Evaluation of the Enterohepatic Circulation after $^3$H-Digoxin Administration in the Rat

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Summary. After intraduodenal administration of $^3$H-digoxin (d) in biliary fistula (b.f.) rats, the total radioactivity in blood and bile is eliminated with $t_1/2$ of 7 h in both fluids. In rats with intact enterohepatic circulation (e.c.), a $t_1/2$ of 13.5 h was observed in blood and of 22 h in bile. To explain the much longer $t_1/2$ in bile than in blood, the pharmacokinetics were studied of all substances, which might participate in e.c. after d administration. E.c. of the water soluble fraction is negligible since almost no absorption was found. Digoxigenin-bis- (b) and monodigitoxoside (m) showed approximately the same absorption kinetics as d. However, the blood levels of radioactivity after i.d. administration of these metabolites in b.f. rats were 5—6 times lower than those after d as a consequence of higher biliary excretion. 90—95% of the absorbed amounts of b and m were extracted in bile within 11 h compared with 61% after d administration. Thus the far longer $t_1/2$ of elimination of radioactivity in bile than in blood after i.d. administration of d in rats with e.c. seemed to be due to a short circuit of b and m between intestine and liver. Evidence for this comes from the chromatographic analysis of the total radioactivity in the bile of these animals which shows that significantly more b is present in the bile of rats with e.c. than b.f. rats. No differences were found in the case of m, which on one hand is formed to a lesser extent and is on the other rapidly converted to polar metabolites, which are not reabsorbed.

Key words: Enterohepatic Circulation — Pharmacokinetics — Digoxigenin-Bis- and Mono-Digitoxoside — Polar Conjugates.

The enterohepatic circulation of digoxin, which is excreted to a large extent in the bile, seems to exert a considerable influence on the pharmacokinetic behaviour of this glycoside in the rat (Rietbrock et al., 1972). Proof of this assumption arose from experiments dealing with the continuous determinations of glycoside levels in both bile and blood of animals with an intact or interrupted enterohepatic circulation. At the same time it was recognized that a complete insight into this problem could only be obtained by a detailed investigation of the absorption, blood concentration, and biliary excretion of all substances which might participate in the enterohepatic circulation after digoxin administration. Therefore a study was undertaken dealing with the pharmacokinetic
behaviour of digoxin, of the digoxigenin-bis- and mono-digitoxosides, and of the water soluble metabolites, all of which have been detected in the bile and urine of rats after the administration of digoxin (v. Bergmann et al., 1972).

Methods and Materials

Female Sprague-Dawley rats (SPF-rats from Mus-Rattus-AG) weighing 200 g were used. The animals were kept under normal laboratory conditions and fed with Altromin standard diet. Food was withdrawn 16 h before the experiment, while water was available ad libitum. Under anaesthesia with urethane (1.2 g/kg i.p.) the common bile duct was cannulated to drain the bile, while another catheter was pushed through the distal part of bile duct into the lumen of the duodenum. This second catheter was used for the i.d. administration of the glycosides as well as for the substitution of bile from a donor rat in order to maintain a constant bile flow in the experimental animal from which the bile was drained. In animals with an intact enterohepatic circulation the proximal catheter was connected with the distal catheter by means of an adapter, which allowed the taking of small aliquots of bile for measurement (for details see Rietbrock and Abshagen, 1972). To gain sufficient material for thin layer chromatographic analysis, the enterohepatic circulation was interrupted for 20—30 min at distinct intervals in one group of animals. Blood was drawn from a catheter in the carotid artery. After the operation the animals were kept in temperature-regulated cages to prevent a reduced bile flow by hypothermia. The bile flow averaged 8 mg/min.

For absorption studies the particular substances were administered into the duodenum via the second catheter and into the colon by another catheter which had been introduced into the colon by means of a small incision. Bile was drained to prevent the re-entry of glycoside into the gut. The animals were killed at distinct time intervals, and the unabsorbed amounts of glycoside in the intestine were determined as well as the biliary and renal excreted radioactivity.

For the determination of radioactivity in the gastrointestinal tract, the small intestine or colon was excised, weighed, and homogenized with 20 ml of ethanol. After centrifugation 0.5 ml of supernate was added to 10 ml scintillation fluid [10% naphathlene, 0.98% diphenyloxazole, and 0.02% 2,2-p-phenylene-bis(5-phenyloxazole) in dioxane]. The blood specimens were weighed, mixed with 2 ml of methanol, and centrifuged; 1 ml of supernate was added to 10 ml of scintillation fluid. To bile and urine specimens scintillation fluid was added directly. The radioactivity of all samples was assayed in a Packard liquid scintillation spectrometer, model 3380. The counting efficiency was determined by the channel ratio method employing an external standard.

The thin layer chromatographic analysis of the biliary excreted radioactivity was carried out by means of partition chromatography on formamide impregnated cellulose plates, developed with chloroform as described by v. Bergmann et al. (1972).

Substances. 12-α-3H-digoxin, specific activity 349 μCi/mg, 12-α-3H-digoxigenin-mono-digitoxoside, specific activity 100.1 μCi/mg, 12-α-3H-digoxigenin-bis-digitoxoside, specific activity 80.12 μCi/mg (Boehringer Mannheim GmbH, Mannheim). The radiochemical purity of the respective glycosides averaged 96%. The total radioactivity per animal amounted to 40 μCi (≈ 0.4 mg mono-, 0.5 mg bis-, 0.12 mg triglycoside), administered as 0.3 ml of 40% ethanol in 0.9% NaCl