Koo Shim

College of Pharmacy, Seoul National University, Seoul 151, Korea

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Abstract□Effect of sodium taurodeoxycholate (TDC) infused intravenously on the pharmacokinetics of methylene blue (MB) was studied in the rat to investigate the role of ion-pair complexation in the body on drug elimination and disposition. Distribution volume (*Vd*) of MB was increased significantly ($p<0.05$) by TDC infusion. Considering together with the fact that apparent partition coefficient (APC) of MB between phosphate buffer (pH 7.4) and n-octanol was increased markedly by TDC, the increase in Vd seemed to be the result of decreased polarity of MB by ion-pair formation with TDC. But total body clearance (*CLt*) and biliary excretion clearance (*CLbil*) of MB were not increased significantly by TDC.

Keywords□Methylene blue, Organic cationic drugs, Taurodeoxycholate, Bile acids, Ion-pair complex, Apparent partition coefficient, Distribution volume, Biliary excretion, Pharmacokinetics, Cholestyramine, Bile flow, Polarity, Lipophilicity.

Biliary excretion is one of the major routes of elimination for drugs administered. Daily bile flow (approximately 0.7-1.0l) is comparable to daily urine flow (approximately 1.5l). Some drugs are actively secreted into bile juice.

Though biliary excretion is a major route of elimination second to urinary excretion, exact mechanism of it is not fully understood. Rollins et al[1] reported active transport of anionic drugs into bile, and Klassen[2] suggested the presence of three different transport systems for acidic, basic and neutral drugs.

Milburn et al[3] thought that molecular weights of drugs should be larger than certain threshold value to be excreted into bile. Levine et al[4-5] thought that complex formation with bile acids is responsible for the biliary excretion of tertiary ammonium drugs which are expected to be eliminated in urine based on their polarity. But Shanker et al[6] reported conflicting results, and Zimmer et al[7] postulated that the inclusion of drugs in mixed micelles of bile acid and lecithin in required for the biliary excretion.

Some cationic drugs e.g. tetrabutylammonium bromide, isopropamide and methylene blue were reported[8-9] to form ion-pair complexes with sodium taurodeoxycholate, a representative bile acid. These drugs are known to be excreted into bile, but some cationic drugs as like tetraethylammonium bromide do not form ion-pair complexes[8-9] and are not excreted into bile.

The fact that basic drugs of larger molecular weight than 200 ± 50[10] are excreted into bile, and form ion-pair complexes with organic anions[11-13] implies the possible role of ion-pair complexation in biliary transport of the drugs.

In this study we tried to elucidate the possible role of ion-pair complexation of organic cationic drugs with bile acids in elimination and distribution of them.

Methylene blue was selected as a model drug of organic cationic drugs, because it was reported to be excreted into bile[14] and it exsist as cationic form in physiological pH. Sodium taurodeoxycholate was selected as a representative bile acid, because it also exists as anionic form in physiological pH and it forms ion-pair complex with methylene blue.[9]

EXPERIMENTS

Materials

Methylene blue trihydrate (MB) of Kokusan Chemical Co. (Japan) was used as a model drug of basic drugs. Sodium taurodeoxycholate (TDC) of Sigma Chemical Co. (USA) was used as a bile acid. Cholestyramine and other reagents were commercially available

and used without further purification.

Animals

Male Wistar or Donryu rats (Experimental Animal Farm of Seoul National University), weighing 250-350g were used. Water and commerical chow (Sam Yang Animal Food Inc., Seoul) were given *ad libitum* on the same condition for more than one week before the experiment.

Partition of MB between n-octanol and phosphate buffer

Partition experiment was performed according to previous reports[8-9]. The phosphate buffer solution (pH 7.4) of MB (5×10^{-5} M) and TDC ($1 \times 10^{-4} \times 10^{-3}$ M) was mixed vigorously with n-octanol of same volume. n-Octanol and phosphate buffer were presaturated mutually before the experiment. After separation of organic phase from water phase by centrifugation, each phase was determined for its MB content. MB in water phase and orgainc phase were determined from the absorbance at 664nm and 660.5nm respectively.

Bile flow and biliary excretion of MB

Male Wistar rats (250 ± 20g) were fasted for 24 hrs before the experiment. But tap water was given *ad libitum*. Cholestyramine powder was suspended in 0.25% (w/v) methylcellulose solution to give 10%(w/v) suspension of it. Cholestyramine suspension was administered orally at a dose of 1.0g/kg (= 10ml/kg) with syringe for oral administration. The needle of the syringe was sufficiently thrust into the stomach of the rat, so that the suspension might not be thrown up. Urethane was injected intraperitoneally at a dose of 1.2g/kg to the rats at 4 hrs later the cholestyramine pretreatment. The femoral vein was cannulated with PE-50 polyethylene tubing for drug administration. The bile duct was cannulated with PE-10 polyethylene tubing. The rats were kept at spine position during the experiment. The body temperature of the rat was kept 37°C using heating lamp. 5%(w/v) MB solution was injected through PE-50 catheter at a dose of 50mg/kg (= 1ml/kg) and bile samples were taken for 5 hours at 15 min intervals.

Disposition of MB

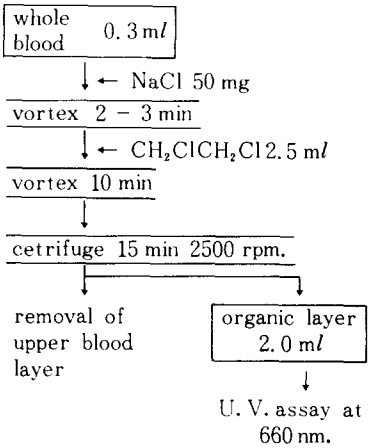
Under light anesthesia, the femoral artery and vein of the male Donryu rats (330-350g) fasted overnight were cannulated with PE-50 polyethylene tubings for drug administration and blood sampling, respectively. The bile collection was performed as mentioned above. After recovery from the anesthesia, 3.9%(w/v) solution of TDC was infused at a constant rate of 0.241ml/hr intravenously through the catheter according to Rollins[1] for 3hrs. 2%(w/v) solution of MB was injected at 30min after the infusion start at a dose of 2ml/kg (40mg/kg as MB trihydrate). Blood samples (0.3ml) were taken at given intervals from the femoral artery through the catheter for 3hrs. To the rats of control group, physiological saline instead of TDC solution was infused in the same manner.

Assay of MB in whole blood

MB was determined spectrophotometrically according to DiSanto[14] after minor modification (Scheme I).

Assay of MB in bile juice

Calibration curve of MB was prepared using bile juice. The method was essentially identical with Scheme I.



Scheme I. : Determination of methylene blue in blood.

Pharmacokinetic and statistical analysis

The average concentrations of MB in blood after i.v. injection were best fitted to conventional 2-compartment model,[15] i.e., $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, with the aid of MULTI program[18] to calculate pharmacokinetic parameters. Some parameters as like *AUC*, *AUMC*, *CLt* and *Vdss*[15] were also calculated model-independently. The difference between the two groups of data was examined for their significance with the Student's *t*-test.

RESULTS AND DISCUSSION

Effect of TDC on the apparent partition coefficient (APC) of MB

Fig. 1 shows that effect of TDC on the partition of MB between phosphate buffer (pH7.4) and n-octanol. APC of MB was increased by TDC in accordance with the increase of TDC concentration. It appears to be due to ion-pair complexation with TDC. In fact the true partition coefficient (TPC) of the complex was very large (56.5)[9]. From that point of view, MB entered into the systemic circulation is expected to form a lipophilic ion-pair complex with endogenous TDC and the pharmacokinetics of MB to be affected by the complexation.

Quantitative analysis of MB in bile juice

Fig. 2 shows the calibration curve of MB in bile juice by the method of Disanto et al.[14] (Scheme I). The absorbance at 660nm exhibited good proportionality to the concentration of MB.

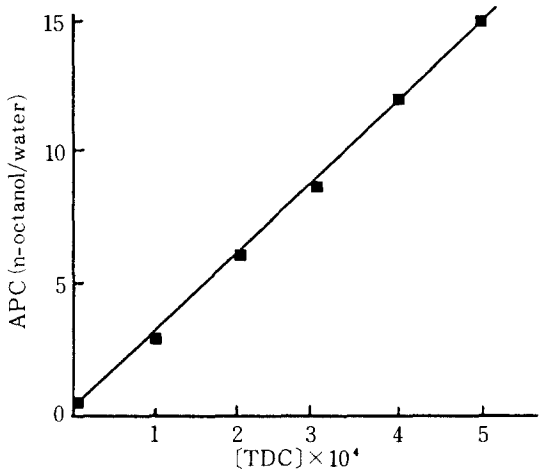


Fig. 1. Effect of sodium taurodeoxycholate (TDC) on apparent partition coefficient (APC) of methylene blue(0.5×10^{-4}M).

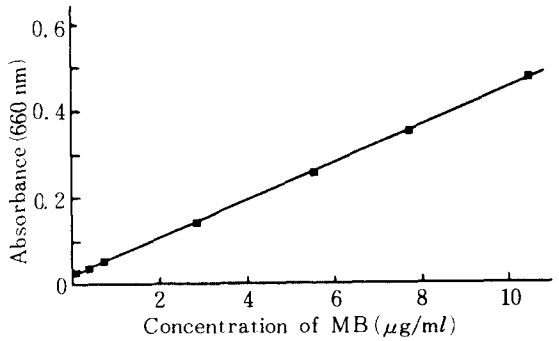


Fig. 2. Calibration curve of methylene blue (MB) in bile.

Effect of cholestyramine pretreatment on the bile flow and the biliary excretion of MB

Fig. 3 and Fig. 4 show bile flow and biliary excretion of MB after adsorption of intraintestinal bile acid by cholestyramine pretreatment for four hours. Cholestyramine has been known to be an anionic resin which is not absorbed in gastrointestinal tract and binds with intraintestinal bile acids to inhibit the hepato-biliary recirculation. Cholestyramine, therefore, decreases the concentration of bile acid in the bile juice. Fig. 3 shows that bile flow is not affected by cholestyramine-pretreatment. This deviates from the reports that cholestyramine decreased the bile flow up to about 15%[16-17]. This might be due to the depletion of intraintestinal bile acids by continuous bile collection over the experiment. The effect of this collection might overwhelm the effect of cholestyramine-pretreatment.

Fig. 4 shows that biliary excretion is not affected by cholestyramine-pretreatment. This implies the possibilities that bile acids do not contribute to biliary excretion of MB,

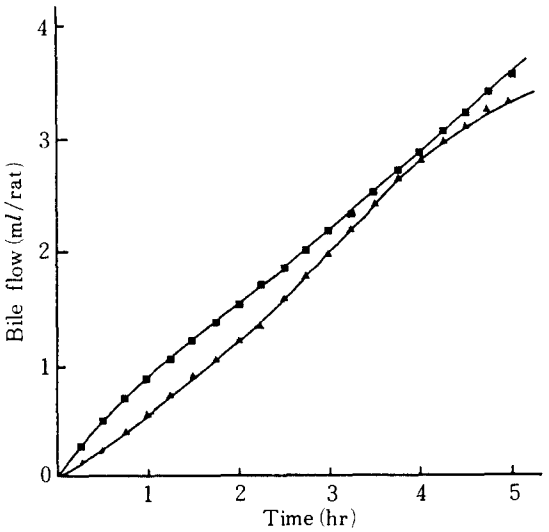


Fig. 3. Effect of cholestyramine pretreatment on the bile flow. Key : —■—Cholestyramine pretreated rat, —▲—Control rat.

Each point represents the mean of three experiments. Mean body weight of the rats used in two groups were adjusted to be same.

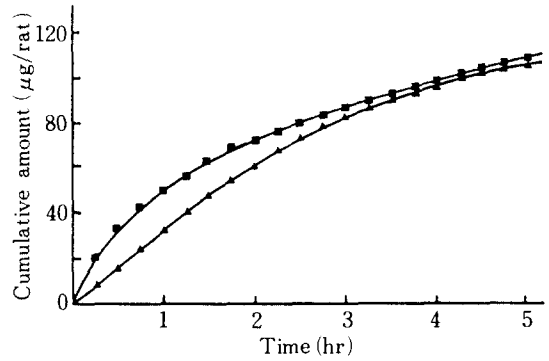


Fig. 4. Cumulative amount of methylene blue excreated in bile. Key: —■—Cholestyramine pretreated rat, —▲— Control rat.

Each point represents the mean of three experiments. Mean body weight of the rats used in two groups were adjusted to be same.

or that minimal concentration of bile acids necessary for the excretion of MB into bile is sufficiciently maintained even after the cholestyramine-pretreatment. Changed concentrations of bile constituents like cholesterol in intraintestinal tract might affect the biliary excretion of MB. More detailed experiments would be necessary to

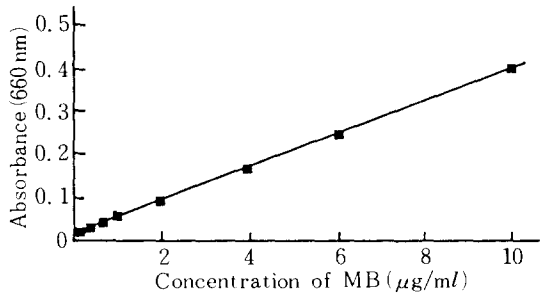


Fig. 5. Calibration curve of methylene blue in blood.

elucidate the possible role of bile acids on the excretion of MB.

Quantitative analysis of MB in whole blood

Fig. 5 shows the calibration curve of MB in blood by the method of Scheme I. A good linearity was recognized for the concentration range examined. Effect of TDC on the absorbance of MB was also examined (Fig. 6) according to scheme I. Presence of TDC up to 10μg/ml did not inhibit the absorbance of MB in blood. Therefore, Scheme I and Fig. 5 seemed to be applicable without further modification to the blood samples taken for the determination of MB administered bolusly to the rats receiving constant TDC infusion.

Effect of TDC infusion on the pharmacokinetics of MB

Fig. 7 shows blood concentration-time profile of MB administered bolusly to the rats receiving constant infusion of TDC. Blood concentrations of MB in TDC-infused rats was lower than those of control rats which received constant infusion of physiological saline instead of TDC in the same manner. But the differences of MB concentrations between two groups were not significant. Solid curves in Fig. 7 were calculated by M-MULTI program[18] using personnel computer (PC-8001, NEC, Japan) to two-compartment model.

Table I shows the pharmacokinetic parameters of two groups obtained by M-MULTI according to two-

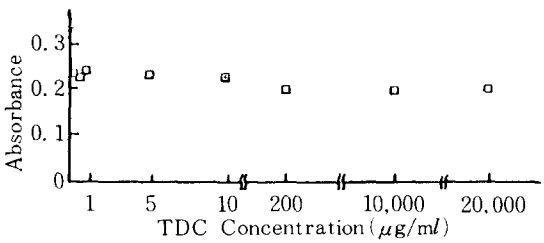


Fig. 6. Effect of sodium taurodeoxycholate (TDC) concentration on the absorbance of methylene blue (2.0 μg/ml).

Absorbance was measured according to Scheme I.

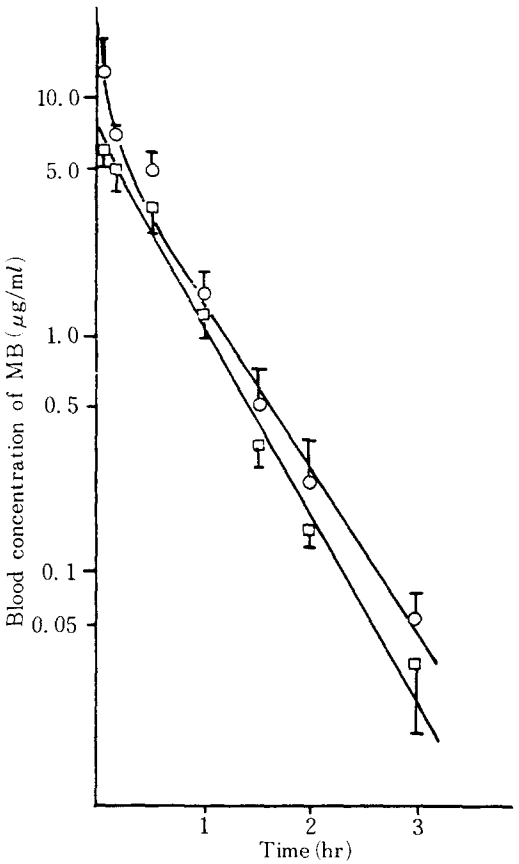


Fig. 7. Blood concentration-time curve of methylene blue (MB) injected intravenously at a dose of 40 mg/kg in the control (-○-) and sodium taurodeorycholate (TDC) infused group (-□-).

Each point represents the mean ± S.E. of three experiments. Solid curves were calculated according to 2-compartment model by M-MULTI. [18]

compartment model. Table II shows model independent parameters of two groups. Table I and II show that both model-dependent and model-independent distribution volume at steady-state, *Vdss*, of MB were significantly ($P<0.05$) increased in TDC group. This might be the result of increased distribution to body tissues due to increased liphophilicity of MB through complexatin with TDC (Fig. 1). Increased volume of central compartment. Vd_1 in Table I implies the increased distribution of MB to highly perfused organ like liver or kidney by ion-pairing with TDC.

Total body clearance, *CLt*, and biliary excretion clearance, *CLbil*, of MB were also increased in TDC group, but not significantly. *CLbil* was negligibly small comparing with *CLt*. Therefore, nonsignificant increase

Table I. Effect of TDC infusion on the pharmacokinetic parameters of MB[a].

Parameters		Control	TDC
A	($\mu g/ml$)	7.65±2.15	2.65±1.40
B	($\mu g/ml$)	7.85±3.92	4.36±1.02
α	(min^{-1})	2.03±1.57	0.04±0.00
β	(min^{-1})	0.03±0.00	0.03±0.00
$Vdss$	(ml/min·kg)	3554.29±283.32 *	6303.86±956.35
Vd_1	(ml/kg)	2841.97±578.00 *	6095.09±1030.65
Vd_2	(ml/kg)	712.31±352.42	208.76±122.11
Vd_β	(ml/kg)	4306.53±884.28 *	6997.07±1027.99
K_{10}	(min^{-1})	0.05±0.00 *	0.03±0.00
K_{12}	(min^{-1})	0.76±0.66	0.00±0.00
K_{21}	(min^{-1})	1.25±0.93	0.03±0.00
AUC	(μg·min/ml)	344.71±74.35	220.01±34.88
CLt	(ml/kg/min)	125.68±22.38	190.10±26.21
$CLbil$	(ml/kg/min)	0.14±0.07	0.20±0.09
$t^{1/2}\alpha$	(min)	6.25±5.79	18.39±1.88
$t^{1/2}\beta$	(min)	24.11±3.20	25.73±2.27

[a] MB was injected intravenously at a dose of 40 mg/kg. Each group consists of three rats. Data were fitted to $Cp = Ae^{-\alpha t} + Be^{-\beta t}$ with the aid of M-MULTI [18]. Results are given as the mean±S.E.. For the meaning of parameters, see the reference [15]. *$p<0.05$.

in CLt may be due to increased urinary excretion of MB by TDC infusion. Increased lipophilicity of MB *in vitro* by ion-pair complexation with TDC did not affect the elimination of it *in vivo* significantly. This might be due to that enough concentration of TDC necessary for the biliary excretion of MB is maintained endogenously. If this is the case, no additional supply of TDC by i.v. infusion will not be necessary for the biliary excretion of MB.

Therefore, a bile acid-deficient state, which may be prepared experimentally, will be effective to visualize the effect of TDC infusion on pharmacokinetics of MB. This will be presented elsewhere.[19]

Table II. Model-independent parameters in control and TDC group[a].

Parameters	Control	TDC
AUC	365.37±61.07	260.15±48.57
$AUMC$	10431.28±2944.66 *	9580.82±2945
$Vdss$	3074.36±185.40 *	5600.16±534.57
CLt	115.00±16.56	163.76±26.98

[a] AUC and $AUMC$ were calculated by trapezoidal rule. $Vdss$ and CLt were calculated by $Vdss = (\text{Dose}) \times (AUMC)/(AUC)^2$ and $CLt = AUC/(\text{Dose})$ respectively. **$p<0.02$.

CONCLUSION

Apparent partition coefficient (APC) of methylene blue (MB) was markedly increased by the presence of sodium taurodeoxycholate (TDC). It seemed to be due to decreased polarity of MB through its ion-pair formation with TDC.

Cholestyramine-pretreatment did not alter the biliary excretion of MB. However, it is not sufficient to conclude that bile acids do not affect the biliary excretion of it, because whether the bile acids was depleted sufficiently by the treatment is not confirmed in this experiment.

TDC infused intravenously at a constant rate increased the distribution volume (Vd) of MB. Considering it together with the result from partition experiment, the increase in lipophilicity due to ion-pair complexation seemed to be the major cause of Vd increase. But total body clearance (CLt) and biliary excretion clearance ($CLbil$) of MB was not altered significantly by the TDC infusion, in spite of possible formation of ion-pair complex with TDC. This may be due to (a) minor contribution of TDC to excretion of intact MB, or to (b) sufficiently high concentration of endogenous TDC in both experimental groups.

More detailed experiments will be necessary to elucidate the exact contribution of ion-pair complexation with bile acids to distribution and excretion of organic cationic drugs.

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REFERENCES

1. Rollins, D.E. and Klassen, C.D.: *Clin. Pharmacokin.* **4,** 368(1979).
2. Klassen, C.D.: *Handbook of Physiology.* pp. 537-553, American Physiological Society, Washington D.C. 1977.
3. Milburn, P., Smith, R.L. and Williams, R.T.: *Biochem. J.* **105,** 1275 (1967).
4. Levine, R.M. and Clark, B.B: *J. Pharmacol. Exptl. Therap* **114,** 63(1955).
5. *ibid,* **121,** 63 (1957).
6. Schanker, L.S. and Solomon, H.M.: *Am. J. Physiol.* **204,** 829 (1963).

7. Zimmer, R.O. and Lindenbaum, S.Jr: *J. Pharm. Sci.* **68,** 581(1979).
8. Shim, C.K., Nishigaki, R., Iga, T. and Hanano, M.: *Int. J. Pharmaceut.* **8,** 143 (1981).
9. Shim, C.K.: *Yakhak Hoeji,* **27,** 125 (1983).
10. Hughes, R.D., Milburn, P. and Williams, R.T.: *Biochem. J.* **136,** 967(1973).
11. Modin, R. and Schill G.: *Acta Pharm. Suecica* **4,** 301 (1967).
12. Persson, B.A. and Schill, G.: *Acta Pharm. Suecica* **3,** 281 (1966).
13. Schill, G.: *Acta Pharm. Suecica* **2,** 12 (1965).
14. DiSanto, A.R. and Wagner, J.G.: *J. Pharm. Sci.* **61,** 1086 (1972).
15. Gibaldi, M. and Perrier, D.: *Pharmacokinetics* 2nd Ed., pp. 215-221, Marcel Dekker Inc., New York and Basel, 1982.
16. Gregus, Z., Fisher, E. and Varga, F.: *Arch. Int. Pharmacodyn.* **245,** 311 (1980).
17. Gregus, Z., Barth, A., Fisher, E., Zaumseil, Z., Klinger, W. and Varga, F.: *Acta Biol. Med. Germ.* **39,** 705 (1980).
18. Shim, C.K. and Chung, S.J.: *Seol University J. of Pharmaceutical Sci.* **8,** 37 (1983).
19. Kwon, O.S., Shim, C.K., Lee, M.H. and Kim, S.K.: *Yakhak Hoeji* **30,** 68 (1986).