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Original scientific paper

Influence of bile acids on the adsorption of lidocaine and verapamil in an *in vitro* experiment

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Abstract: The work is concerned with the influence of the structure of bile acids (cholic, deoxycholic, chenodeoxycholic, and their keto derivatives) on the *in vitro* adsorption of lidocaine and verapamil from an aqueous phase to rat's intestine membrane. Transport of lidocaine from an aqueous medium to the rat's intestine membrane was significantly increased ($p < 0.05$) only by 7-ketodeoxycholic acid, whereas an analogous increase in verapamil transport was produced only by cholic acid. It appeared that, of all the tested bile acids, these two acids form the most stable complexes (by hydrogen bonds) with the respective drug.

Keywords: lidocaine; verapamil; bile acids.

INTRODUCTION

Bile acids belong to a special group of surface active molecules, so-called amphiphilic molecules. They are planar polar molecules in which the hydrophobic and hydrophilic surfaces are separated.[1–4] Thanks to this property, bile acids show several pharmacological features, such as relaxation of smooth muscles of the endothelium,[5] lowering of the glucose blood level[6] and the like. In addition, bile acids exhibit a promotive effect in the action of some drugs (insulin, quinidine, morphine, *etc*.).[6,7]

In previous investigations,[8] 7-ketodeoxycholic and 12-ketochenodeoxycholic acids, as well as cholic acid, showed promotive effects on the anesthetic action of lidocaine in infiltrational tail anesthesia of rats. ^{1}H-NMR measurements[9] showed that lidocaine in deuterated chloroform forms hydrogen-bond complexes with some bile acids (keto derivatives of cholic, deoxycholic and chenodeoxycholic acids). Kinetic studies revealed a positive correlation between the forma-

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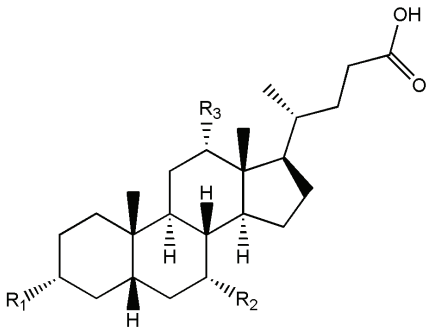
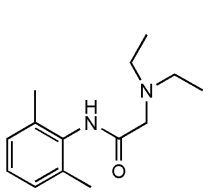
tion constant of the bile acid–lidocaine complexes and the promotive action of the bile acids on the transfer of lidocaine from an aqueous phase to chloroform (model of cell membrane) if the bile acids were present in the organic phase (pretreatment model).[9] In the mechanism of the promotive action of bile acids on the transport of drugs through the cell membrane, their interaction with membrane phospholipids plays an important role.[10,11]

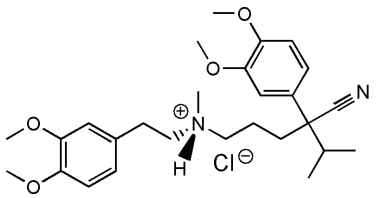
In view of the above facts, the aim of this work was to investigate the effect of bile acids (cholic, deoxycholic and chenodeoxycholic, and their keto derivatives) (Fig. 1, Table I) on the transfer of lidocaine from an aqueous phase to the membrane of rat intestine (*in vitro*) pretreated with the corresponding bile acid solution. Namely, the objective was to establish whether bile acids exhibit the same structure–activity relationship as in the transfer of lidocaine from an aqueous phase to chloroform, *i.e.*, how well can chloroform model a biological membrane. In addition, the aim was to examine the action of bile acids on the transport of verapamil to the rat intestine membrane. Namely, in contrast to the lidocaine molecule, which has one proton-donor group (amide group) and one proton-acceptor group (tertiary amine group), the verapamil molecule has only proton-acceptor groups (methoxy, nitrile and tertiary amine groups). The application of verapamil may indicate the importance of hydrogen bonds in the formation of bile acid complexes, and thus in the promotive action of bile acids.



structure of tested bile acids



lidocaine



verapamil hydrochloride

Fig. 1. Structures of tested bile acids and lidocaine and verapamil hydrochloride.

TABLE I. The tested bile acids

Position of hydroxyls and keto groups (Fig. 1)	Bile acid
$R_1 = R_2 = R_3 = OH$	3α,7α,12α-trihydroxy-5β-cholanoic acid (cholic acid)
$R_1 = R_3 = OH$; $R_2 = H$	3α,12α-dihydroxy-5β-cholanoic acid (deoxycholic acid)
$R_1 = R_2 = OH$; $R_3 = H$	3α,7α-dihydroxy-5β-cholanoic acid (chenodeoxycholic acid)
$R_1 = R_3 = OH$; $R_2 = O$	3α,12α-dihydroxy-7-keto-5β cholanoic acid (7-ketodeoxycholic acid)
$R_1 = R_2 = OH$; $R_3 = O$	3α,7α-dihydoxy-12-keto-5β-cholanoic acid (12-ketochenodeoxycholic acid)
$R_3 = OH$; $R_1 = R_2 = O$	12α-hydroxy-3,7-diketo-5β-cholanoic acid
$R_1 = OH$; $R_2 = R_3 = O$	3α-hydroxy-7,12-diketo-5β-cholanoic acid (7,12-diketolitocholic acid)
$R_1 = R_2 = R_3 = O$	3,7,12-triketo-5β-cholanoic acid
$R_1 = OH$; $R_3 = O$; $R_2 = H$	3α,-hydroxy-12-keto-5β-cholanoic acid (12-ketodeoxylitocholic acid)
$R_1 = R_3 = O$; $R_2 = H$	3,12-diketo-5β-cholanoic acid
$R_1 = OH$; $R_2 = O$; $R_3 = H$	3α-hydroxy-7-keto-5β-cholanoic acid (7-ketolitocholic acid)
$R_1 = R_2 = O$; $R_3 = H$	3,7-diketo-5β-cholanoic acid

EXPERIMENTAL

Material

Cholic, deoxycholic and chenodeoxycholic acids (Sigma, New Zealand, 98 %) were used as the starting compounds for the synthesis of their keto derivatives. The syntheses of keto derivatives of all the three bile acids, and their transformation to sodium salts were performed as described in previous articles.[1,3,4]

Determination of the influence of sodium salts of bile acids on the in vitro *adsorption of lidocaine and verapamil in the small intestine*

The small intestine (5 cm) was taken from an ether-anesthetized Wister rat and prepared according to Al-Salami *et al*.[12] Then, the intestine was cut into small pieces and placed in Tyrode solution (pH 7.4 $H_2PO_4^-/HPO_4^{2-}$) containing the dissolved sodium salt of a bile acid, so that the bile acid concentration was identical to the critical micellar concentration[3,4] (the control was Tyrode solution without bile acid). The intestine was soaked in Tyrode solution with bile acid for 24 h under a constant flow of oxygen and stirring (50 rpm) – this phase of the experiment served to pretreat the intestine with bile acid. Subsequently, the intestine was removed from the bile acid solution and washed with Tyrode solution (25 ml). Then, the intestine pieces were transferred to Tyrode solution containing either lidocaine hydrochloride (10 ml, 40 mM) or verapamil hydrochloride (10 ml, 40 mM) and left in contact with the given drug (magnetic stirrer, 50 rpm) for 5 min. After that, a sample of the solution (750 µl) was taken to determine spectrophotometrically the concentration of the tested drug (the blank was Tyrode solution, Agilent 8453). Lidocaine was measured at a wavelength of 263 nm and verapamil at 280 nm, using the corresponding calibration graph.

Data treatment

The results were treated (paired-samples *t*-test) using the SPSS10.0 package for Windows.

RESULTS AND DISCUSSION

In pharmacodynamic investigations of the promotive action of bile acids on drug transport, it is customary to treat experimental animals first with the bile acid and then with the drug.[6,7,13] Hence, the test of the influence of bile acids on the resorption of lidocaine and verapamil in the intestine membrane was preceded by treatment with solutions of the sodium salts of bile acids and the decrease in the concentration of the tested drug was measured after 5 min. Namely, the effect of the tested bile acid on the drug concentration in the solution after intestine soaking for 10 min did not differ from that for the blank, as the greatest change in the drug concentration occurred in the first 5 min.

The concentrations of the tested drugs measured 5 min after the application in the *in vitro* experiment are given in Table II, from which it can be seen that a higher degree of resorption after 5 min was registered for verapamil in the control experiment than for lidocaine, which could be expected based on the higher lipophilicity of verapamil (lidocaine, log P = 2.26; verapamil, log P = 3.45).[14,15] The decrease in the lidocaine concentration was significant in the presence of 7-ketodeoxycholic acid. Namely, lidocaine and 7-ketodeoxycholic acid form a hydrogen-bonded complex with a larger formation constant (K = 14.22 dm^3 mol^{-1}) compared to the other tested bile acids, the complex formation constants of which are in the range 1–9 dm^3 mol^{-1}.[9] A significant decrease in the verapamil concentration was evidenced when the intestine membrane was pretreated with sodium cholate. Namely, in contrast to lidocaine, the verapamil molecule contains only proton-acceptor groups (methoxy, nitrile and tertiary amine groups), which means that verapamil forms hydrogen-bonded complexes with those bile acids which

TABLE II. Concentrations of tested drugs measured 5 min after application in the *in vitro* experiment (starting concentrations: *c* (lidocaine) = 40mM; *c* (verapamil) = 40 mM)

Bile acid	*c*(lidocaine)[a] / mM	*c*(verapamil)[a] / mM
Control	35.12±4.74	31.78±3.31
Deoxycholic	31.70±4.09	28.38±3.52
Chenodeoxycholic	32.02±3.50	28.91±4.12
Cholic	30.17±3.98	25.80±3.19[b]
12-Ketolitocholic	34.09±2.61	32.00±2.50
3,12-Diketo-5*β*-cholanoic	36.00±2.50	34.36±4.41
7-Ketolitocholic	30.84±5.47	30.51±2.98
3,7-Diketo-5*β*-cholanoic	33.98±7.04	32.37±4.60
12-Ketochenodeoxycholic	29.42±6.80	31.36±2.52
7-Ketodeoxycholic	27.76±6.30[b]	30.90±2.76
7,12-Diketolitocholic	33.00±2.98	32.84±2.71
12*α*-Hydroxy-3,7-diketo-5*β*-cholanoic	34.73±4.00	31.93±2.16
3,7,12-Triketo-5*β*-cholanoic	32.90±3.75	33.25±3.04

[a]Each value represents mean ± *sd* (n = 7); [b]values which are significantly different (p < 0.05) from the corresponding control

have hydroxyl groups. It is evident from Table II that the greatest effect on the decrease of verapamil concentration in the aqueous phase was induced by cholic acid, followed by deoxycolic acid and then by chenodeoxycholic acid. Namely, cholic acid molecule has three hydroxyl groups on the α side of the steroid skeleton, which form a binding region for the proton-acceptor groups of the verapamil molecule. As can be seen from Fig. 2, the 2-cyano-2-(3,4-dimethoxyphenyl)--2-isopropyl-ethyl segment (region) of the verapamil molecule fits the triangle formed by the three OH groups of cholic acid and hence this bile acid forms the most stable complex with verapamil. The molecules of deoxycholic and chenodeoxycholic acids have smaller numbers of hydroxyl groups; hence, their interaction with verapamil is less pronounced. The bile acids whose molecules contain only keto groups, such as 3,7,12-triketo-5β-cholanoic, 3,12-diketo-5β-cholanoic and 3,7-diketo-5β-cholanoic acids, do not affect verapamil transport since they, like verapamil, have only proton-acceptor groups.

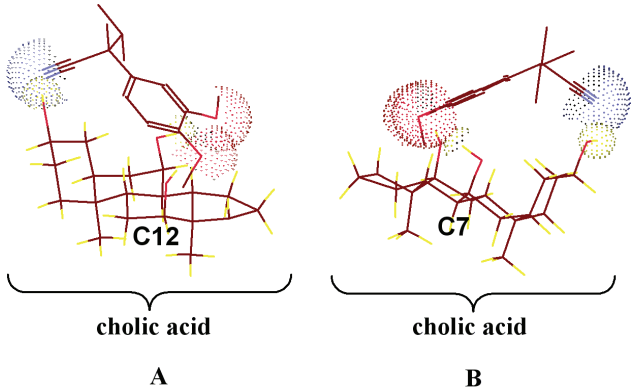


Fig. 2. Hydrogen-bonded complex formed between verapamil and cholic acid; the proton-acceptor groups: methoxy, nitrile and tertiary amine groups coincide sterically with the triangle formed by the three hydroxyl groups of cholic acid (A-view from C12 of cholic acid; B-view from C7 of cholic acid).

In order to form a complex with lidocaine (1:1 stoichiometry), a bile acid should have in its molecular structure either two OH groups or one keto (or carbonyl group from COOH) and one OH group at an appropriate mutual distance (from 4.4 to 5.5 Å).[9] Of the tested bile acids, 7-ketodeoxycholic acid provoked the strongest decrease of the lidocaine concentration. Namely, the molecule of this bile acid may bind with lidocaine in three different ways involving 1:1 stoichiometry (Fig. 3). At the same time, because of steric repulsions, 7-ketodeoxycholic acid can bind only one molecule of lidocaine; however, the complex 7-ketodeoxycholic acid–lidocaine (1:1) can be realized *via* three microstates. The same structural analysis shows that the other tested bile acids form their complexes with lidocaine *via* one microstate only. In other words, formation of the

complex 7-ketodeoxycholic acid–lidocaine (1:1) is three times more probable than the formation of such a complex with the other bile acids.

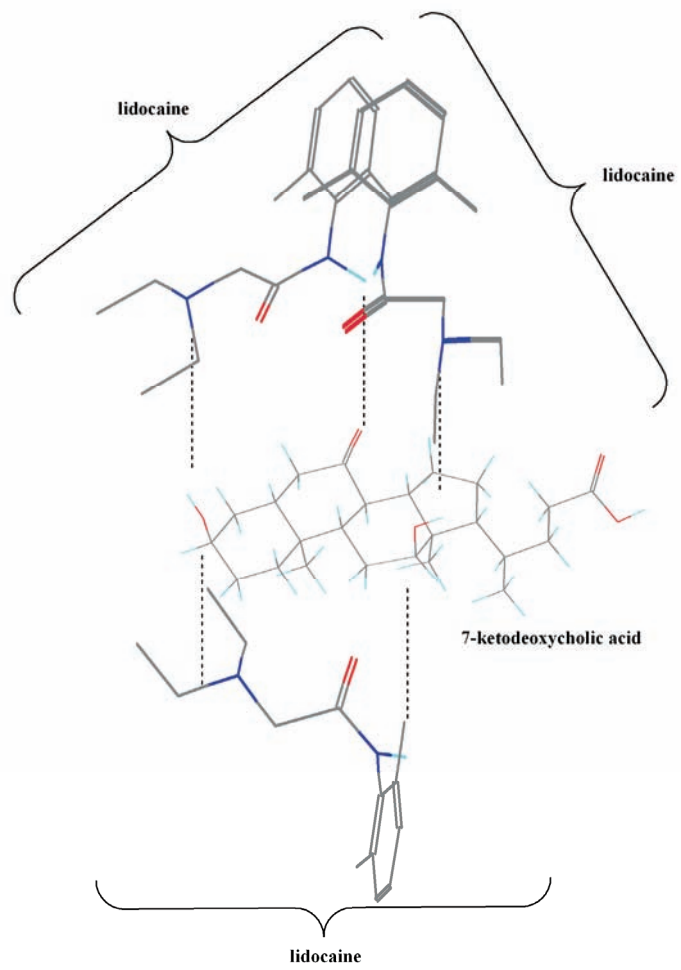


Fig. 3. Possible interactions (hydrogen bonds) between lidocaine and 7-ketodeoxycholic acid.

Deoxycholic and chenodeoxycholic acids, as markedly hydrophobic molecules,[4] induced no significant increase in the resorption of lidocaine (Table II).

Based on this experiment, it can be concluded that the formation of a mixed micelle of cell membrane phospholipids from the small intestine with a hydrophobic bile acid does not influence resorption of the tested drug, that is, no "holes" are formed in the cell membrane. However, in order to have a faster drug resorption, the cell membrane should form a complex with the drug, which then becomes more hydrophobic (Fig. 4). Namely, only the unprotonated lipophilic form

of lidocaine or verapamil is able to penetrate the cell membrane.[14,15] In the cell membrane, the corresponding drug and bile acid can form hydrogen-bonded complexes (such complexes between lidocaine and bile acids in chloroform were proven by NMR measurements[9]) which then shifts the ionization equilibrium (aqueous solution) to the formation of molecular lidocaine or verapamil (Fig. 4).

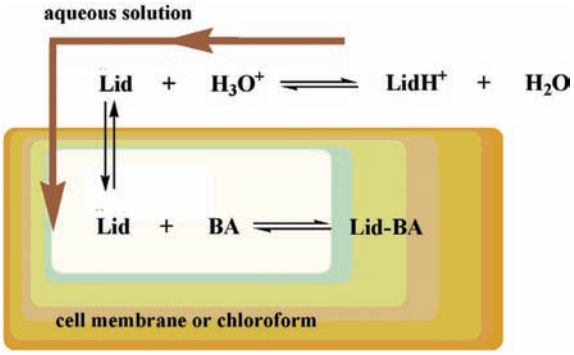


Fig. 4. General mechanism proposed for the promotive action of bile acids (BA) on the increase of resorption of lidocaine and verapamil. Lid–BA = hydrogen-bonded complex.

From Table II, it can also be seen that structural differences in the bile acids influenced lidocaine transfer from the aqueous medium to the membrane, and that this effect was not the same as when this drug was transferred to chloroform.[9] Namely, in the transfer of lidocaine to chloroform, each of the tested bile acids showed a promotive effect with respect to control at a significance of $p < 0.01$. On the other hand, if the transfer lidocaine to the small intestine is considered, then only 7-ketodeoxycholic acid showed a significant promotive action.

CONCLUSIONS

The transport of lidocaine from an aqueous medium to the intestine membrane is significantly increased ($p < 0.05$) by 7-ketodeoxycholic acid, whereas verapamil transport is influenced by cholic acid. Namely, of all the tested bile acids, these two acids form the most stable hydrogen-bonded complexes with the corresponding drug.

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И З В О Д

УТИЦАЈ ЖУЧНИХ КИСЕЛИНА НА АДСОРПЦИЈУ ЛИДОКАИНА И ВЕРАПАМИЛА У *IN VITRO* ЕКСПЕРИМЕНТУ

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Циљ овог рада је да се испита утицај структуре жучних киселина (холна, деоксихолна и хенодеоксихолна киселина као и њихови кето-деривати) на адсорпцију лидокаина и верапа-

мила из водене фазе у зид танког црева пацова *in vitro*. Пре испитивања адсорпције одговарајућих лекова цревна мембрана је третирана раствором жучних киселина чије су концентрације одговарале њиховим критичним мицеларним концентрацијама. Од свих испитиваних жучних киселина једино је 7-кетодеоксихолна имала знатан утицај ($p < 0.05$) на адсорпцију лидокаина из водене фазе у зид танког црева. На адсорпцију верапамила у зид танког црева једино је холна киселина имала знатан утицај ($p < 0.05$). Сматра се да је овакав ефекат наведених жучних киселина на адсорпцију испитиваних лекова последица формирања комплекса са максималним бројем водоничних веза између жучних киселина и верапамила односно лидокаина.

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