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The effect of digoxin dosage on the digoxin–quinidine interaction in the bile duct-cannulated rat

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Pretreating anaesthetized bile duct-cannulated rats with 9 mg kg^{-1} quinidine significantly decreased the cumulative biliary excretion of digoxin and its metabolites after 10 or 100 μg kg^{-1} [^{3}H]digoxin, although the effect was more marked in animals receiving the high dose of digoxin. In contrast, however, although quinidine pretreatment raised plasma radioactivity levels by 50–80% in animals given the higher dose of digoxin, no significant effect on circulating plasma levels was observed in rats receiving 10 μg kg^{-1} digoxin. Generally, quinidine had no statistically significant effect on other aspects of digoxin disposition, although with both digoxin doses there were trends towards a reduction in the direct intestinal secretion and urinary excretion of digoxin-derived radioactivity with an increase in tissue levels of radioactivity (apart from the small intestine wall where concentrations were reduced). The radioactivity in the bile after 100 or 10 μg kg^{-1} digoxin comprised about 25 and 33% of digoxin and digoxigenin bis-digitoxoside, respectively, as well as appreciable amounts of the mono-digitoxoside and a highly polar component. This metabolite profile was unaffected by quinidine. The influence of cardiac glycoside dosage shown by the present work indicates that the digoxin–quinidine interaction and possibly analogous interactions involving other cardiac glycosides, may not always be readily detectable from plasma concentration data.

It is well established in man that quinidine given concurrently with digoxin will significantly elevate plasma digoxin concentrations. The main mechanisms for this interaction are thought to be inhibition of renal and extra-renal digoxin clearance and possibly a relative impairment of digoxin binding to tissues (for a review see Pederson 1985). There have been a number of studies dealing with the tissue concentration/binding (Doherty et al 1980; Kim et al 1981; Geiger et al 1982; Sato et al 1983; Jogestrand et al 1984; Okudaira et al 1986) and renal clearance (Leahey et al 1981; Schenck-Gustafsson & Dahlqvist 1981; Sato et al 1983; Pedersen et al 1983) aspects of the interaction. As far as non-renal clearance is concerned, laboratory animals have been used to investigate the direct intestinal secretion (George & Renwick 1982; Schafer et al 1984, 1985) and biliary elimination (Sato et al 1983; Ben-Itzhak et al 1985) of digoxin. The study of Sato et al (1983), using unanaesthetized guinea-pigs, was useful in that, apart from intestinal secretion, it examined non-renal and renal mechanisms together in the same animal.

In the present study, using an anaesthetized bile duct-cannulated rat model, we aimed to obtain a similar overview of the suggested mechanisms contributing to the digoxin–quinidine interaction. However, we found that the effect of quinidine on plasma levels of digoxin-derived radioactivity was influenced by the digoxin dosage, with a lower digoxin dosage resulting in this aspect of the interaction being less detectable. We also examined the effect of quinidine on the biliary digoxin metabolite profile.

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Materials and methods

Materials. 12α-[^{3}H]Digoxin (15·4 Ci $mmol^{-1}$) was obtained from New England Nuclear, Boston, MA. Digoxigenin-bis-digitoxoside (D_2), Digoxigenin-mono-digitoxoside (D_1) and digoxigenin (D_0) were obtained from Uniscience Ltd, London, UK. Digoxin and quinidine sulphate (Analar) were bought from the Sigma Chemical Co. (Poole, Dorset) and BDH Ltd (Poole, Dorset), respectively.

Animals. Female Sprague-Dawley rats (200–250 g) with free access to food and water were used.

Disposition of radioactivity in anaesthetized animals. The rats were anaesthetized with pentobarbitone (60 mg mL^{-1}, 60 mg kg^{-1} i.p.), and a carotid artery (collection of blood), femoral vein (injection of drugs) and the bile duct (collection of bile) were cannulated essentially as described by Klaassen & Strom (1978). Rectal temperature was maintained at 37 °C by means of a heat-lamp regulator device (Yellow Springs Instrument Company, Yellow Springs, OH). Experimental animals were given quinidine sulphate (9 mg kg^{-1} i.v.) 10 min before [^{3}H]digoxin (either 100 or 10 μg kg^{-1}, 12·5 μCi kg^{-1} i.v.). Control rats were given an equivalent volume of 0·9% NaCl (saline) instead of the quinidine sulphate. Bile, and blood (0·4 mL), samples were collected at the times indicated in Results. Saline was given i.v. to replace fluid lost as bile or blood. Blood was centrifuged (3000*g* for 10 min) to obtain plasma. After 2 h the rats were killed and the tissues and biological material as indicated in Table 1 were removed. The intestines, 'small intestine' (jejunum and ileum), caecum and colon were weighed before and after removing the contents. Samples of the small intestine were taken about 5 cm from the caecum, while samples of caecum and colon were taken from areas

about mid-way along their lengths. Skeletal muscle was obtained from the 'thigh' area. Urine voided during the 2 h and that remaining in the bladder was collected. Radioactivity present in plasma, bile, urine, gut contents (homogenized using a test-tube and plastic pestle before assay) and tissue samples (finely minced with scissors before assay) were determined by standard liquid scintillation counting methods as detailed by Griffiths et al (1984). Total radioactivity, which may have comprised metabolites as well as unchanged digoxin was expressed as 'digoxin equivalents'.

Quantitation of [^{3}H]digoxin metabolites in bile. Bile (0·01–0·02 mL, containing about 0·05 μCi of radioactivity) was applied to silica-gel plates (Eastman Chromagram 13174, with fluorescent indicator) and a mixture of chromatographic standards (digoxin, D_2, D_1 and D_0) was added to the same area on the baseline. The solvent system used was cyclohexane–acetone–acetic acid (49 : 29 : 2 v/v) (Storstein 1976) in which digoxin, D_2, D_1 and D_0 had R_F values of 0·39, 0·43, 0·48 and 0·55, respectively. In preliminary experiments, radioactivity was located using a radiothin layer scanner (Panax Equipment Ltd, Redhill, Surrey, UK). Subsequently chromatographic loci were visualized under UV light (wavelength, 245 nm) using a Chromatolite lamp (Hanovia Ltd, Slough, Bucks, UK), cut out and transferred to 5 mL NE260 scintillant (Nuclear Enterprises Ltd, Edinburgh, UK) for liquid scintillation counting.

Statistics. All experimental data are means ± s.e. and data were analysed using a non-paired Student's *t*-test with a probability of $P < 0·05$ being taken as significant.

Results

Quinidine pretreatment significantly decreased the cumulative biliary excretion of digoxin and its metabolites at both digoxin doses. This effect was more marked in animals receiving 100 μg kg^{-1} digoxin (Fig. 1a, b). In contrast, however, although quinidine pretreatment raised plasma radioactivity levels by 50–80% in the higher-dose digoxin animals, no significant effect on circulating plasma levels was observed in rats receiving 10 μg kg^{-1} (Fig. 1c, d).

As far as direct intestinal secretion is concerned, most of the digoxin-derived radioactivity was found in the small intestine (contents) where quinidine seemed to inhibit secretion, significantly so in rats receiving the lower digoxin dose (Table 1). With the smaller amounts of radioactivity secreted into the colon and caecum the situation was less clear, quinidine even significantly increasing caecal secretion in the rats receiving the higher dose of digoxin. With the urinary excretion of digoxin-derived radioactivity, although mean values were lower with quinidine pretreatment, these differences were not significant (Table 1).

While generally quinidine had no significant effect on tissue concentrations of radioactivity, the trend was for mean tissue concentrations to increase with pretreat-

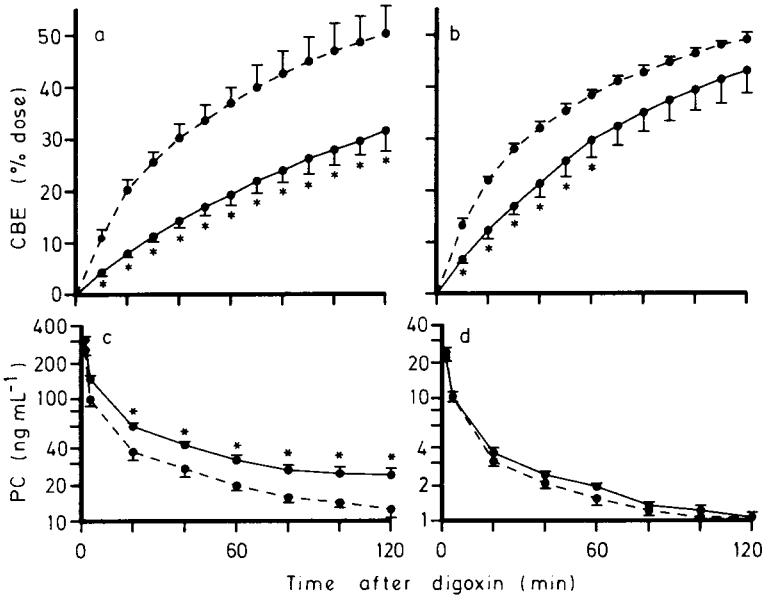

FIG. 1. The effect of quinidine on the plasma concentrations (PC) and cumulative biliary excretion (CBE) of digoxin-derived radioactivity in rats. The rats were anaesthetized (pentobarbitone 60 mg kg^{-1} i.p.), bile duct-cannulated and injected with either 100 or 10 μg kg^{-1} (a, c and b, d, respectively) [^{3}H]digoxin (12·5 μCi kg^{-1} i.v.). Quinidine-treated animals (solid lines) received quinidine sulphate (9 mg min^{-1} i.v.) 10 min before the digoxin. Concentrations of digoxin-derived radioactivity are expressed as digoxin equivalents. Asterisks indicate that quinidine had a significant effect ($P < 0·05$).

Table 1. The effect of quinidine on the distribution of radioactivity 2 h after the administration of [^{3}H]digoxin in the rat. Rats were anaesthetized (pentobarbitone 60 mg kg^{-1}, i.p.), bile duct-cannulated and injected with [^{3}H]digoxin (12·5 μCi kg^{-1} i.v.). Quinidine-treated animals received quinidine sulphate (9 mg kg^{-1} i.v.) 10 min before the digoxin. Concentrations of radioactivity have been converted to 'digoxin equivalents'.

	Concentration/amount of radioactivity			
	High-dose digoxin (100 μg kg^{-1})		Low-dose digoxin (10 μg kg^{-1})	
Tissue/material	Digoxin alone	Digoxin-quinidine	Digoxin alone	Digoxin-quinidine
Adrenal	3840 ± 1505	5395 ± 662	262 ± 29	306 ± 16
Liver	275 ± 101	398 ± 33	15 ± 2·8	21 ± 2·9
Small intestine	236 ± 34	174 ± 37	13·2 ± 1·6	8·0 ± 1·3*
Caecum	161 ± 22	197· ± 30	10·2 ± 2·2	11 ± 0·6
Colon	167 ± 64	164 ± 37	6·5 ± 1·6	8·1 ± 2·4
Skeletal muscle	96 ± 1·4	97 ± 8·7	6·1 ± 0·6	6·3 ± 0·7
Kidney	61 ± 15	75 ± 11	3·7 ± 0·5	4·6 ± 1·3
Heart (ventricle)	60 ± 11	76 ± 8·0	5·6 ± 0·8	6·7 ± 0·9
Lung	43 ± 7	64 ± 11·6	3·0 ± 0·51	3·3 ± 0·14
Brain	4·3 ± 1·2	5·2 ± 1·5	0·1 ± 0·03	0·2 ± 0·1
Small intestine[a] (contents)	4·0 ± 1·4	2·7 ± 0·4	6·0 ± 0·4	2·9 ± 0·4*
Caecum[a] (contents)	1·3 ± 0·15	2·0 ± 0·16*	1·5 ± 0·14	1·4 ± 0·08
Colon[a] (contents)	0·32 ± 0·17	0·4 ± 0·07	0·7 ± 0·22	0·4 ± 0·2
Total[a] (gut contents)	5·62	5·1	8·2	4·7
Urine[a]	7·4 ± 2·8	3·6 ± 0·7	6·3 ± 1·7	5·0 ± 1·4

[a] Amounts expressed as % dose, otherwise units are ng g^{-1}. Means are given ± s.e. (n = 4). * Significantly different from corresponding 'digoxin alone' value ($P < 0·05$).

ment, except for the small intestine wall. With the latter, quinidine pretreatment reduced the concentration, significantly so in the case of the low-dose digoxin-treated rats (Table 1).

With the 100 μg kg^{-1} dose of digoxin, about 33 and 25% of the biliary radioactivity obtained during the first 20 min comprised D_2 and digoxin, respectively. There were lesser amounts of D_1 and only traces of D_0 (Fig. 2).

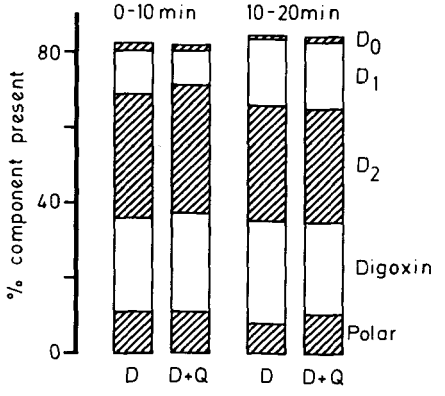

FIG. 2. Digoxin biliary metabolite profiles in rats receiving digoxin alone (D) and digoxin plus quinidine (D + Q). The bile (0–10 and 10–20 min samples) came from the '100 μg kg^{-1}' digoxin experiment illustrated in Fig. 1. The 'polar' component comprised radioactive material remaining on the baseline. Quinidine pretreatment had no significant effect on the metabolite profile. For clarity, s.e. values have been omitted.

Roughly 10% of the radioactivity comprised 'polar' material and remained on the baseline of the chromatogram. Although there appeared to be a slight increase in the proportion of D_1 with time, quinidine pretreatment did not significantly affect the biliary digoxin metabolite patterns at the two time periods (0–10 and 10–20 min) examined (Fig. 2). With the 10 μg kg^{-1} digoxin dose, the biliary metabolite profile was similar to that with the high dose of digoxin and again was not significantly affected by quinidine treatment (results not shown).

Discussion

The findings with the high dose of digoxin (100 μg kg^{-1}) are similar to those of Sato et al (1983) who administered 250 μg kg^{-1} digoxin to guinea-pigs along with 25 mg kg^{-1} quinidine. Those workers also found that quinidine treatment induced higher but roughly parallel plasma drug levels when compared with control values (as in Fig. 1a). This type of plasma elimination profile suggests that after quinidine, the apparent volume of distribution is decreased and the elimination half-life is essentially unaffected, which in turn indicates that quinidine reduces the plasma clearance of the other drug. A reduction in plasma digoxin clearance has been found with many studies in man (Pedersen 1985). In rats receiving the higher dose of digoxin, the lower clearance appears primarily due to a quinidine-induced reduction of its biliary elimination, but decreased direct intestinal secretion and renal excretion probably also contribute. A marked reduction in biliary digoxin elimination along with a less marked decrease in urinary excretion was also noted by Sato et al (1983).

The minimal quinidine-induced elevation of digoxin-

derived plasma radioactivity associated with the low ($10\,\mu g\,kg^{-1}$) digoxin dose was unexpected, but seemed to be linked with a less marked inhibition of biliary elimination (Fig. 1). The lesser effect on the lower digoxin dose was surprising since if quinidine were competing for digoxin elimination it would be expected that the higher dose would be less affected.

The biliary excretion data of the present study resemble in some respects the results of Ben-Itzhak et al (1985) who examined the biliary elimination of digoxin in the rat isolated perfused liver and found that quinidine decreased digoxin excretion and increased hepatic digoxin concentration. However, in contrast to the present work, those workers reported that lowering (halving) the digoxin dose increased the effect of quinidine on biliary excretion.

While quinidine has an inhibitory effect on the total amount of digoxin and its metabolites excreted in the bile, the metabolite pattern is not altered. Perhaps this indicates that before any biliary excretion of digoxin and its metabolites is possible a certain degree of metabolism must occur. The effect of quinidine on digoxin metabolite profile has been examined in human urine (Schenck-Gustafsson & Dahlqvist 1981). The quinidine appeared to have little effect, the proportion of unchanged digoxin remaining at about 85%. The digoxin biliary metabolite profile obtained in the present study differs somewhat from that reported by Kitani et al (1985) (using a $100\,\mu g\,kg^{-1}$ dose of digoxin but a different strain of female rats) mainly in that we found a greater and lesser proportion of D_2 and digoxin, respectively.

Although the present experiments were not designed to examine the direct intestinal secretion of digoxin in detail, our findings are in line with those of George & Renwick (1982) who showed that quinidine inhibited the active transport of digoxin across the wall of the rat small intestine. There is also agreement with the findings of Schafer et al (1985) who perfused guinea-pig intestine in-situ. Those workers found that quinidine inhibited the secretion of digoxin into the lumen of the small intestine (jejunum) as well as reducing the digoxin concentration in the small intestine wall. The overall reduction in the elimination of digoxin and its metabolites caused by quinidine seems to be associated with a general trend for higher concentrations of tissue radioacitivity even with the lower digoxin dose where the effect on plasma levels and biliary elimination is less marked. The only clear exception to this trend is in the small intestine, where as found by Schafer et al (1985), lower tissue concentrations of drug are associated with quinidine treatment. The tendency for quinidine to raise tissue levels of digoxin and its metabolites in experimental animals has been reported by other workers (Doherty et al 1980; Sato et al 1983) and man (Jogestrand et al 1984).

In conclusion, while it is not clear why the digoxin-quinidine interaction should be less apparent in terms of changes in digoxin-derived plasma radioactivity with a lower digoxin dose, there are some possible consequences of such a dose-dependency. For instance, the interaction may not always be readily observable in terms of elevated plasma drug levels, although there may still be some elevation in tissue levels and enhancement of digoxin effect. Also, the equivocal results regarding the presence or absence of an analogous digitoxin-quinidine interaction (Kim et al 1981; Kuhlmann et al 1984; Schafer et al 1985) could be due to different relative cardiac glycoside doses used.

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