Drug Interactions in Intestinal Absorption of ^{3}H-Digitoxin in Rats

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Summary. The absorption of ^{3}H-digitoxin from perfused rat small intestine was inhibited by probenecid (1.0×10^{-2} *M*), ethacrynic acid (0.5×10^{-3} *M*), and mersalyl (8.0×10^{-3} *M*) indicating that digitoxin absorption is at least partly an active process.

In recent studies on the in vitro absorption of digitoxin, the contribution of an active transport was assumed by the following findings: saturable and uphill transport from mucosa to serosa[1]; dependency on oxygen and energy supply[2]; and functional asymmetry concerning mucosal and serosal administration of digitoxin[3]. Moreover the absorption of ^{3}H-digitoxin was inhibited by probenecid, ethacrynic acid, and mersalyl[2]. The present study has been performed in order to investigate the drug effects obtained in vitro on the intestinal absorption of ^{3}H-digitoxin in vivo in rats.

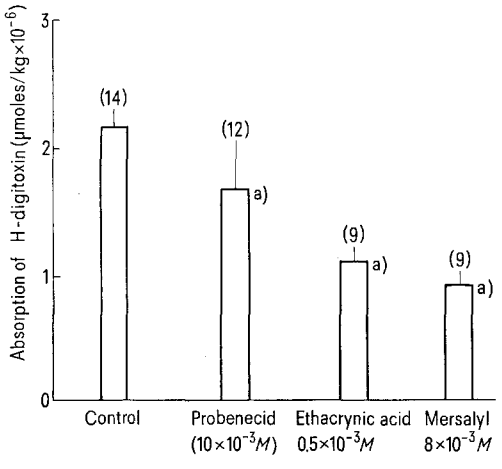

Fig. 1. Effect of probenecid, ethacrynic acid, and mersalyl on the intestinal absorption of ^{3}H-digitoxin in vivo in rats in urethane anaesthesia (1.2 g/kg). The amount of ^{3}H-digitoxin absorbed after 40 min is given in µmoles/kg body wt. Number of experiments in brackets.

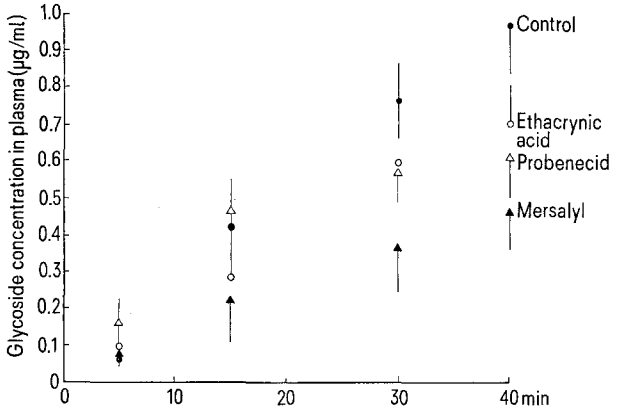

Fig. 2. Glycoside plasma levels during 40 min single pass perfusion of the small intestine of anaesthetized rats (1.2 g/kg urethane) with Krebs-Ringer-solution containing 2×10^{-5} *M* ^{3}H-digitoxin in controls and under probenecid, ethacrynic acid, and mersalyl. Results are given as the mean ± SD of 6 experiments.

Methods. The small intestine of male Wistar rats (200 ± 50 g body weight, fasted for 36 h) was perfused in vivo under 1.2 g/kg urethane anaesthesia in single pass perfusion according to Schanker et al.[4]. In 40 min experiments, 50 ml of Krebs-Ringer-solution (m*M*: NaCl 118.0, KCl 4.7, $CaCl_2$ 2.2, $MgSO_4$ 1.05, $NaHCO_3$ 24.9, KH_2PO_4 1.18) were perfused with a constant rate of 1.25 ml per min at a pH 7.4 and 37 °C, the intraluminal pressure never exceeding 15 cm of water. ^{3}H-digitoxin (NEN, Dreieichenhain/GFR) and the inhibitors used – probenecid and ethacrynic acid (Sharp & Dohme, München/GFR) and mersalyl (Hoechst, Frankfurt/GFR) – were added to the perfusion fluid.

Blood samples (0.4 ml) were withdrawn from the carotid artery at 5, 15, 30, and 40 min. Tissue samples of liver, kidney, and skeletal muscle (200–300 mg) were removed at the end of the experiments. For determination of the glycoside concentration (digitoxin including its metabolites), radioactivity in plasma and tissues was measured by liquid scintillation counting as described earlier[5]. The amount of ^{3}H-digitoxin absorbed was calculated from changes in volume and glycoside content of the perfusion fluid. The fluid volume was determined by ^{14}C-inulin (NEN, Dreieichenhain/GFR) as a marker as well as volummetrically.

Results. The drug effects on the absorption of ^{3}H-digitoxin are shown in Figure 1. In controls 2.2 µmoles/kg body wt. of ^{3}H-digitoxin were absorbed from the small intestine of rats in 40 min. With probenecid (1.0×10^{-2} *M*), ethacrynic acid (0.5×10^{-3} *M*), and mersalyl (8.0×10^{-3} *M*), the digitoxin absorption decreased significantly to 1.75, 1.1, and 0.50 µmoles/kg body wt. per 40 min.

From the results shown in Figure 2, it can be concluded that the glycoside concentration measured during the period of absorption in the rat plasma is diminished significantly by probenecid (0.58 µg/ml), ethacrynic acid (0.71 µg/ml), and mersalyl (0.46 µg/ml) compared with the controls (1.08 µg/ml).

In the Table the glycoside contents in plasma, liver, kidney, and skeletal muscle reached at the end of the experiments are summarized. Corresponding to the decreased rate of digitoxin absorption, the inhibitors impair the glycoside concentration in plasma as well as in tissues.

Discussion. The results obtained show that probenecid, ethacrynic acid, and mersalyl inhibit the absorption of ^{3}H-digitoxin in vivo in rats, as already demonstrated in vitro in mice. The inhibitors significantly reduce the

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Glycoside content in plasma, liver, kidney, and skeletal muscle of anaesthetized rats (1.2 g/kg urethane) after 40 min single pass perfusion of the small intestine in vivo with Krebs-Ringer-solution containing 2×10^{-5} *M* ^{3}H-digitoxin in controls and under probenecid, ethacrynic acid, and mersalyl

Experiments	Glycoside concentration after 40 min perfusion with ^{3}H-digitoxin				
	Plasma (μg/ml)	Liver (μg/g)	Kidney (μg/g)	Muscle (μg/g)	*n*
Controls	1.08 ± 0.15	6.96 ± 1.50	1.85 ± 0.48	0.66 ± 0.17	5
Probenecid (1.0×10^{-2} *M*)	0.59 ± 0.12[a]	4.19 ± 1.40[a]	1.30 ± 0.31	0.45 ± 0.10[a]	5
Ethacrynic acid (0.5×10^{-3} *M*)	0.71 ± 0.19[a]	4.26 ± 1.55[a]	1.23 ± 0.47	0.40 ± 0.21	6
Mersalyl (8.0×10^{-3} *M*)	0.46 ± 0.14[a]	4.79 ± 0.80[a]	1.06 ± 0.33[a]	0.32 ± 0.14[a]	6

Results are given as the mean ± SD; *n* = number of experiments; [a] indicates significant difference from control values according to Student's *t*-test, $p < 0.01$.

amount of ^{3}H-digitoxin absorbed from the intestine as well as the glycoside concentration in plasma and tissues reached at the end of 40 min single pass perfusion. But, as shown in Figure 2, the inhibitory effects are significantly different from the controls not before a perfusion period of 30 min indicating a rather long latent period of the drug actions. The time course of the glycoside concentration in the plasma remained linear with ethacrynic acid and mersalyl, whereas in the inhibition with probenecid characteristics of saturation are obvious. In view of the mechanism of the drug actions, it should be mentioned that in a previous study the effects had been explained by inhibition of energy dependent processes which mediate in part the absorption of digitoxin[2]. In the case of ethacrynic acid and mersalyl specific effects on the Na^+, K^+ activated membrane, ATPase might be an appropriate explanation for the inhibition of digitoxin absorption[6,7]. It was shown by DAMM and WOERMANN[2] earlier that, with these drugs, the inhibition of digitoxin correlated strongly with the inhibition of the intestinal absorption of sodium, water and glucose. Probenecid is known as an inhibitor of organic anion transports[8,9] as well as for non-specific metabolic inhibitions[10–12]. The latter effect only seems to be involved in the inhibition of digitoxin absorption. In all, as demonstrated in vitro, digitoxin absorption in vivo can hardly be interpreted by simple diffusion; the present results indicate that active processes are at least partly involved in this transfer.

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Effect of Alcohol Ingestion on the Epithelial Cell Population in Rat Small Intestine

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Summary. Chronic administration of alcohol to well-nourished rats led to striking changes in the small intestinal cell population. The present experiments corroborate the view that alcohol is directly toxic to the small intestine.

Diarrhea, disturbances of digestion, and malabsorption of nutrients are frequently associated with chronic alcohol intake. Light and electron microscopic changes[2,3] and functional alterations[4–10] of the small intestine have been reported in alcoholic patients and experimental animals. The causes, however, are still unknown. Malnutrition and direct enterotoxic effect of alcohol have been assumed as pathogenic mechanisms of such abnormalities. The present investigation was undertaken to study the effect of a prolonged period of alcohol ingestion on the small intestinal cell population in otherwise well-nourished rats.

Materials and methods. 5 Wistar strain male rats (body weight 30–40 g) were allowed to drink only alcohol for 12 weeks. A 32% (v/v) ethyl alcohol solution in 25% (w/v) sucrose in tap water was used. 5 animals from the same stock were kept as controls and given no alcohol. All rats were fed on a solid semi-synthetic diet supplemented with large amounts of vitamins and lipotropes[11,12].

Tissues from both control and experimental groups were processed in exactly the same fashion. To determine the percentage of cells in division in the small intestine during a given time interval, the colchicine technique was applied[13,14]. The rats were injected s.c. with colchicine in a dose of 0.1 mg per 100 g of body weight 3 h prior to sacrifice. The animals were sacrificed by exsanguination under light ether anesthesia, and pieces of proximal jejunum and distal ileum were rapidly removed, flattened on cardboard, fixed in Bouin's solution for 72 h, and embedded in paraffin. Longitudinal sections were cut at 4 μm and stained with hematoxylin-eosin and PAS-hematoxylin. Areas of sections showing crypts cut through their length were selected for counting the total number of nuclei and the number of mitosis in metaphase and prophase. At least 10 crypts per section were counted in the particular tissue from each animal. The length of villi