No effect of probenecid on the renal and biliary clearances of digoxin in man

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- 1 The cardiac glycoside digoxin is subject to a number of pharmacokinetic interactions. This study concerns the influence of the anionic transport inhibitor probenecid on the steady-state kinetics of digoxin.
- 2 Six healthy young men were enrolled in the study. After an administration period of 6 days with digoxin only (0.5 to 1 mg p.o. day⁻¹) or digoxin in combination with probenecid (2 g p.o. day⁻¹; 8 days), digoxin was administered intravenously (0.7 oral dose) on day 7. Plasma and urine samples were taken over 48 h. The biliary clearance of digoxin was measured during day 8 by a duodenal perfusion technique.
- 3 Probenecid did not affect the plasma clearance (mean $\pm$ s.d.: 255 $\pm$ 80 vs 266 $\pm$ 40 ml min⁻¹), renal clearance (166 $\pm$ 17 vs 155 $\pm$ 10 ml min⁻¹), biliary clearance (106 $\pm$ 40 vs 111 $\pm$ 50 ml min⁻¹), elimination half-life (34.4 vs 35.2 h) or volume of distribution (538 $\pm$ 241 vs 566 $\pm$ 60 l) of digoxin.
- 4 Our results suggest that different systems exist in man for the renal and biliary secretion of probenecid and digoxin.

Keywords digoxin probenecid interaction renal and biliary clearance

Introduction

The actions of the anionic drug probenecid (pKa 3.4) are largely confined to inhibition of the transport of organic anions across epithelial barriers. This effect is most important in the renal tubule, where the secretion of many drugs, metabolites and endogenous compounds is inhibited (Weiner *et al.*, 1964). Probenecid has also been shown in animals to inhibit the biliary elimination of a number of drugs, including indomethacin (Duggan *et al.*, 1977), methotrexate (Kates & Tozer, 1976), rifamycin antibiotics (Kenwright & Levi, 1973) and to decrease the non-renal clearance in man of some biliary excreted drugs (Baber *et al.*, 1978; Stoeckel *et al.*, 1988). In the rat, probenecid itself is secreted into bile by a dose-dependent and saturable process, subject to inhibition by, for example, phenolphthalein (Guarino & Schanker, 1968).

The cardiac glycoside digoxin—an uncharged steroid—is secreted both in the renal tubules and into bile (Beermann *et al.*, 1972; Caldwell & Cline, 1976; Steiness, 1974), and these processes can be inhibited by drugs with different molecular properties. Quinidine and verapamil,

both basic compounds, are the clinically most important inhibitors of digoxin clearance. They produce a significant increase in the plasma concentration of digoxin (Belz *et al.*, 1981; Ejvinsson, 1978) owing to inhibition of its renal (quinidine) and biliary elimination (Angelin *et al.*, 1987; Dahlqvist *et al.*, 1980; Hager *et al.*, 1979; Hedman *et al.*, 1990, 1991).

Secretion of drugs into urine and bile is considered to occur by selective transport systems for organic anions, cations or uncharged steroids (Kupferberg & Schanker, 1968; Rennick, 1981; Schanker, 1968; Sperber, 1959). However, there seem to be overlapping inhibition of these secretory systems (Eaton & Klaassen, 1978). Probenecid is reported to have a modest inhibitory effect *in vitro* on the uptake of another glycoside, ouabain, in rat liver slices (Erttmann & Damm, 1974) and in rat isolated hepatocytes (Eaton & Klaassen, 1978) as well as being able to lower the renal clearance of cimetidine in healthy volunteers (Gisclon *et al.*, 1989). Hence, the question arises whether an anion like probenecid may affect secretion and thus the kinetics of digoxin in man.

Methods

Subjects

The study was carried out in six healthy male volunteers between 25 and 35 years of age (mean $\pm$ s.d.: 29 ± 4) and with weights ranging from 67 to 90 kg (mean: 79 ± 8). All subjects had normal results following clinical, biochemical and haematological examinations as well as normal ECG before the study. No other medication was allowed during the study periods. Approval of the study was obtained from the Ethics Committee of Huddinge University Hospital and each subject gave informed consent.

Study procedures

The study comprised two cross-over periods, each continuing for 9 days. *Control period:* Digoxin (0.5 to 1 mg day^{-1} p.o.; Lanacrist®, Astra Läkemedel AB, Södertälje, Sweden) alone was administered during the first 6 days, with the aim of achieving steady-state plasma concentrations of approximately 1 nmol l^{-1} , followed by an intravenous digoxin dose (0.7 oral dose) administered on the seventh day. *Combination period:* Digoxin (0.5 to 1 mg day^{-1}) was administered as in the control period, but in combination with probenecid (Probecid®, Astra Läkemedel AB, Södertälje, Sweden) 1 g twice daily for 8 days. In both periods the plasma concentration of digoxin was checked after at least 4 days and the dose was adjusted if necessary. The volunteers received the combination treatment within 1 month after the initial control period.

Blood and urine sampling After the intravenous dose on day 7 the concentration of digoxin in plasma was measured over 48 h. Blood samples were taken at the following times: 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 h (day 7) and during day 8 at each hour from 24 h until 32 h, the last sample being obtained at 48 h (day 9). Urine was collected in approximately 6 h fractions over the first 24 h, thereafter hourly during day 8, with a final sample from 32 h to 48 h.

A modified duodenal-marker-perfusion technique (Arvidsson *et al.*, 1982; Nilsell *et al.*, 1983) was used to study the biliary excretion of digoxin. In the morning of day 8, a triple lumen polyvinyl tube was inserted under fluoroscopic control in the fasting state. An infusion mixture was prepared of 5% glucose (w/v) and amino acids (Vamin®, KabiVitrum AB, Stockholm, Sweden), to which essential amino acids (L-methionine, 40 mM; L-valine, 25.8 mM; and L-phenylalanine, 18.2 mM) and sulphobromophthalein (BSP) as a non-absorbable marker had been added. This mixture was administered at a constant rate of 5 ml min^{-1} through the tube into the duodenum over 8 h. The tube was positioned in the duodenum so that the infusion site was at the level of the ampulla of Vater and the proximal (bile lipid sampling) and distal (drug and bile lipid sampling) collecting ports were 2 and 12 cm distal.

Bile sampling: Bile was aspirated continuously for 8 h, and after an initial equilibration period of three hours, it was sampled (10 ml) in 1 h fractions, in parallel with the urine collections.

Analyses

Digoxin was measured in plasma, urine and bile aspirates by radioimmunoassay (Pharmacia Digoxin RIA, Pharmacia AB, Sweden) after dilution with blank plasma. Endogenous constituents of bile and probenecid (1 mmol l^{-1}), did not interfere with the assay. The inter-assay variations for the means of duplicate digoxin determinations in plasma were 4.0% (1.3 nmol l^{-1} ; $n = 92$) and 4.3% (3.2 nmol l^{-1} ; $n = 94$), and the intra-assay variations were 3.5% (1.0 nmol l^{-1} ; $n = 20$) and 4.9% (4.2 nmol l^{-1} ; $n = 20$).

Plasma sampling for the measurement of probenecid was performed simultaneously with that for the measurement of digoxin and the concentrations of probenecid in plasma were assayed by a specific h.p.l.c. method, originally developed for analysis of ceftriaxone (Trautmann & Haefelfinger, 1981). Duplicate determinations of quality control samples analysed concomitantly with the plasma samples resulted in inter-assay variations of 3.4% ($170 \text{ } \mu\text{mol l}^{-1}$) and 7.3% ($424 \text{ } \mu\text{mol l}^{-1}$), and the intra-assay variations of determinations on spiked plasma samples were 0.8% ($78 \text{ } \mu\text{mol l}^{-1}$; $n = 6$) and 3.0% ($313 \text{ } \mu\text{mol l}^{-1}$; $n = 6$).

Creatinine in plasma and urine was analysed by the method of Bergman & Öhman (1980). Concentrations of probenecid in plasma was found not to interfere with the determination of creatinine.

Biliary secretion rates of cholesterol, bile acids and phospholipids were measured as described by Nilsell *et al.* (1983).

Pharmacokinetic calculations

Plasma A biexponential function was fitted to the intravenous digoxin data using the NLIN regression programme (SAS Institute Inc., Cary, NC, USA). The goodness-of-fit was based on confidence intervals of the parameter estimates and visual inspection of the plots and of the residual plots.

The pharmacokinetic parameters were calculated according to standard methods (Gibaldi & Perrier, 1982). The area under the plasma digoxin concentration-time curve after the intravenous dose was calculated for one dose interval AUC(0,24) using the linear trapezoidal method and was adjusted to a dose of 0.36 mg of digoxin. The plasma clearance of digoxin was determined as the intravenous dose of digoxin divided by AUC(0,24). The distribution volume at steady-state (V_{ss}) was estimated from:

$$D_{i.v.} \cdot \frac{\text{AUMC}}{\text{AUC}^2},$$

after subtracting the plasma drug concentrations from the previous dose, estimated from the slope of the concentration-time curve on days 7 and 8.

The time-average mean steady-state plasma concentration of probenecid in each subject was obtained from AUC(0,12) (after extrapolation of the concentration at 12 h) divided by the dosing interval.

Urine The renal clearance of digoxin during one dose interval (day 7) was calculated by dividing the cumulated amount of digoxin excreted into the urine

over 24 h by the corresponding AUC. The renal clearance of digoxin on day 8 was determined from the mean of the fractional renal excretion rates divided by the corresponding plasma concentration of digoxin.

In both the control and combination treatment periods, creatinine clearance was measured in each subject over the initial 6 h period on day 7.

Bile The measurement of biliary digoxin clearance was started at 24 h after the intravenous dose (day 8). The biliary excretion rate of digoxin was calculated from:

$$\frac{C_{\text{dig}}}{C_{\text{BSP}}} \times \text{rate of infusion of BSP}$$

where C_{dig} and C_{BSP} are the concentrations of digoxin (nmol l^{-1}) and BSP ($\mu\text{mol l}^{-1}$) in the same distal bile aspirate.

The biliary clearance of digoxin was calculated by dividing its biliary excretion rate by the plasma drug concentration at the midpoint of each interval. The bile fractions obtained over the first 3 h were excluded as this time was needed to establish an equilibrium in the rate of appearance of digoxin in the bile. Individual mean biliary clearance values were based on determinations of excretion rates in at least 4 hourly fractions collected during the 8 h investigation period.

Statistics

The results are given as mean $\pm$ s.d. Student's *t*-test for paired observations was used to test the significance of differences. A *P* value of < 0.05 was considered significant.

The ability (statistical power) of the study to detect a difference of 25% in renal and biliary clearance of digoxin was 99% and 70%, respectively, at a significance level of 5%.

Results

The administration of digoxin and probenecid to young healthy men did not cause any adverse reactions except for temporary slight nausea in two cases, one after

digoxin administration and the other during probenecid treatment.

Figure 1 shows the semilogarithmic mean plasma digoxin concentration-time plots (and the fitted non-linear regression curve) after the intravenous dose at steady state—alone and with simultaneous administration of probenecid. The plasma digoxin concentrations are corrected for dose (0.36 mg i.v.). Probenecid did not significantly change these concentrations. The average trough concentrations of digoxin in plasma (corrected for dose) were 0.84 ± 0.24 (day 7) and $0.85 \pm 0.20 \text{ nmol l}^{-1}$ (day 8) during the control period, and 0.80 ± 0.16 and $0.92 \pm 0.23 \text{ nmol l}^{-1}$ during combination treatment.

The mean ($\pm$ s.d.) steady-state concentration of probenecid in plasma varied between subjects from 289 to $423 \mu\text{mol l}^{-1}$, the average being $363 \pm 53 \mu\text{mol l}^{-1}$.

The simultaneous administration of probenecid did not alter the steady-state clearance (Table 1) or other pharmacokinetic parameters of digoxin; the dose-adjusted AUC(0,24) of digoxin being (mean $\pm$ s.d.) 32.2 ± 8.5 when given alone and $29.4 \pm 4.3 \text{ nmol l}^{-1} \text{ h}$ together with probenecid, the elimination half-life 34.4 and 35.2 h, respectively and V_{ss} 538 ± 241 and $566 \pm 60 \text{ l}$,

Figure 1 Mean plasma concentrations of digoxin (nmol l^{-1}) in six healthy young men after administration of intravenous digoxin (0.36 mg) alone (■) and in combination with probenecid ($2 \text{ g p.o. day}^{-1}$) (○).

Table 1 Steady-state plasma, renal and biliary clearances (ml min^{-1}) of digoxin alone (D) and in combination with probenecid (D+P)

Subject	Plasma clearance		Renal clearance day 7*						Renal clearance day 8**				Biliary clearance	
	D	D+P	D		D+P				D		D+P		D	D+P
			CL_{cr}	Q_{ur}	CL_{cr}	Q_{ur}			Q_{ur}	Q_{ur}				
1	270	299	192	173	1.7	155	143	1.6	199	2.8	188	2.1	163	186
2	175	225	172	161	1.1	162	119	1.1	169	2.1	197	3.0	72	76
3	405	317	160	149	1.3	162	ND	1.4	181	2.3	147	2.8	ND	117
4	234	253	147	187	0.6	137	148	0.6	163	1.0	167	1.0	86	48
5	247	235	174	196	0.8	157	144	1.4	158	3.3	142	1.9	77	92
6	202	ND	152	140	0.9	ND	126	0.9	175	3.0	145	1.4	133	145
Mean	255	266	166	168	1.1	155	136	1.2	174	2.4	164	2.0	106	111
$\pm$ s.d.	80	40	17	22	0.4	10	13	0.4	15	0.8	24	0.8	40	50
vs control (D)	NS		NS		NS		NS		NS		NS		NS	
vs day 7					$P < 0.01$		NS		NS	$P < 0.01$	NS	$P < 0.05$	NS	

* day 7: one dose interval (24 h); ** day 8: mean of 1 h fractional clearance; ND = not determined; NS = not significant; CL_{cr} = creatinine clearance (ml min^{-1}); Q_{ur} = urine flow (ml min^{-1}); Statistical comparison was performed by Student's paired *t*-test

respectively. The urinary 24 h recovery of digoxin in per cent of intravenous dose was $70 \pm 19\%$ without and $59 \pm 8\%$ with probenecid (NS). Creatinine clearance (Table 1) was significantly lower during combination treatment with probenecid ($136 \pm 13 \text{ ml min}^{-1}$) than in the control period ($171 \pm 22 \text{ ml min}^{-1}$, $n = 5$; $P < 0.01$).

Mean biliary excretion rates of cholesterol, bile acids and phospholipids were not significantly different between the control (50 ± 18 , 1960 ± 1230 and $206 \pm 81 \text{ } \mu\text{mol h}^{-1}$, respectively) and combination treatment period (60 ± 20 , 2235 ± 1281 and $407 \pm 287 \text{ } \mu\text{mol h}^{-1}$, respectively).

In order to investigate a possible effect of the duodenal perfusion technique, the renal clearance of digoxin was determined separately on days 7 and 8 (Table 1). During both the control and the combination period, the urine flow rate was higher on day 8 compared to the preceding day (2.4 ± 0.8 vs $1.1 \pm 0.4 \text{ ml min}^{-1}$, $P < 0.01$; control period and 2.0 ± 0.8 vs $1.2 \pm 0.4 \text{ ml min}^{-1}$, $P < 0.05$; combination treatment). The increased flow rate however, did not influence the renal clearance of digoxin significantly, being 174 ± 15 (day 8) and $166 \pm 17 \text{ ml min}^{-1}$ (day 7), and 164 ± 24 and $155 \pm 10 \text{ ml min}^{-1}$, respectively. The urine flow rates were not different between the control and the combination treatment periods within the corresponding day.

Discussion

The results of this study indicate no important interaction of probenecid with the renal elimination of digoxin. In contrast, creatinine clearance (day 7) was 22% lower during combination treatment with probenecid than in the control period. This finding is difficult to explain, although two mechanisms are possible—a reduction of the glomerular filtration rate (GFR) by probenecid or an inhibition of the secretory component of creatinine elimination. It has been reported (Gisclon *et al.*, 1989) that probenecid decreases GFR (inulin clearance) in man by 29%. This decrease in GFR is comparable with the extent of impairment of creatinine clearance found in our study, as well as with the results reported in two other studies in humans where probenecid reduced inulin clearance by 22% and 27% (Odland *et al.*, 1983a,b). The

similarity of these results is striking, but in the light of all the studies with negative findings in relation to the effect of probenecid on GFR, this explanation is less probable. Furthermore, if GFR were decreased, the renal clearance of digoxin should also have been lower but this was not the case. In the study by Gisclon *et al.* (1989), probenecid was also proposed to lower the renal secretion of the organic cation cimetidine in healthy males. Cimetidine has previously been shown to decrease the renal secretion of creatinine (Burgess *et al.*, 1982) implying that creatinine is transported in man by the organic base pump. Because probenecid is an anion, such an explanation would at first sight seem unlikely. However, the tubular handling of creatinine is complex and zwitterionic properties of creatinine may be suggested as both inhibitors of the acid and base systems depress its secretion (Weiner, 1973). Hence, the more plausible explanation for our findings seems to be an impaired tubular secretion of creatinine caused by probenecid.

In agreement with the renal findings, no significant effect was seen of probenecid treatment on the biliary clearance of digoxin. In our study both bile acid as well as cholesterol and phospholipid excretion rates were unaffected by probenecid, signifying no influence of probenecid on the secretion of bile lipids or on bile-acid-dependent flow in man. The sum of measured biliary and renal clearances of digoxin on day 8 was similar to the independently determined plasma clearance on day 7, suggesting that the duodenal perfusion technique provided a good estimate of the biliary clearance of digoxin.

In summary, investigation of the combination of the anion transport inhibitor probenecid and digoxin in six healthy young men, indicated no pronounced effects on the renal and biliary clearance of digoxin. Thus, our results suggest that different secretory pathways exist for digoxin and probenecid.

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