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Note

The influence of bile salts and mixed micelles on the pharmacokinetics of quinine in rabbits

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Abstract

The bioavailability of orally administered drugs can be influenced by interactions with food components and by physico-chemical conditions in the upper gastrointestinal tract. Normally, bile salts enhance the transport of lipophilic drugs across mucosal membranes. Bile salts are able to form stable mixed micelles consisting of fatty acids and phospholipids. Conventional micellar systems are known to solubilize lipophilic drugs having a low bioavailability. The influence of bile salts and mixed micelles on the pharmacokinetics of the lipophilic drug quinine was investigated in rabbits. Female rabbits were given intraduadenally quinine (5 mg/kg body weight) without and with incorporation into the micellar or mixed micellar systems. Blood was collected every 30 min for 6 h. In plasma, concentration of quinine was measured using HPLC. The plasma concentration–time profiles of quinine were significantly lower within the first 2 h after administration in presence of both the sodium salt of glycodeoxycholic acid (above the critical micellar concentration) as well as of mixed micellar systems consisting of glycodeoxycholic acid and palmitic acid and/or lecithin. The pharmacokinetic parameters AUC (relative bioavailability) and c_{max} of quinine were significantly decreased by micellar systems in rabbits. These mixed micellar systems lower and not as expected, increase the absorption of quinine in vivo. Therefore, quinine should be orally administered at least 1 h before food intake, particularly before fat intake.

Keywords: Quinine pharmacokinetics; Bile salts; Mixed micelles; Rabbits

1. Introduction

The bioavailability of orally applied drugs can be influenced by interactions with food or food components such as pectins [1–3] or by the physico-chemical conditions in the gastrointestinal tract [4].

The main products of the hepatic biotransformation of cholesterol are the detergent-like bile salts (BS) such as the used sodium salt of the glycodeoxycholic acid (GDCA). In the small intestine, particularly during lipid digestion, BS are capable to form stable mixed micelles consisting of fatty acids such as palmitic acid (PA) and phospholipids such as lecithin (LE). They normally enhance the transport of lipophilic drugs across biological membranes [5]. Conventional micellar systems are known to improve the solubility of extremely lipophilic drugs having a low bioavailability [6].

Quinine (QU) was chosen as a model drug. In different studies, pharmacokinetics and bioavailability of the antimalaria drug QU has been determined in humans, e.g. [7]. Orally administered QU is rapidly absorbed by passive diffusion in the upper parts of the small intestine. Up to date, only limited data is available on the effects of micellar systems present in intestine on the pharmacokinetics of lipophilic drugs such as QU. In some studies, it was found

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that PA can enhance the absorption of quinine in absence of BS [8]. Using micellar electrokinetic affinity chromatography, Schwarz et al. [9] found that particularly lipophilic basic drugs such as QU or propranolol interact strongly with BS or mixed micelles.

In the present study, therefore, interactions between QU and BS or mixed micelles consisting of BS and a fatty acid and/or a phospholipid have been investigated in vivo in female rabbits.

2. Material and methods

2.1. Materials

Quinine hydrochoride: Caesar & Loretz (Hilden, Germany); Pentobarbital inj.: SPOFA United Pharmaceutical Works (Prague, Czech Republic); sodium salt of glycodeoxycholic acid, lecithin from egg and palmitic acid: Sigma (Deisenhofen, Germany); xylazin (Rompun®): Bayer (Leverkusen, Germany); ketamine (Velonacron®): Berlin-Chemie (Berlin, Germany); heparin (Heparin-Natrium-25000 ratiopharm®): Ratiopharm GmbH (Ulm, Germany); isotonic saline solution: Braun (Melsungen, Germany).

All solvents (HPLC grade) were from Merck (Darmstadt, Germany). Water was double-distilled.

2.2. Animal experiment

Four female New Zealand White rabbits, body weight approximately 3.5 kg, were supplied by Charles River Deutschland (Sulzfeld, Germany) and maintained at 20 ± 1 °C in air-conditioned rooms on a 12-h cycle of light and dark. The animals were housed individually in stainless steel metabolic cages ($0.5 \times 0.5 \times 1$ m) and received the complete diet for rabbits-maintenance 'Ssniff® K-H' from Spezialdiäten GmbH (Soest, Germany) and tap water at libitum.

At the beginning, the rabbits were anesthetized by intravenous administration of pentobarbital sodium dissolved in isotonic saline (50 mg/kg body weight). The narcosis was maintained by a continuous infusion of diluted solution of pentobarbital sodium (18 mg/h/animal). The rabbits were placed on a surgical board equipped with a heating panel to maintain the body temperature. To prevent clotting and to stabilize the blood circulation, an infusion of heparinised isotonic saline (36 ml/h) was administrated via the femoral vein by an infusion pump. The arterial blood pressure was recorded continuously from the cannulated carotid artery. The blood pressure of the rabbits was constant during the experiment. The rabbits received single doses of the QU preparations intraduodenally via catheter implanted in the duodenum at a dose of 5 mg/kg alone or in combination with the micelles. GDCA was used in a concentration of 15 mM, that means above the critical micellar concentration (CMC). PA was applied in a concentration of 1.8 mM and LE in a concentration of 1 mg/ml.

Blood samples (2 ml) were collected from the cannulated carotid artery every 30 min for 6 h. The collected blood samples were mixed with 2 ml of citrate solution (3.0%, v/v) and centrifuged (10 min at $3000 \times g$). The supernatant was stored at −20 °C. QU was extracted from the plasma samples with acetonitrile before analysis.

2.3. Analysis of quinine

Quinine was analyzed using HPLC system of Merck-Hitachi (Darmstadt, Germany) consisting of the autosampler AS-4000, the pump L-6200 A using a flow of 1 ml/min and an injection volume of 20 μl, a Lichrospher 100 RP18 (5 μm) column (Knauer, Berlin, Germany), a F-1080 fluorescence detector (excitation 350 nm, emission 450 nm) and the software D-6200 A Interface. The mobile phase was acetonitrile/0.05 M KH_2PO_4 (1:1, v/v), containing 0.2% conc. H_3PO_4.

2.4. Pharmacokinetic analysis

Pharmacokinetic parameters such as peak plasma concentration (c_{max}) and time of its maximum occurrence (t_{max}) were read directly from the individual plasma concentration–time profiles. The other pharmacokinetic parameters, e.g. biological half life ($t_{1/2}$), mean residence time (MRT) and the area under the curve ($AUC_{0-360\ min}$) were calculated using the program 'TOPFIT 2.0 Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC'.

2.5. Statistical analysis

The values are given as arithmetic mean ± SD. Data were analyzed by the Student's *t*-test for non-paired samples. When variances were non-homogenous, the Mann–Whitney-test was used. Further, the non-parametric confidential interval method of Diletti–Steinijans was applied. Differences with $P < 0.05$ were considered to be significant.

2.6. Ethical considerations

Principles of laboratory animal care were followed as well as the current German law on the protection of animals. All procedures were approved by the Animal Welfare Commission of the Thuringian state government (Germany) for animal research (Chinin-Bioverfügbarkeit; No.: 03-58/98).

3. Results and discussion

The pharmacokinetics of QU were investigated in presence of BS and mixed micelles in rabbits. GDCA was chosen as a typical conjugated BS present in human bile in a concentration above the CMC. Furthermore, mixed micelles

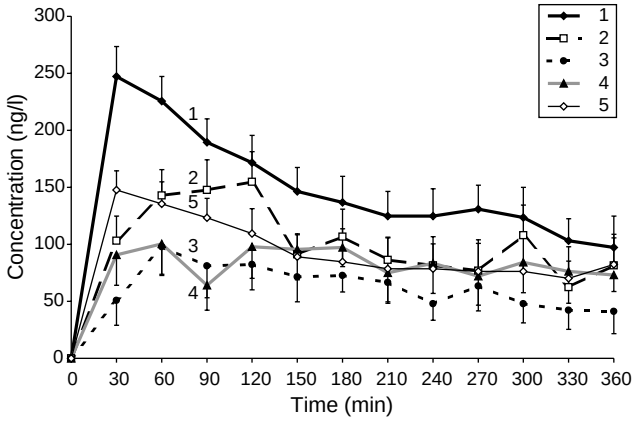

Fig. 1. Plasma concentration-time profile of quinine after intraduodenal administration alone (1) or in presence of the sodium salt of glycodeoxycholic acid (GDCA) (2) as well as in presence of mixed micelles consisting of GDCA and lecithin (3), GDCA and palmitic acid (4) or GDCA, lecithin and palmitic acid (5) in rabbits. Values are mean ± SD; $n = 4$.

were prepared from GCDA and LE, from GCDA and the fatty acid PA as well as from GCDA, LE and PA.

After intraduodenal administration of QU, the typical pharmacokinetics of QU are shown in Fig. 1 (curve 1). The effect of the entero-hepatic circulation appeared between 240 and 360 min as shown by the slight increase of the QU concentration in plasma. Maximum plasma levels of QU were measured in rabbits within the first hour of the experiment, whereas this maximum appears in humans between the first and the third hour.

The plasma levels of QU were distinctly decreased if the drug was administrated in presence of GCDA above the CMC (Fig. 1, curve 2). Furthermore, the maximum of the plasma level c_{max} of the drug occurred later (QU alone: 30 min; QU with BS: 120 min). The effect of entero-hepatic circulation (after 300 min) of QU was more distinct in presence of BS. It is supposed that there are prolonged and strong associations between the QU molecules and the GCDA micelles. Related results were also found in previous in vitro experiments. Thus, the transport of quinine across both artificial lipid membranes and ileum of male Wistar rats (everted-sac technique) was decreased in presence of BS above the CMC. But this effect was not observed if the BS was used below the CMC [1,2].

Because different micellar systems are present in the duodenum during lipid digestion, the influence of binary and ternary mixed micelles on the plasma levels of QU was also studied in rabbits. After single administration of QU incorporated into the micelles, the time-plasma concentration profile QU was lower compared with the administration of the drug without micelles (Fig. 1). Using binary micelles, a strong decrease in plasma level of QU and a change in the pharmacokinetics were observed (curves 3 and 4). The maximum was reduced to less then the half. Likewise, the application of the ternary micellar system resulted in a significant lower plasma level (curve 5). But the course of the curve was similar to that obtained without micelles (curve 1). Later, the differences between the variants tested were smaller because of the elimination of the drug and the metabolisation of the micelle forming components.

The lower plasma levels of QU in the binary GDCA-PA system (compared with GDCA-LE micelle) may be a result of the formation of ion-pairs between the anionic PA and the cationic QU retarding the drug absorption. On the other hand, the higher plasma level of QU in presence of the ternary micelle within the first period of the experiment is evidently caused by the increased solubility and, therefore, by the thermodynamic activity of QU incorporation into the micelles. The effects of entero-hepatic circulation on the plasma levels were particularly seen in the experiment with the GDCA-LE micelles (Fig. 1, curve 3).

The pharmacokinetic parameters AUC and c_{max} of QU were markedly decreased in presence of mixed micelles (Table 1). The changes in the AUC up to 360 min show clearly the effect of the different micelles on the inhibition of the absorption of QU and in consequence on its bioavailability. The quotients $AUC_{quinine}/AUC_{quinine+micelle}$ were 1.6, 3.9, 2.4 and 1.8, when the drug was applied in order of GDCA, GDCA/LE, GDCA/PA and GDCA/LE/PA, respectively. The plasma half-life time $t_{1/2}$ was significantly higher if QU was administrated in presence of both PA

Table 1
Pharmacokinetic parameters obtained after intraduadenal administration of quinine alone or in presence of mixed micelles

Parameter	Group				
	QU	QU/BS	QU/BS/LE	QU/BS/PA	QU/BS/LE/PA
$AUC_{0-360\ min}$ (μg min/ml)	56.92 ± 1.75	36.33 ± 1.14*	14.44 ± 0.83*	23.15 ± 0.96*	30.87 ± 0.94*
MRT (min)	161 ± 0.1	168 ± 8.4*	165 ± 8.7	183 ± 12.8*	167 ± 4.6*
$t_{1/2}$ (h)	4.5 ± 0.5	5.8 ± 0.7*	4.8 ± 0.1	10.3 ± 0.7*	6.2 ± 0.6*
c_{max} (ng/nl)	248.8 ± 36.6	154.4 ± 40.5*	100.9 ± 17.4*	104.7 ± 23.4*	146.7 ± 19.3*
t_{max} (min)	30	120	60	60	30
Cl_{tot} (ml/min)	55.1 ± 4.4	64.5 ± 3.6*	120.0 ± 5.2*	51.0 ± 6.8	61.7 ± 2.9

Values are mean ± SD; $n = 4$; *$P \leq 0.05$. QU, quinine (5 mg/kg body weight); BS, bile salt (glycodeoxycholic acid) (15 mM); PA, palmitic acid (1.8 mM); LE, lecithin (1 mg/ml); AUC, area under the curve; MRT, mean residence time; $t_{1/2}$, half life; c_{max}, peak plasma concentration; t_{max}, time of plasma maximum concentration; Cl_{tot}, total clearance.

containing systems as shown previously. In the literature it could be demonstrated that this effect was caused by ion-pair formation [14]. The prolonged MRT of QU in both PA-containing systems can be explained by ionic interactions between the drug and the fatty acid (additionally to its incorporation into the micelles). Furthermore, the highest value for the total clearance as well as the lowest plasma concentration maximum c_{max} as well the lowest AUC were observed in presence of the binary GDCA-LE micelle. This shows that QU has a very high affinity to GDCA-LE micelles.

Altogether it was shown that mixed micellar systems lower and not—as expected—increase the absorption of QU in vivo. The affinity of QU to micellar systems, determined also by micellar electrokinetic affinity chromatography [9] and in in vitro transport models [1], is still influencing the bioavailability of lipohilic drugs such as QU in vivo. The absorption of QU from the gastrointestinal tract may be influenced by interactions with stable mixed micellar systems which can be formed in the small intestine.

For some lipophilic drugs such as propranolol [10], bioavailability is enhanced in presence of BS. Reasons for this effect may be a decrease in the barrier function of the mucosa, transcellular mechanisms and an increase in membrane fluidity, or a paracellular transport as a result of high bile salt concentrations and micellar structures in the intestine [11]. On the other hand, absorption of some drugs such as cefadroxil was decreased in the presence of BS or mixed micelles. Reasons for the decreased bioavailability may be a reduced thermodynamic activity of the drugs after incorporation into or adsorption at the micellar systems or a decreased active transport as compared to the increase of passive transport [12]. The lower absorption of the drugs by micelles or mixed micelles is considered to be caused by the decreased thermodynamic activity of drugs after their incorporation into the micelles in dependence of the partition coefficient of the drug used. It also depends on the diffusion of the micelles through the unstirred layer [6,13]. Beside the high affinity of the QU molecules to micellar BS systems measured by micellar electrokinetic affinity chromatography [9], interactions between QU and phospholipids should also be considered.

The solubility of QU is increased by addition of BS. But the high affinity of the lipophilic drug QU to micellar systems overlaps the improved solubility in aqueous solutions. With increasing lipophilicity of the drugs, their solubilization in aqueous solution is the rate limiting step of the absorption process. In such cases, incorporation of drugs into micelles might enhance the absorption of these drugs. Other interactions between drugs and components of the micelle may increase the bioavailability, for instance the ion-pair formation between propranolol and taurodeoxycholic acid when this BS is used below the CMC [10].

In conclusion, the incorporation of QU into mixed micellar systems reduced markedly the plasma level and, therefore, the bioavailability of QU of intraduodenally administered drug in rabbits. Quinine should be orally administered at least 1 h before food intake, particularly before fat intake.

Because of the complex conditions in the human intestine, e.g. the influence of food constituents, the absorption of drugs may be influenced depending on the lipophilicity of the drug used. Therefore, it is necessary to study the drug absorption in presence of micellar systems simulating the digestion process in human intestine.

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References

[1] B. Fritzsch, R.H.H. Neubert, G. Dongowski, J. Giessler, Influence of nutrition factors forming stable mixed micelles on permeation of quinine in vitro using everted sac technique, Pharmazie 54 (1999) 473–474.
[2] B. Fritzsch, R. Neubert, G. Dongowski, L. Heinevetter, Interactions between food components and drugs. Part 8: effect of pectins and bile salt preparations forming stable micelles on transport of quinine in vitro, Pharmazie 55 (2000) 59–61.
[3] P.G. Welling, Drug–food interactions affecting drug absorption in: P.F. D'Arcy, J.M. McElnay, P.G. Welling (Eds.),, Mechanisms of Drug Interactions, Springer, Berlin, 1996, pp. 45–123.
[4] D. Hörter, J.B. Dressman, Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract, Adv. Drug Deliv. Rev. 25 (1997) 3–14.
[5] J.R. O'Reilly, O.I. Corrigan, C.M. O'Driscoll, The effect of mixed micellar systems, bile acid/fatty acids, on the solubility and intestinal absorption of clofazimine (B663) in the anesthetized rat, Int. J. Pharm. 109 (1994) 147–194.
[6] F.G.J. Ploema, J.J. Tucker, D.J.A. Crommelin, The role of bile salts in the intestinal absorption of drugs, Acta Pharm. Technol. 36 (1990) 43–52.
[7] A. Sowunmi, L.A. Salako, Effect of dose size on the pharmacokinetics of orally administrated quinine, Eur. J. Clin. Pharmacol. 49 (1996) 383–386.
[8] J. Scott-Moncrieff, Z. Shao, A. Mitra, Enhancement of intestinal in situ absorption by bile salt fatty acid mixed micelles in dogs, J. Pharm. Sci. 83 (1994) 1465–1469.
[9] M.A. Schwarz, K. Raith, G. Dongowski, R.H.H. Neubert, Effect of partition equilibrium of various drugs by the formation of mixed bile salt/phosphatidylcholine/fatty acid micelles. A characterization by micellar affinity capillary electrophoresis. Part IV, J. Chromatogr. A 809 (1998) 219–229.
[10] M. Gasco, M. Trotta, M. Eandi, The influence of bile salts on the absorption in vitro and in vivo of propranolol, J. Pharm. Biomed. Anal. 2 (1984) 425–439.
[11] T. Sugiyama, A. Yamamoto, Y. Kawabe, T. Uchiyama, Y.S. Quan, S. Muranishi, Effects of various absorption enhancers on the intestinal absorption of water soluble drugs by in vitro using chamber method: correlation with an in situ absorption experiment, Biol. Pharm. Bull. 20 (1997) 812–814.
[12] C. Ibanez, B. del Val, A. Rius, M. Villodre, Antiemetics, gastrointestinal drugs, urologic drugs—experimental influence of

surfactants on intestinal absorption of drugs—cefadroxil as model drug and bile salt as natural model surfactant: studies in rat colon and rat duodenum, Arzneimittelforschung 49 (1999) 44–50.

[13] V.S. Chadwick, T.S. Gaginella, G.L. Carlson, J.C. Debongnie, S.F. Phillips, A.F. Hofmann, Effect of molecular structure on bile acid-induced alterations in the absorptive function, permeability, and morphology in the perfused rabbit colon, J. Lab. Clin. Nutr. 94 (1979) 661–674.

[14] R. Neubert, W. Fürst, S. Schneider, H. Müller, H.-J. Loh, M. Jirka, Einfluß der Ionenpaarbildung auf die pharmakokinetischen Eigenschaften von Arzneistoffen. 1. Mitt: Einfluß der α-Methylpalmitinsäure auf die Pharmakokinetik von Chinin am Kaninchen, Pharmazie 42 (1987) 29–30.