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Micellar Solubilization of Cholesterol, Cholesteryl Myristate and Gallstones by Synthetic Surfactants

Suk Kyu Han and Nam Hong Kim

College of Pharmacy, Pusan National University, Busan 607

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Abstract—Solubilization of cholesterol, its fatty acid esters and gallstones were undertaken employing nonionic, anionic and cationic surfactants. Cholesterol was effectively solubilized by all of these surfactants. Cationic surfactants were most effective. However, cholesteryl myristate was not solubilized at all. In the dissolution test of gallstones in surfactant solutions, cationic surfactant solutions were exceptionally effective in dissolving gallstones. These results suggest the possible existence of interaction between alkylammonium radical and cholesterol on micellar surface.

Cholesterol and its fatty acid esters are insoluble but are solubilized in bile by mixed micelles of bile salts and phospholipids such as lecithin[1]. The limits of cholesterol solubility in solutions of bile salts and lecithin have been studied extensively[2~8]. Bile salts are the most important physiological surfactants. They are the mixture of sodium salts of dihydroxy- and trihydroxycholanic acids, which are usually conjugated with glycine or taurine[1]. The concentration of bile salts in human bile is not sufficient to solubilized cholesterol normally present in the bile. However, the bile contains other components besides cholanic acid derivatives, such as fatty acid salts and phospholipids that greatly increase the solubilization power. When the amount of cholesterol in a patient's bile exceeds that which can be dissolved by the available bile salts and lecithin, the bile is termed lithogenic, and cholesterol gallstone seeds are precipitated and start to grow. Gallstones are classified as cholesterol gallstones and bilirubin gallstones, and cholesterol gallstones are reported as 90% pure cholesterol[9]. Moreover, the high concentration of cholesterol and its fatty acid esters in blood is largely responsible for development of atherosclerosis. The most important alteration in the intima of arteries observed in the development of atherosclerosis is the accumulation of lipids, of which cholesterol and its esters are important components[10,11]. The main problem with cholesterol in human body is their extremely low solubility in aqueous system, The cholesterol has been known to be less soluble than 0.25 mg per 100 ml of water. Its esters should be far less soluble than this value. However, normal human blood contains approximately 200 mg total

cholesterol per 100 ml of blood, of which about 55 mg is free cholesterol and 145 mg is esterified cholesterols[12]. Phospholipid micelles are a vehicle for solubilizing cholesterol in blood[13]. An understanding of the increased solubility of cholesterol in phospholipid micelles might explain how phospholipids or phosphoproteins containing phospholipids serve as carriers of water-insoluble substances in the body. The relationship between cholesterol and atherosclerosis has been discussed in detail by Van Belle[14]. Keeser[15] has suggested that as blood con- a cause of atherosclerosis the solubility of cholesterol in blood is more important than the centration of cholesterol. Studies of compounds which change the solubility behavior of cholesterol are essential for understanding and possibly overcoming these disorders.

The ability of surfactants solutions to dissolve cholesterol has been known for a long time, and recently reviewed by Sjoeblem[14]. So far, the solubilization of cholesterol in physiological surfactants has been investigated extensively, but work with synthetic surfactants are relatively rare. Sodium caprylate, sodium dodecyl sulfate, and some polyoxyethylated nonionic surfactants are reported to solubilize cholesterol[17,18]. Gemant developed a method for evaluating the ability of compounds to solubilize cholesterol by paper chromatography[19,20]. This method roughly simulates the conditions in the body. As for the study of dissolution of gallstones *in vitro*, the artificial synthetic biles and quaternary nicotinic acid derivatives are reported to dissolve gallstones[21,22]. The purpose of this research is to undertake more systematic investigation of solubilization of cholesterol and gallstones by synthetic surfactants, employing nonionic, cationic and anionic surfactants and to compare their solubilizing capacities.

EXPERIMENTAL

Material—Sodium dodecyl sulfate, technical grade of Matheson Colemen and Bell, was twice recrystallized from a 1 : 1 mixture of ethyl alcohol and isopropyl alcohol. Another anionic surfactant employed was sodium *n*-dodecylbenzene sulfonate, supplied by Tokyo Kasei Co., Japan. Tween 80 was supplied by Hayashi Pure Chemical Industries Ltd, Japan and other two nonionic surfactants were Triton X-100 of Rohme and Haas Co., a branched octylphenol adduct containing an average 9, 5 ethylene oxide units and Brij 35 of Wako Pure Chemical Co., which was dodecyl alcohol adduct of average 10 ethylene oxide units. The cationic surfactants employed were Zephiramine chloride which is tetradecyldimethylbenzylammonium chloride and *n*-alkylamine hydrochlorides, supplied by Tokyo Kasei Co., Japan. They were used without further purification. Cholesterol and cholesteryl myristate were supplied by Eastman Kodak Co. All other reagents used were reagent grades.

Method of solubilization- One hundred mg of cholesterol or its esters and 50 ml of the surfactants solution of the desired strength were put into 100 ml Erlenmyer flask. The flask was kept in a constant temperature bath at 37° with occasional shaking for 10 days. The period was long enough to reach the equilibrium state. The content of the bottle was filtered through a Milipore filter with pore size 0.22 micron. The filtrate was used for assay of solubilized cholesterol.

Dissolution of cholesterol gallstones—Cholesterol gallstones obtained from gallbladder and weighing approximately 500 mg each were soaken in water. Each day the gallstone was removed from the water, blotted dry, and weighed in a small covered dish. The procedure was repeated until the weight varied no more than 0.5 mg. Stones prepared in this manner were dried to weigh and added to 50 ml of test solution formed from 1.0% of test compound. The flasks were stoppered and incubated for 10 days at 37°. The stones left undissolved after incubated period were washed with water and dried. The change in weight was recorded.

Assay of cholesterol—The method of Zlatkis *et al.*[23] was used for quantitative determination of solubilized cholesterol and its esters.

RESULTS AND DISCUSSION

The limits of solubility of cholesterol were significantly increased by the presence of nonionic, anionic and cationic surfactants as shown in Fig. 1-3. Among the three different types

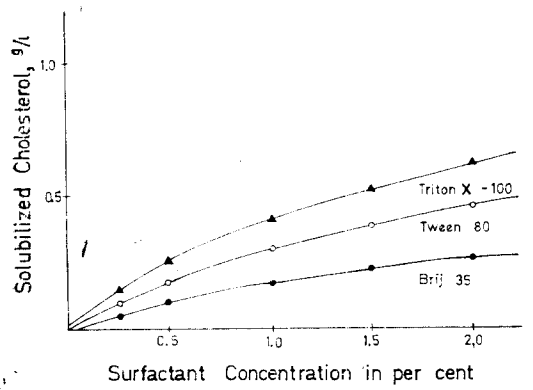

Fig. 1—Solubilities of cholesterol in aqueous solution of nonionic surfactants at 37°.

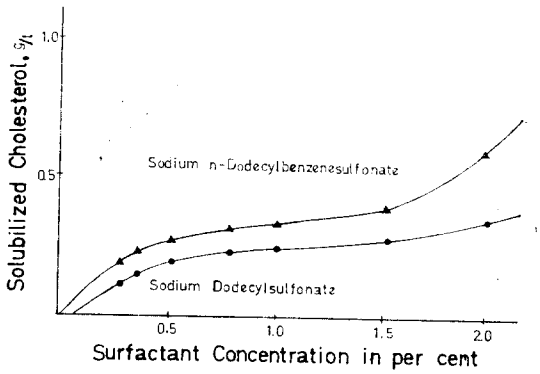

Fig. 2—Solubilities of cholesterol in aqueous solutions of anionic surfactants at 37°.

of surfactants, cationic surfactants showed the most pronounced increases in solubility of cholesterol. Anionic and nonionic surfactants were less effective than their cationic counterparts. However, big differences between the solubilization by these two types of surfactants were not observed. Ethylamine, *n*-propylamine, *n*-butylamine, *n*-hexylamine and *n*-octylamine hydrochlorides did not show any solubilization of cholesterol, even though *n*-hexylamine and *n*-octylamine hydrochlorides are supposed to be surface active. *n*-Decylamine and *n*-dodecylamine

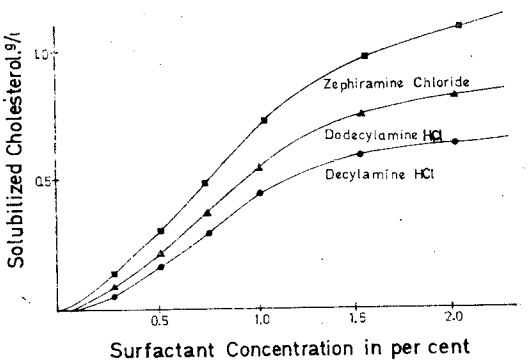

Fig. 3— Solubilities of cholesterol in aqueous solution of cationic surfactants at 37°.

hydrochlorides showed good solubilization of cholesterol. Zephiramine chloride was found to have the most effective solubilizing activity than any other surfactants examined. Tetramethylammonium chloride and tetra-*n*-propylammonium chloride did not show any solubilization of cholesterol. From above experimental results, it is clear that as the longer alkyl chain of the hydrophobic moiety of the surfactant is, the more effective solubilization of cholesterol was observed. However, there is a limit to the length of the alkyl chain, since compounds with long alkyl chain have limited solubility in water. For example, haxadecylamine hydrochloride has its kraft point, 42°, which is above the experimental temperature. The requirement of large hydrophobic moiety of the surfactant structure suggested that large micellar sizes are required for solubilization of cholesterol. This phenomena were also observed with solubilization of cholesterol with nonionic and anionic surfactants. Sodium *n*-dodecylbenzene sulfonate was more effective than sodiuml dodecyl sulfate, and Triton X-100, Tween 80 and Brij 35 are the decreasing order of nonionics to solubilize cholesterol. On the contrary to the moderate solubilization of cholesterol by the synthetic surfactants, cholesterol myristate was not appreciably solubilized by any surfactants examined in this research. It is very interesting to note that when cholesterol is esterified by saturated fatty acid, its solubility in surfactant solutions are dramatically decreased. This decreased solubility of cholesterol esters should be ascribed to the increased molecular size and lipophilicity. The result of dissolution rates of gallstones in surfactant solutions are tabulated in Table I. Clearly, it was shown that the cationic surfactants, dodecylamine hydrochloride and zephiramine chloride were far more effective in dissolving gall than anionic and nonionic surfactants. It is interesting to note that *n*-dodecylamine hydrochloride is more effective than zephiramine chloride. That result is the reverse of the result of cholesterol solubilization experiments. The anionic and nonionic surfactants were only slightly effective in dissolving gallstones. Sodium deoxycholate, one of

Table I—Dissolution of gallstones by 1% solution of surfactants

Surfactant	Weight before incubation, mg	Weight after incubation, mg	Dissolution %
Brij 35	486	462	5.0
Triton X-100	445	429	3.6
Tween 80	476	467	1.9
Sodium dodecyl sulfate	557	560	2.9
Dodecylamine hydrochloride	544	257	52.7
Zephiramine chloride	602	495	17.7
Sodium deoxycholate	573	570	0.4
Tetra-*n*-propylammonium bromide	531	531	—
Tetramethylammonium chloride	496	496	—
Sodium deoxycholate +0.5% Tetra-*n*-propylammonium bromide	533	330	38.1
Sodium deoxycholate +0.5% Tetramethylammonium chloride	611	306	50.0

the physiological surfactants, was included in the dissolution test of gallstones for comparison with other surfactants. The dissolution of gallstones with this compound was disappointing. Tetramethylammonium chloride and tera-*n*-propylammonium bromide did not show any appreciable dissolution of gallstones. However, when these quaternary ammonium compounds added to the solution of sodium deoxycholate, the dissolution is greatly enhanced. This effect might be ascribed to the formation of comicelle of tetraalkylammonium compounds with sodium deoxycholate. Strictly speaking, the methodology employed in the gallstone dissolution is not a complete dissolution. It is rather a disintegration than a dissolution. Great part of the materials in gallstones is dispersed in medium solution. However, It is very convenient and useful method to evaluate the dissolution of gallstone-solubilizing agents *in vitro*. It is worthwhile to note that the cationic surfactants, alkylamine hydrochlorides and quaternary ammonium compounds were especially effective in solubilizing cholesterol and dissolving gallstones. Quaternary nicotinic acid derivatives of which cholesterol-solubilizing activities were reported[22] are quaternary ammonium compounds and cholestyramine[24] which binds neutral steroids is a polymer of quaternary ammonium structure. The results strongly suggest the possible existence of interaction between alkyl ammonium radical on and cholesterol micellar surface. Unfortunately, the mechanism of the interaction is not clear, and further works to elucidate it are required.

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