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In-vitro enhancement of cholesterol dissolution by commonly used drugs

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A rotating disc apparatus was used to study the dissolution of cholesterol in sodium cholate solutions and ox bile. Drugs with structures that render them capable of lowering interfacial resistance were tested and shown to increase cholesterol dissolution rates in both systems. In sodium cholate solutions, loperamide (3×10^{-4} M) increased the rate of dissolution by over six times, and a similar effect was observed with amitriptyline (3×10^{-3} M), diphenhydramine (3×10^{-3} M), dicyclomine (3×10^{-3} M) and propantheline (3×10^{-3} M). These drugs are as effective as benzalkonium chloride at these concentrations. Amitriptyline, propantheline, dicyclomine and diphenhydramine also increased cholesterol dissolution rates into ox bile. If these drugs are excreted into human bile in sufficient quantities and in an active form they may be able to enhance the speed of cholesterol gallstone dissolution therapy.

Medical dissolution therapy for cholesterol gallstones using chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) has produced disappointing results. In the National Co-operative Gallstone Study (Schoenfield & Lachin 1981) which included 916 patients, a complete gallstone dissolution rate of only 13·5% was achieved after 2 years treatment with CDCA. Although dosages of the drug used in this trial were probably less than optimal (Way 1983), drug therapy has had little clinical impact (Bouchier 1984) and it clearly needs to be more effective if it is to replace surgery.

Given bile which is desaturated, the simplest model predicts that a small (less than 25 mm diameter) cholesterol gallstone should dissolve in a matter of days (Higuchi et al 1973a). This does not occur either in-vivo or in-vitro, probably because of an interfacial resistance to dissolution (Higuchi et al 1973b; Igimi & Carey 1981). The physical basis of this is seen as a charge repulsion between the negatively charged cholesterol crystals of the gallstone and the negatively charged bile micelles (Prakongpan et al 1976).

In-vitro cholesterol dissolution rates can be greatly increased by the addition of cationic amphiphilic substances such as benzalkonium chloride (Higuchi et al 1973c) which probably reduce the charge repulsion (Patel & Higuchi 1980; Patel et al 1980; Feld et al 1982) and lower interfacial resistance.

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In this study we have screened some commonly used drugs for their ability to enhance in-vitro dissolution of cholesterol discs.

MATERIALS AND METHODS

Materials

[^{14}C]Cholesterol, labelled at the C_4 position (Amersham International), was obtained as a toluene solution and mixed with unlabelled cholesterol (Sigma-purity equivalent to United States Pharmacopeia 1980) as described below. The following drugs were gifts: pirenzepine dihydrochloride (Boots), loperamide hydrochloride (Janssen) and dicyclomine hydrochloride (Merrell-Dow). Sodium cholate and all other drugs were obtained from Sigma, including benzalkonium chloride which was predominantly the $C_{12}H_{25}$ homologue. Sodium phosphate buffer, 0·1 M, pH 7·4 (Gomori 1969) was made up daily from stock solutions. Picofluor 15 (Packard) was used as a scintillation fluid. Ox bile was supplied by a local abattoir and stored frozen in 500 ml aliquots.

Methods

Cholesterol monohydrate preparation. A stream of nitrogen was used to evaporate the toluene from a vial containing radiolabelled cholesterol, which was then redissolved in 10 ml of ethanol at 60 °C. 7·5 g of unlabelled cholesterol was dissolved in 500 ml of 90% ethanol at 60 °C, filtered and allowed to cool to room temperature (20 °C). Crystals were collected by filtration 48 h later and air dried at room

temperature for 24 h. This was repeated three times. On the fourth cycle, the radioactive cholesterol solution was added at the first filtration stage. After collection and drying of crystals (as above) they were ground using an agate mortar and pestle, sieved to give a uniform particle size, and stored in a water filled humidifier in the dark at 4 °C. Differential scanning calorimetry and thermogravimetric analysis of an unlabelled sample showed results consistent with the monohydrate form of cholesterol. Final specific activities of cholesterol preparations used were approximately 100 μCi g^{-1}.

Apparatus. Discs were made by filling the central recess (1·3 cm in diameter) of a stainless steel die with about 0·15 g of radioactive cholesterol monohydrate powder. The die was placed in the bottom of a stainless steel cylinder, a plunger inserted down the copper lined shaft and compressed using a hydraulic laboratory press (RICC Ltd) at 10 tonnes for 5 min.

The die, containing the compressed cholesterol disc, was then screwed onto the end of a double bearing drive shaft in a custom-built dissolution apparatus. This employed a single speed a.c. electric motor and drive belt system to rotate four such discs at speeds variable between 50 and 500 rev min^{-1}. Dissolution medium was contained in four 100 ml glass vessels immersed in a waterbath at 37 °C.

Radioactivity was measured by liquid scintillation counting, using an LKB Rackbeta machine with an automatic quench correction facility based on the external standards ratio method.

Dissolution studies. After preliminary studies on the effect of varying rotation speeds on dissolution rates of both benzoic acid discs into 0·01 M HCl and cholesterol discs into sodium cholate solutions, a single rotation speed of 115·2 rev min^{-1} was chosen for all the drug screening experiments. Each experiment was run for 3 h with at least five 1 ml samples being pipetted from the dissolution vessel. Each disc was used for approximately 15 experiments but was discarded before 10% (i.e. 15 mg) of original cholesterol had been dissolved. Each batch of experiments included at least four cholate only controls, interspersed between drug runs. Discs were stored in distilled water at room temperature when not in use.

Calculation of 'C values'. To evaluate the effect of drugs on cholesterol dissolution rate, a number representing the increase in dissolution rate with the drug over the dissolution rate in sodium cholate only (C value) was calculated. In most instances the C value is the mean slope obtained with the drug over the mean slope obtained in control experiments with the same disc. In a few experiments, drugs were soluble enough to perform two stage experiments, where a concentrated solution of drug was added to the dissolution vessel and rates before and after addition were directly comparable. Unpaired *t*-tests were performed between the slopes obtained with the drug and all the cholate controls in that batch of experiments.

Solubility determination. Equilibrium cholesterol solubility was determined by adding an excess of finely ground radioactive cholesterol to the test solution (2% sodium cholate in 0·1 M sodium phosphate buffer pH 7·4, with or without drug) and agitating continuously at 37 °C. Every 24 h, samples were filtered through a 0·22 μm filter (Flow Ltd) and counted. Equilibrium solubility was judged to have been reached when counts remained constant over 24 h.

RESULTS

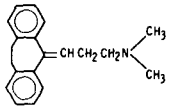
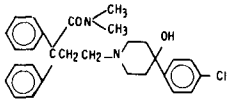
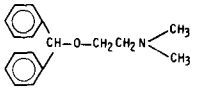
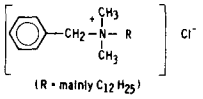
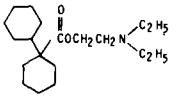
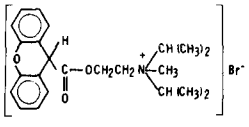
Table 1 shows that, in the cholate system (2% sodium cholate in 0·1 M sodium phosphate buffer, pH 7·4, in the rotating disc apparatus at 115·2 rev min^{-1} and 37 °C), amitriptyline (3×10^{-3} M), loperamide (3×10^{-4} M), diphenhydramine (3×10^{-3} M), dicyclomine (3×10^{-3} M) and propantheline (3×10^{-3} M) are all as effective as benzalkonium chloride (3×10^{-3} M), increasing cholesterol dissolution rates by roughly 6-fold. The effect of amitriptyline in the cholate system was dose-dependent, with a doubling of dissolution rate being achieved at approximately $0·5 \times 10^{-3}$ M amitriptyline.

Amitriptyline (3×10^{-3} M), propantheline (3×10^{-3} M), dicyclomine (3×10^{-3} M) and diphenhydramine (3×10^{-3} M) also significantly increased dissolution rates in ox bile. C values ± s.e.m. were 1·922 ± 0·14 ($P < 0·001$), 1·670 ± 0·12 ($P < 0·01$), 1·453 ± 0·09 ($P < 0·01$), and 1·196 ± 0·014 ($P < 0·05$), respectively; n = 4 in all cases. Although the increase in dissolution rates (vs controls) is lower in this system, it should be noted that absolute control rates are much higher than in the cholate system. Ox bile contains phospholipids and conjugated bile acids but unlike human bile is very desaturated with respect to cholesterol with a saturation index of approx. 0·3.

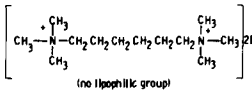
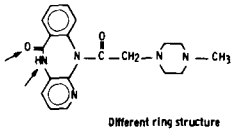
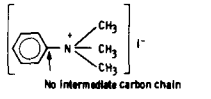
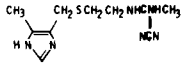
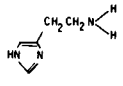
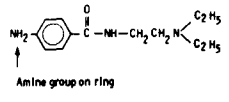
None of the drugs increased equilibrium cholesterol solubility in sodium cholate.

Table 1. Structural formulae of the most (A) and least (B) active drugs compared with benzalkonium chloride. C values greater than 1 indicate enhanced dissolution. Dissolution rate measurements into 2% sodium cholate in 0·1 M sodium phosphate buffer pH 7·4 in rotating disc apparatus at 115·2 rev min^{-1} and 37 °C. All drugs were tested, at 3×10^{-3} M, except loperamide, which was tested at 3×10^{-4} M.

A.

Drug/compound	Structure	C value ± s.e.m.
Amitriptyline		6·75 ± 0·69 ($P < 0·001$)
Loperamide		6·50 ± 0·14 at 3×10^{-4} M ($P < 0·001$)
Diphenhydramine		6·41 ± 0·43 ($P < 0·001$)
Benzalkonium chloride		6·33 ± 0·35 ($P < 0·001$)
Dicyclomine		5·91 ± 0·44 ($P < 0·001$)
Propantheline bromide		5·01 ± 0·38 ($P < 0·001$)

B.

Drug/compound	Structure	C value ± s.e.m.
Hexamethonium bromide		1·37 ± 0·08 ($P < 0·001$)
Pirenzepine		1·35 ± 0·07 ($P < 0·001$)
Trimethyl phenyl ammonium iodide		1·20 ± 0·14 ($P > 0·05$)
Cimetidine		1·11 ± 0·11 ($P < 0·50$)
Histamine		0·99 ± 0·02 ($P > 0·95$)
Procainamide		0·72 ± 0·11 ($P > 0·60$)

DISCUSSION

None of the drugs tested increase equilibrium cholesterol solubility in the cholate system. They do, however, increase the rate of incorporation of cholesterol molecules into bile salt micelles. One possibility is that they achieve this by acting at the interface in the same way as benzalkonium chloride, that is, by reducing micellar charge and allowing more efficient contact between cholesterol crystals and bile salt micelles. Support for this hypothesis derives from structural similarities between the most active compounds (Table 1A). All possess a lipophilic ring structure separated from a tertiary or quaternary ammonium group by a short intermediate chain. In contrast, the relatively ineffective drugs shown in Table 1B have a variety of different structures. The amphiphilic structural features common to the active drugs and benzalkonium chloride may allow the molecule to reduce surface micellar charge and increase the probability of solid phase cholesterol becoming incorporated into the micelle.

Other possible explanations for the effects of these drugs include; (a) a direct relationship to the surface activity of the drug (amitriptyline, diphenhydramine, dicyclomine and propantheline are all sufficiently surface active to form micelles at higher concentrations—Attwood & Florence 1983) or (b) a mechanism involving adsorption of the drug to the crystal/solution interface.

It has been independently proposed that the structural features listed above are also necessary for a cationic compound to be actively excreted into bile

(Schanker 1965). This would greatly increase the therapeutic potential of these drugs since they may achieve high concentrations in bile. Although data on biliary excretion of drugs is relatively limited, one study recovered 47·2% of a 0·8 mg kg^{-1} i.v. dose of [^{14}C]amitriptyline from a 6 h bile collection in rats (Ryrfeldt 1973). Miyazaki et al (1979) reported that 42% of an oral 1 mg kg^{-1} dose of [^{14}C]loperamide could be recovered from rat bile over 48 h. In both studies the drugs were extensively metabolized before excretion into bile. Whether metabolites are as effective as the parent compounds and whether sufficient concentrations of active drug can be achieved in bile are being assessed using patients with T-tubes in-situ.

In view of the long treatment periods necessary for gallstone dissolution using bile acid therapy, adjuvant drugs having even a relatively small effect may be worthwhile. The striking effects on dissolution rates shown by the drugs reported here makes them potential candidates for this role.

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