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MICELLAR INTERACTION OF INDOMETHACIN AND PHENYLBUTAZONE WITH BILE SALTS

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SUMMARY

In the previous report, it was confirmed that bile salt micelles display a significantly higher affinity for indomethacin as compared to phenylbutazone in pH 7.3 phosphate buffer at 37°C. The extent of micellar solubilization seemed to be influenced by the nature of the solubilized molecule. The difference between indomethacin and phenylbutazone in micellar interaction was investigated. This difference could not be explained on the basis of the lipid solubility and molal volume. It was suggested that the mechanism responsible for the solubilization of indomethacin may be due to the formation of mixed micelles of the bile salts and the drug. On the other hand, the lower degree of interaction of phenylbutazone with bile salt micelles can probably be attributed to its carbon acid.

INTRODUCTION

In the previous reports of this series (Miyazaki et al., 1979, 1980), it was confirmed that bile salts markedly enhance the dissolution and absorption of the non-steroidal anti-inflammatory drugs, indomethacin and phenylbutazone, which are very poorly soluble in water. The mechanism of interaction between the two drugs and bile salts was also studied. The results indicated that the enhancement of the dissolution of indomethacin in the presence of bile salts was mainly due to micellar solubilization. On the other hand, the enhanced dissolution of phenylbutazone may be due to the wetting effect. Based on the saturation ratios found for the two solubilizates, it can be seen that bile salt micelles display a significantly higher affinity for indomethacin as compared to phenylbutazone. The present investigation was undertaken to clarify the difference between indomethacin and phenylbutazone in micellar interaction.

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MATERIALS AND METHODS

Materials

The following materials were used: indomethacin [1], phenylbutazone [1], mefenamic acid [2], oxyphenbutazone [3], phenindione [1], sodium deoxycholate [4], sodium taurodeoxycholate [1], and sodium lauryl sulfate [5]. They were used without further purification except for mefenamic acid.

Apparent partition coefficients

Six ml of the buffered drug solution and 6 ml of organic solvent previously saturated with the same buffer solution were placed into a glass-stoppered test tube. Samples were equilibrated by shaking the tubes at 37°C in an incubator. The separated aqueous phase was analyzed for indomethacin and phenylbutazone by the fluorometric (Hucker et al., 1966; Moriyama et al., 1971) and UV spectrophotometric (Burns et al., 1953) procedure, respectively. The apparent partition coefficient of solute was calculated from the initial and final concentrations of the drug in the aqueous phase.

Apparent molal volumes

The densities of saturated solutions were determined at 25°C with a 50 ml Wadon pycnometer. Apparent molal volumes were calculated using the equation described by Lee and Hyne (1968).

Solubility studies

The solubilities of drugs were determined in 1/15 M phosphate buffer of pH 7.3 containing various concentrations of bile salts at 37°C. Excess amounts of samples were suspended in 2 ml of the bile salt solutions. These suspensions were shaken for 24 h in the incubator. Aliquots were filtered with a Millipore filter (0.45 μm) and assayed spectrophotometrically.

Micellar interaction by the molecular sieve method

The interaction between the bile salt and the drugs was studied by the molecular sieve method (Ashworth and Heard, 1966).

NMR measurements

Sodium deoxycholate (20 mM) was dissolved in the pH 7.3 phosphate buffer, in ^2H_2O. An excess of the drugs was equilibrated with the bile salt solution by the described procedure for solubility studies. Aliquots filtered were examined in JEOL PS-100 NMR spectrometer using DSS as internal reference. All runs were carried out at 37°C.

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[1] Sigma Chemicals, St. Lowis, Mo.
[2] Sankyo, Tokyo.
[3] Fujisawa Pharmaceuticals, Osaka.
[4] Tokyo Kasei, Tokyo.
[5] Wako Pure Chemical Industries, Osaka.

RESULTS AND DISCUSSION

In the previous report (Miyazaki et al., 1979), data on the micellar solubilization of indomethacin and phenylbutazone in 0–40 mM aqueous solutions (pH 7.3) of the sodium salts of cholic, deoxycholic, taurocholic and glycocholic acids at 37°C were presented. The interaction of the drugs with the other conjugated bile salt, sodium taurodeoxycholic acid, and the typical synthetic anionic surfactant, sodium lauryl sulfate, were also studied by the solubility method. The slope of the linear portion of the solubilization curve represents the ratio of micellar drug to micellar bile salt and is termed the saturation ratio (Hofmann, 1963). As shown in Table 1, the saturation ratio for indomethacin in the bile salt solution is many times greater than that of phenylbutazone, indicating that bile salt micelles display a significantly higher affinity for indomethacin. A similar difference in micellar interaction was also found for sodium lauryl sulfate. Lovering and Black (1974) observed a similar poor solubility of phenylbutazone in micelles of bile salts. These differences between indomethacin and phenylbutazone in micellar interaction are examples of the well established fact that the extent of micellar solubilization is influenced by the nature of the solubilized molecule.

The structural formulae of indomethacin and phenylbutazone are presented in Table 2. Indomethacin has a carboxylate anion, whereas phenylbutazone has a bulky heterocyclic molecule with a negative charge. The pK_a of both drugs is 4.5 (Newton and Kluza, 1978) and they are essentially ionized at pH 7.3.

According to a pseudo two-phase model, the solubilizate molecule is partitioned between an aqueous phase and a micellar phase. This partitioning is similar to that observed for a poorly water-soluble drug between a non-polar solvent and water. In order to examine the differences in their lipid solubility, we measured the apparent partition coefficient at pH 7.3 using benzene and cyclohexane as non-polar solvents. It is clear in Table 2 that the apparent partition coefficients of phenylbutazone were greater than those of indomethacin. No correlation between the apparent partition coefficient and micellar solubili-

TABLE 1

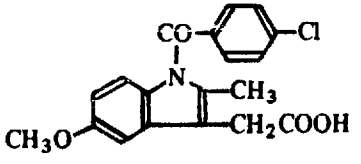
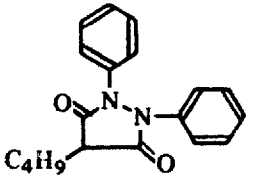
SATURATION RATIOS OF BILE SALTS FOR INDOMETHACIN AND PHENYLBUTAZONE

Bile salts	Saturation ratio * $\left(\frac{\text{mol of micellar drug}}{\text{mol of micellar bile salts}}\right)$	
	Indomethacin	Phenylbutazone
Sodium deoxycholate **	0.271	0.085
Sodium cholate **	0.247	0.023
Sodium glycocholate **	0.213	−0.006
Sodium taurocholate **	0.234	−0.015
Sodium taurodeoxycholate	0.154	0.023
Sodium lauryl sulfate	0.158	0.023

* Slope of the linear portion of the solubilization curve determined by the least-squares method.
** Values obtained from the previous report (Miyazaki et al., 1979).

TABLE 2

STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF INDOMETHACIN AND PHENYLBUTAZONE

Drugs	Structure	pK_a	Apparent partition coefficient		Molal volume (ml/mol)
			Benzene/buffer	Cyclohexane/buffer	
Indomethacin		4.5	2.30	1.38	328.41
Phenylbutazone		4.5	23.67	1.93	276.70

zation could be demonstrated. Consequently, it seems to be difficult to explain the difference in micellar interaction between indomethacin and phenylbutazone on the basis of the lipid solubility.

It can be rationalized that the molal volume of solubilizate is usually one of the important factors determing their degree of solubilization (Collett and Koo, 1975). Volume available within the micelle for the solubilizate molecule is limited. Therefore, the apparent molal volume measurements of both drugs were carried out at pH 7.3. It is clear in Table 2 that the apparent molal volume of indomethacin was greater than that of phenylbutazone. This suggests that the molal volume of the drugs contributes little to the difference in their micellar interaction.

Micellar solubilization is broadly classified into 3 types (Klevens, 1950): first, incorporation in the hydrocarbon center of the micelle; second, incorporation by penetration into the palisade layer of the micelle with the solubilizate oriented in approximately the same manner as is the surfactant molecule in the micelle; and third, adsorption on the surface of the micelle. Small et al. (1969) proposed that cholate and deoxycholate micelles are formed by hydrophobic association of the hydrocarbon backs of the rigid steroid nuclei in such a way that the hydrophilic sides, containing the hydroxy groups and the negatively charged ionic groups, are exposed to water. At pH 7.3, bile salts are negatively charged due to the protonation of the carboxylate groups. As previously indicated, indomethacin has also a carboxylate anion and the ionic form carries a relatively large hydrophobic portion in the molecule. There is an electronic similarity between bile salts and indomethacin; both have polar groups (carboxylate groups) at each end of their molecules. It would appear that this was being incorporated predominantly into the palisade layers of the bile salt micelles, resulting in a mixed micelle formation (Bates et al., 1966; Thakkar, 1970). This may explain why indomethacin enters the bile salt micelles. On the other hand, it seems reasonable to assume that the lower degree of interaction of phenylbutazone with bile salt micelles can probably be attributed to the carbon acid. Stella (1975) showed that phenylbutazone has a hindered dissolution at pH

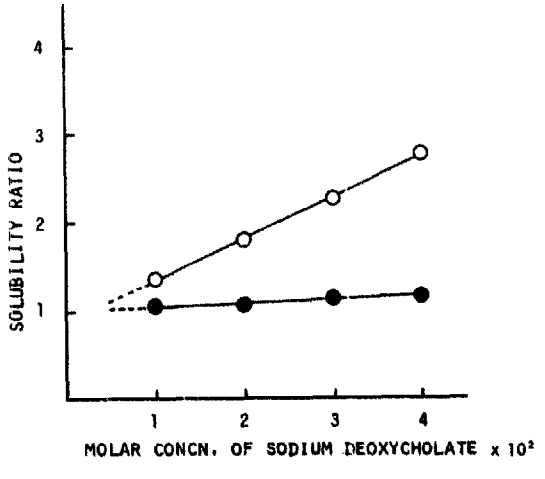


Fig. 1. Effect of sodium deoxycholate on the solubilities of mefenamic acid (o) and oxyphenbutazone (•) in pH 7.3 phosphate buffer at 37°C.

values greater than its pK_a because of non-instantaneous ionization kinetics. Non-instantaneous ionization is a characteristic of most carbon acids. In addition, the possible species of phenylbutazone that can exist in a pH 7.3 solution is the so-called mesomeric anion (Stella and Pipkin, 1976). The electrostatic repulsion between the bulky heterocyclic molecule with a negative charge (the mesomeric anion) and the negatively charged carboxyl groups of bile salts may be considered to impede the incorporation of phenylbutazone into the micelles. Therefore, the effect of drug ion was investigated using mefenamic acid and oxyphenbutazone, $pK_a = 4.2$ and 4.7 (Newton and Kluza, 1978), as the other normal carboxylic acids and carbon acids, respectively.

The interaction of the drugs with bile salts was studied by the solubility method. Since sodium deoxycholate had the greatest effects on the solubility and dissolution of indomethacin and phenylbutazone, it was selected for further study. The equilibrium solubilities of mefenamic acid and oxyphenbutazone in the bile salt solution at various concentrations are shown in Fig. 1. The solubilities are expressed as the ratio of solubility in the bile salt solution to its solubility in pH 7.3 buffer. It is clear that the solubility of mefenamic acid is increased in the presence of sodium deoxycholate, whereas a slight increase in the solubility was observed for oxyphenbutazone. The saturation ratios were found to be 0.236 and 0.094 for mefenamic acid and oxyphenbutazone, respectively.

The interactions of the drugs with the bile salt were also studied by the molecular sieve method. Fig. 2 shows the micellar interaction between the drugs and sodium deoxycholate. As might be expected, mefenamic acid formed a micellar complex, and the fraction of this increased as the concentration of the bile salt increased. On the other hand, oxyphenbutazone forms a micellar complex with the bile salt only to a small extent. The difference between mefenamic acid and oxyphenbutazone in micellar interaction was similar to that in indomethacin and phenylbutazone.

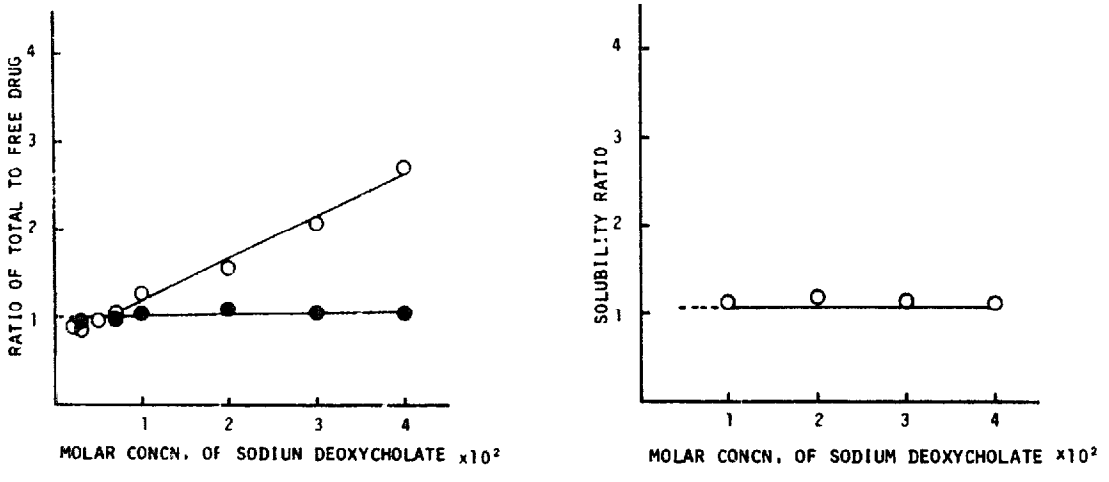


Fig. 2. Plots showing the ratio of total to free mefenamic acid (○) and oxyphenbutazone (●) in pH 7.3 phosphate buffer containing various concentrations of sodium deoxycholate at 37°C.

Fig. 3. Effect of sodium deoxycholate on the solubility of phenindione in pH 7.3 phosphate buffer at 37°C.

TABLE 3

^{1}H-CHEMICAL SHIFTS FOR SODIUM DEOXYCHOLATE IN THE PRESENCE AND ABSENCE OF THE DRUGS IN ^2H_2O

Composition	Chemical shifts, τ (ppm)			
	C-18H	C-19H	C-21H	C-23H
Sodium deoxycholate alone	9.30	9.08	9.01	7.90
Sodium deoxycholate in the presence of indomethacin	9.41	9.20	9.05	7.91
Sodium deoxycholate in the presence of phenylbutazone	9.30	9.11	9.05	7.89

Further evidence for the effect of drug ion on the extent of micellar interaction was obtained when the authors carried out an investigation of the solubility of the other typical carbon acid, phenindione ($pK_a = 4.09$) (Stella and Gish, 1979). Its solubility profile is shown in Fig. 3; the solubility of phenindione was not changed by sodium deoxycholate. The lower degree of interaction with bile salt micelles seems to be characteristic of most carbon acids.

NMR spectroscopic measurements have provided useful information about location of the solubilized drug molecules in the bile salt micelle. NMR chemical shifts for certain hydrogens of sodium deoxycholate both alone and in the presence of indomethacin and phenylbutazone are shown in Table 3. Proton assignments were adapted from those reported for bile salts (Small et al., 1969).

An upfield shift in the C-18 and C-19 methyl protons of ~0.1 ppm occurred by the addition of indomethacin. The signals from the C-21 methyl group and C-23 protons were essentially unchanged, as were other protons not listed in Table 3. Hydrophobic bonding is the suggested mechanism of aggregation of bile salt monomers into micelles and the C-18 and C-19 methyl groups are on the hydrophobic side of the steroid nucleus (Small et al., 1969). These observations suggest that the hydrophobic side of the bile salt molecule containing the C-18 and C-19 methyl groups interacts with the hydrophobic portion of the indomethacin molecules. Such interaction may enhance the solubilization process.

In the case of phenylbutazone, no appreciable shifts of the protons were observed, indicating a poor interaction of phenylbutazone with the bile salt micelle.

The results obtained in this work indicate that the mechanism responsible for the solubilization of indomethacin is due to the formation of mixed micelles of the bile salts and the drug. On the other hand, the lower degree of interaction of phenylbutazone with the bile salt micelles is probably associated with the carbon acid, that is, the drug ion. It is suggested that the carbon acids which form the mesomeric anion have this bulky charge group and have difficulty being incorporated into the micelle. However, further studies should be made to obtain a complete understanding of the mechanism.

REFERENCES

Ashworth, R.W. and Heard, D.D., The evaluation of a molecular sieve technique to determine the interaction between a preservative and a surfactant. J. Pharm. Pharmacol., 18 (1966) 98S–102S.

Bates, T.R., Gibaldi, M. and Kanig, J.L., Solubilizing properties of bile salt solutions II: Effect of inorganic electrolyte, lipids, and a mixed bile salt system on solubilization of glutethimide, griseofulvin, and hexestrol. J. Pharm. Sci., 55 (1966) 901-906.

Burns, J.J., Rose, R.K., Chenkin, T., Goldman, A., Schulbert, A. and Brodie, B.B., The physiological disposition of phenylbutazone (Butazolodin) in man and a method for its estimation in biological material. J. Pharmacol. Exp. Ther., 109 (1953) 346–357.

Collett, J.H. and Koo, L., Interaction of substituted benzoic acids with polysorbate 20 micelles. J. Pharm. Sci., 64 (1975) 1253–1255.

Hofmann, A.F., The function of bile salts in fat absorption. Biochem. J., 57 (1963) 57–68.

Hucker, H.B., Zacchei, A.G., Cox, S.V., Brodie, D.A. and Cantwell, N.H.R., Studies on the absorption, distribution and excretion of indomethacin in various species. J. Pharmacol. Exp. Ther., 153 (1966) 237–249.

Klevens, H.B., Solubilization. Chem. Rev., 47 (1950) 1–74.

Lee, I. and Hyne, J.B., Partial molal volumes of tetraalkylammonium chlorides in ethanol–water mixtures. Can. J. Chem., 46 (1968) 2333–2339.

Lovering, E.G. and Black, D.B., Drug permeation through membranes III: Effect of pH and various substances on permeation of phenylbutazone through everted rat intestine and polydimethylsiloxane. J. Pharm. Sci., 63 (1974) 671–676.

Miyazaki, S., Inoue, H., Yamahira, T. and Nadai, T., Interaction of drugs with bile components I: Effect of bile salts on the dissolution behavior of indomethacin and phenylbutazone. Chem. Pharm. Bull., 27 (1979) 2468–2472.

Miyazaki, S., Yamahira, T., Inoue, H. and Nadai, T., Interaction of drugs with bile components II: Effect of bile on the absorption of indomethacin and phenylbutazone in rats. Chem. Pharm. Bull., 28 (1980) 323–326.

Moriyama, M., Saito, M., Awazu, S., Hanao, M. and Nogami, H., Biopharmaceutical studies on indomethacin I: Analysis of concentration time course in rabbit plasma after intravenous administration and relationship between dosage form and absorption after intraduodenal administration. Yakugaku Zasshi, 91 (1971) 1217–1222.

Newton, D.W. and Kluza, R.B., pK_a values of medicinal compounds in pharmacy practice. Drug Intell. Clin. Pharm., 12 (1978) 546–554.

Small, D.M., Penkett, S.A. and Chapman, D., Studies on simple and mixed bile salt micelles by nuclear magnetic resonance spectroscopy. Biochim. Biophys. Acta, 176 (1969) 178–189.

Stella, V.J., Nonclassical phase transfer behavior of phenylbutazone. J. Pharm. Sci., 64 (1975) 706–708.

Stella, V.J. and Pipkin, J.D., Phenylbutazone ionization kinetics. J. Pharm. Sci., 65 (1976) 1161–1165.

Stella, V.J. and Gish, R., Ionization kinetics of the carbon acid phenindione. J. Pharm. Sci., 68 (1979) 1042–1047.

Thakkar, A.L., Solubilization of some steroid hormones in aqueous solutions of bile salts. J. Pharm. Sci., 59 (1970) 1499–1501.