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THE INFLUENCE OF DIGOXIN-SPECIFIC ANTIBODY FRAGMENTS ON DIGOXIN DISPOSITION IN THE RAT

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Abstract—Pentobarbitone-anaesthetized bile duct-cannulated female rats were injected intravenously with an equimolar dose of digoxin-specific sheep antibody fragments (DS-Fab) at 2 or 60 min after a dose of [^{3}H]digoxin. The plasma drug levels were promptly elevated by 7-fold or 12–30-fold when the DS-Fab were given at 2 or 60 min respectively. When tissue drug concentrations were measured 2 min after a dose of DS-Fab (given 60 min after digoxin) which caused a 30-fold increase in plasma concentration, reductions could be detected if corrections were made for the presence in the tissues of high plasma concentrations of DS-Fab-bound drug. For instance, reductions in the heart, liver and small intestine were 63, 58 and 48% respectively. However, by 120 min after digoxin injection the only detectable effects on tissue drug concentration were in the kidney, where concentrations had increased 14-fold or 7-fold when the DS-Fab were given at 2 or 60 min respectively. Over the 120 min period the urinary excretion of digoxin-derived radioactivity was enhanced, and in the case where DS-Fab were given at 2 min, a 3-fold increase in urinary excretion was seen, which resulted in a net increase in the overall drug elimination. This greater urinary elimination was accompanied by a marked increase in the amount of bound drug in the urine (control and experimental values were 4 and 36% respectively). The cumulative biliary excretion of radioactivity seemed to be slightly reduced by DS-Fab administration at 2 or 60 min, although this was not statistically significant. A lack of significant drug-specific binding in the bile suggested that the liver is not involved in the elimination of hapten-DS-Fab complexes. There was little effect on the intestinal secretion of the drug.

DS-Fab†, hapten-binding fragments derived from antibodies raised in sheep, can be used to treat life-threatening overdosage associated with digitalis glycosides [1], having been recently marketed for this purpose (Digibind®, Wellcome). DS-Fab attract the drug away from cardiac and other tissue receptors to sequester it in the interstitial fluid and plasma [2]. Subsequently the DS-Fab-bound drug is filtered through the kidney glomerulus prior to urinary excretion [3]. The manufacturers recommend that an equimolar dose of DS-Fab can be initially used in the reversal of either digoxin or digitoxin toxicity. The effectiveness in the latter case is based on the fact that although during manufacture a digoxin–protein conjugate is used as an immunogen [4, 5], and the affinity of the antibodies produced is 10 times less for digitoxin than for digoxin, the affinity is still sufficiently high for use in digitoxin poisoning [6, 7].

In man, digitoxin undergoes more extensive hepatic metabolism than digoxin, with biliary elimination playing an important role [8, 9], and it has been suggested [6] that DS-Fab treatment diverts digitoxin elimination away from slow hepatic disposal towards rapid urinary excretion. Digoxin handling in the rat is intermediate between that of digoxin and digitoxin in man, with the unanaesthetized animal excreting about half of an administered dose of digoxin by non-urinary routes [10]. It thus appears suitable to use a rat/digoxin model to ascertain whether DS-Fab administration has any "diversionary" influence on cardiac glycoside elimination. Also, as there have been few studies describing post-DS-Fab tissue digoxin concentrations, a further reason for using a small animal model was to facilitate the measurement of tissue concentrations, particularly in the heart and excretory organs.

MATERIALS AND METHODS

Materials. 12α-^{3}H-digoxin (15.4 Ci/mmol) was obtained from New England Nuclear, Boston, MA. Tissue solubilizer (TS-1) was purchased from Koch-Light Ltd, Haverhill, Suffolk and liquid scintillant (NE260) was purchased from Nuclear Enterprises (Edinburgh, U.K.). Dextran (T-70) and digoxin, and D_0, D_1, D_2 were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.) and Uniscience Ltd (London, U.K.) respectively. All other reagents were obtained from British Drug Houses (Poole, Dorset, U.K.) and were of analytical grade.

Digoxin-specific Fab fragments were provided by Mrs Mary Andrews having been manufactured at the Regional Laboratories, Glasgow and West of Scotland Blood Transfusion Service (Law Hospital, Lanarkshire, UK) with the help of Dr. R. Fraser. They were prepared using methods largely based on those of previous workers [5, 11]. Digoxin-specific

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† Abbreviations: DS-Fab, digoxin-specific Fab fragments; Fab, fragment antigen binding; Fc, fragment crystallisable; D_0, digoxigenin; D_1, digoxigenin monodigitoxoside; D_2, digoxigenin bisdigitoxoside.

whole antiserum was obtained from sheep which had been previously immunised with a digoxin–human serum albumin conjugate. Immunoglobulin G was separated by the method of Steinbuch and Audran [12] and digested with papain to give Fab fragments. DS-Fab were separated by adsorption onto digoxin-sepharose. The Fab fragments were released from the digoxin-sepharose by treatment with KSCN which was in turn removed by dialysis with buffered saline. The Fab fragment preparation was shown by standard procedures (immunoelectrophoresis and sodium dodecylsulphate gel electrophoresis) to contain traces of Fc fragment but no immunoglobin G. The digoxin binding capacity and the mean affinity constant were about 8.6 μg/mg and 1 nM respectively, as determined by equilibrium dialysis [4]. The DS-Fab were stored as 1 ml aliquots in phosphate-buffered saline (pH 7.4) at −20° until required.

Methods

Disposition of radioactivity in anaesthetized bile duct-cannulated rats. Female Sprague–Dawley rats (200–270 g) with free access to food and water were used. The animals were anaesthetised with pentobarbitone (60 mg/ml, 60 mg/kg, i.p.) and after cannulating a carotid artery and jugular vein, the bile duct was cannulated essentially as described by Klaassen and Strom [13]. Rectal temperature was maintained at 37° by means of a heat-lamp regulator device (Yellow Springs Instrument Company, Yellow Springs, OH). All the rats were given [^{3}H]digoxin (10 μg/kg, 12.5 μCi/kg, i.v.). Experimental rats were given an equimolar dose of DS-Fab (0.5 ml/kg, 1.84 mg/ml, i.v.) at 2 or 60 min after the digoxin. Control animals were given diluted non-immune sheep serum (0.5 ml/kg, i.v.) at the respective times. Bile, and blood (0.25 ml), samples were collected at the times indicated in Results. In all experiments, the first blood samples after DS-Fab administration were taken 2 min after injection of the antibody. Saline was given i.v. to replace fluid lost as bile or blood. Blood was centrifuged (3000 *g* for 10 min) to obtain plasma.

In initial studies rats were killed 120 min after digoxin. However, in a subsequent study designed to examine tissue drug concentrations when DS-Fab appeared to be having its greatest effect on plasma levels, animals were killed 62 min after digoxin (and 2 min after DS-Fab). Tissues and biological material as indicated in Tables 1 and 2 were removed. Lymph nodes (facial, bronchial, mesenteric and popliteal) were pooled. The intestines ("small intestine", caecum and colon) were weighed before and after removing the contents (homogenised prior to assay). The "small intestine" was removed distal to the bile duct and therefore comprised jejunum and ileum with a small segment of duodenum. Samples (finely minced with scissors) of the "small intestine" were taken about 10 cm from the caecum, while samples of caecum and colon were taken from areas about mid-way along their lengths. Both urine voided during 2 hr and that remaining in the bladder was collected. To obtain a further estimate of urinary drug excretion, it was assumed that the proportion of the digoxin dose in the kidney, after subtracting the amount present in the plasma, represented the proportion of the dose in the urine remaining within the kidney. The values for plasma volumes for kidney and other tissues in the female rat were taken as those published by Everett *et al.* [14]. Radioactivity present in plasma, bile, urine, gut contents and tissues were determined by standard liquid scintillation counting methods as detailed previously [15]. Total radioactivity (which may have included digoxin metabolites along with unchanged digoxin) was expressed as digoxin equivalents.

Quantitation of [^{3}H]*digoxin metabolites in bile and urine.* 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 base line. The solvent system used [16] was ethylacetate, chloroform, acetic acid; 90, 5, 5 by volume, in which digoxin, D_2, D_1, and D_0 had R_f values of 0.52, 0.48, 0.42 and 0.38 respectively. In preliminary experiments, radioactivity was located using a radio-thin layer scanner (Panax Equipment Ltd., Redhill, Surrey, UK). Subsequently, chromatographic loci were visualised under u.v. light (wavelength, 245 nm) using a "Chromatolite" lamp (Hanovia Ltd, Slough, Bucks, UK), cut out and transferred to 5 ml NE260 scintillant for liquid scintillation counting.

With urine samples where radioactivity levels were lower, an organic solvent extraction/concentration procedure was required. Urine was extracted three times with 3 volumes of chloroform. Aliquots (0.05 ml) of the chloroform extracts and the final residual aqueous phase were taken for radioactivity determination to ascertain extraction efficiency. The combined chloroform extracts were evaporated to dryness and the residue taken up in 0.01 ml of a chloroform:methanol (1:1) mixture. This mixture and the residual aqueous phase were subjected to chromatography as described for the bile samples.

Determination of bound [^{3}H]*digoxin-derived radioactivity.* Aliquots (0.025 ml) of bile or urine were diluted with phosphate-buffered (pH 7.4) saline (0.475 ml) and 0.5 ml of dextran-coated charcoal (1.25% activated charcoal and 0.125% dextran in phosphate-buffered saline containing 0.1% gelatin) were added. The suspension was mixed and allowed to stand for 5 min and then centrifuged (2000 *g* for 15 min). Aliquots (0.3 ml) of the supernatant were taken for scintillation counting to determine radioactivity corresponding to bound digoxin. The percentage bound was calculated using the value for bound drug and the previously determined value for total radioactivity.

Statistics. All experimental data are means ± SE and data were analysed using a non-paired Student's *t*-test with a probability of $P < 0.05$ being taken as significant.

RESULTS

In initial experiments, DS-Fab given 60 min after [^{3}H]digoxin caused a twelve-fold increase in the plasma concentration of radioactivity with the levels still seven times higher than for controls after 120 min (Fig. 1A). This administration of DS-Fab appeared

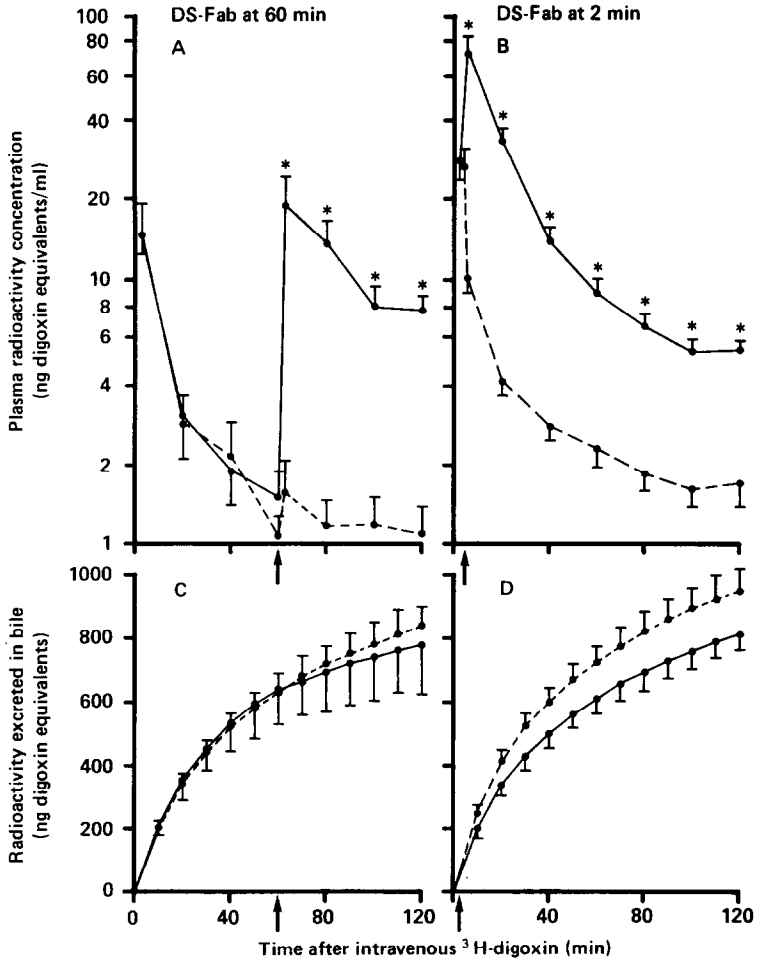

Fig. 1. The effect of DS-Fab on the plasma concentrations and cumulative biliary excretion of [^{3}H]digoxin-derived radioactivity in anaesthetised bile duct-cannulated rats. An equimolar dose of DS-Fab was given i.v. (indicated by arrows) 60 or 2 min after digoxin (10 μg/kg, 12.5 μCi/kg). The data points are means ± SE from 5 and 8 rats for the 60 and 2 min experiments respectively. Solid and dashed lines represent DS-Fab treated or control rats. Asterisks indicate a significant difference from control values ($P < 0.05$). The respective weights for experimental and control rats were 263 ± 26 and 261 ± 28 g (60 min experiment), and 236 ± 6 and 238 ± 12 g (2 min experiment).

Table 1. The effect of DS-Fab on the tissue concentrations of [^{3}H]digoxin-derived radioactivity after intravenous administration in the rat

	Concentration of radioactivity 120 min after digoxin (ng/g)†			
	DS-Fab at 60 min		DS-Fab at 2 min	
Tissue	Control	DS-Fab	Control	DS-Fab
Adrenal cortex	581 ± 125	611 ± 99	1090 ± 170	839 ± 220
Adrenal medulla	479 ± 110	740 ± 16	682 ± 58	527 ± 120
Liver	26.2 ± 7.3	20.3 ± 2.9	30 ± 3.4	32 ± 2.8
Small intestine	17.2 ± 3.9	19.5 ± 3.3	32.0 ± 7.3	25.7 ± 6.9
Caecum	13.4 ± 2.6	11.9 ± 1.6	23.8 ± 2.8	19.2 ± 2.0
Colon	15.2 ± 3.8	7.7 ± 2.4	14.3 ± 2.5	15.8 ± 4.5
Heart	7.9 ± 1.2	7.3 ± 0.6	8.8 ± 0.9	9.1 ± 1.0
Lymph nodes	5.8 ± 1.4	5.7 ± 1.2	6.4 ± 1.9	5.1 ± 1.1
Kidney	14.2 ± 9.7	102 ± 37*	5.8 ± 0.8	83 ± 7.9*
Lung	4.7 ± 1.7	7.1 ± 0.8	6.8 ± 1.0	8.3 ± 0.7
Brain	0.24 ± 0.07	0.28 ± 0.08	0.5 ± 0.1	0.4 ± 0.06

An equimolar dose of DS-Fab was given (i.v.) 60 or 2 min after [^{3}H]digoxin (10 μg/kg, 12.5 μCi/kg) to anaesthetised bile duct-cannulated rats (same animals referred to in Fig. 1). The means ± SE are from 5 and 8 rats for the 60 and 2 min experiments respectively. †Calculated in terms of digoxin equivalents. *Significant difference from control ($P < 0.05$).

Table 2. The plasma and tissue concentrations of [^{3}H]digoxin-derived radioactivity 2 min after intravenous DS-Fab administration in the rat

Tissue	Concentration of radioactivity 62 min after digoxin (ng/g)† Control	DS-Fab (at 60 min)
Plasma	1.7 ± 0.03	53.5 ± 2.9*
Adrenal cortex	308 ± 21.3	321 ± 25
Adrenal medulla	332 ± 26.5	321 ± 29
Liver	39.4 ± 6.1	26.3 ± 1.8
Small intestine	17.9 ± 2.9	11.0 ± 1.5
Caecum	11.9 ± 1.6	12.7 ± 1.9
Colon	13.3 ± 3.8	6.3 ± 1.2
Heart	11.0 ± 0.8	12.5 ± 0.8
Kidney	6.4 ± 0.5	25.6 ± 3.1*
Lung	9.0 ± 2.0	14.4 ± 2.1
Brain	0.53 ± 0.13	0.77 ± 0.14

An equimolar dose of DS-Fab was given (i.v.) 60 min after [^{3}H]digoxin (10 μg/kg, 12.5 μCi/kg) to anaesthetised bile duct-cannulated rats. At 62 min the rats were killed and plasma and tissue samples taken. The means ± SE are from 5 rats.

† Calculated in terms of digoxin equivalents. *Significant difference from control ($P < 0.05$).

to slightly decrease the biliary elimination of digoxin-derived radioactivity (Fig. 1C) although the change was not statistically significant. Giving the DS-Fab 2 min after the [^{3}H]digoxin elevated the already high concentrations of plasma radioactivity seven-fold (Fig. 1B) and after 120 min the concentrations were still three times higher than for controls. Injecting the DS-Fab at the earlier time appeared to further decrease the cumulative biliary excretion of digoxin-derived radioactivity (Fig. 1D), but again the reduction was not significant. At no time did DS-Fab administration affect the rate of bile flow (results not shown).

Apart from in the kidney DS-Fab, whether given at 60 or 2 min after [^{3}H]digoxin, appeared to have little effect on tissue concentrations of radioactivity at 120 min (Table 1). In the case of the kidney, concentrations were raised 7–14-fold by DS-Fab administration. The highest concentrations of radioactivity were found in the adrenal gland, with no significant difference being detected between the concentrations in the cortex and medulla.

In animals given DS-Fab 60 min after digoxin administration and killed 2 min later, the general tissue drug distribution at 62 min (Table 2) was similar to that at 120 min (compare "DS-Fab at 60 min" data, Table 1), with DS-Fab apparently only significantly changing the drug concentration in the kidney (4-fold increase). This was in spite of a 30-fold DS-Fab-induced elevation in plasma radioactivity.

Table 3 shows the effect of DS-Fab on the proportion of the digoxin dose eliminated during the 120 min experimental period. Without DS-Fab administration a major part of the dose was eliminated in the bile, with lesser and roughly similar portions being excreted in the urine and intestinal contents. The DS-Fab given at 60 min had no effect on the proportion of the digoxin dose excreted in the urine collected during 120 min, while DS-Fab given earlier (at 2 min) increased this about three-fold. In both experiments however, DS-Fab increased the percentage of the digoxin dose estimated to be present in urine within the kidney by 16–20-fold. Although DS-Fab administration had no significant effect on the amount of radioactivity eliminated via the bile or intestinal secretion, there seemed to be a trend for the antibody fragments to reduce elimination by these routes, particularly in the case of the bile. With the DS-Fab given at 2 min however, this trend was more than compensated for by the increased urinary excretion.

In the experiment in which DS-Fab were given at 2 min, bound radioactivity was measured in the bile and urine. In the urine collected, the amount of radioactivity bound was significantly higher in experimental compared with control rats; the values were 36 ± 4.5 and 3.9 ± 0.2% respectively. For the pooled bile collected during 2 hr, the corresponding values were 7.3 ± 0.5 and 7.9 ± 0.5%. Analysis of individual bile samples taken at 10 min intervals during the course of the experiment revealed no changes in the proportion bound with time.

For the same experiment, the radioactivity in the urine, and the 10 and 20 min bile samples was analysed by thin-layer chromatography. Of the urinary radioactivity, about 90% was extractable into chloroform, the other 10% comprising unidentified polar material which remained on the baseline of the chromatogram. The percentage of unchanged digoxin in the urine of both experimental and control rats was approximately 88%. In the bile the amounts of unchanged drug and polar metabolites were about 78 and 12% respectively, with no differences being detected between the control and experimental rats, or between the 10 and 20 min samples. Apart from unchanged digoxin and the polar material, the radioactivity in the urine and bile comprised mainly D_2 with only traces of D_1 and D_0 being detected.

DISCUSSION

The rapid and striking elevations in the concentrations of plasma digoxin-derived radioactivity (Fig. 1, Table 2) after DS-Fab injection reflect the presumed redistribution and equilibriation process occurring between tissue-bound and DS-Fab-bound

Table 3. The effect of DS-Fab on the elimination of [^{3}H]digoxin-derived radioactivity after intravenous administration in the rat

Route of elimination	Percentage of digoxin dose eliminated after 120 min			
	DS-Fab at 60 min		DS-Fab at 2 min	
	Control	DS-Fab	Control	DS-Fab
Biliary	33.4 ± 2.9	29.5 ± 4.2	40.5 ± 3.2	34.5 ± 1.8
Urinary (collected urine)†	8.7 ± 1.0	8.4 ± 1.3	5.0 ± 0.8	16.3 ± 1.5*
Urinary (urine within kidney)‡	0.26 ± 0.03	5.36 ± 2.0*	0.29 ± 0.03	4.88 ± 0.5*
Intestinal secretion	6.5 ± 0.7	5.1 ± 0.6	7.0 ± 1.0	6.5 ± 1.0
Total	49	48	53	62

An equimolar dose of DS-Fab was given (i.v.) 60 or 2 min after [^{3}H]digoxin (10 μg/kg, 12.5 μCi/kg) to anaesthetised bile duct-cannulated rats (same animals referred to in Fig. 1 and Table 1). †Calculated from urine collected during 2 hr. ‡Estimated from kidney concentrations after subtracting radioactivity present in plasma (see Materials and Methods). The means ± SE are from 5 and 8 rats for the 60 and 2 min experiments respectively). *Significant difference from control ($P < 0.05$).

drug. Such changes in plasma concentration have been well documented for digoxin in man [17, 18], dogs [3] and guinea pigs [19]; and for digitoxin in man [7, 20, 21], dogs and rhesus monkeys [6]. The roughly parallel concentration data for the experimental and control rats seen in Fig. 1B suggest that DS-Fab do not drastically affect the plasma digoxin elimination half-life. This agrees with findings in some other species. For instance in man, the plasma half-life of the DS-Fab-digoxin complex has been reported to be 14–30 hr [18, 22] while that of digoxin is about 36 hr [9]. Furthermore in the mouse, plasma digoxin half-lives with and without DS-Fab are 4.3 and 2.9 hr respectively [15]. In contrast, DS-Fab reduces the plasma half-life of digitoxin to 5–10% of its normal values in man [20, 23] and rhesus monkeys [6].

The DS-Fab induced enhancement of urinary digoxin excretion most clearly seen when the antibody fragments were given at 2 min (Table 3), has also been observed in guinea pigs [19]. On the other hand, DS-Fab appear not to significantly increase the rate of excretion in dogs [3] and in man the situation has not been clearly established [18, 22]. With digitoxin, however, DS-Fab will markedly increase the urinary excretion rate in man [20] or rhesus monkeys [6].

While the amount of digoxin-derived radioactivity excreted in the urine was increased some three-fold by giving DS-Fab at 2 min (Table 3), the protein-bound material only represented about one-third of the radioactivity in the urine. To account for the degree of enhancement seen, roughly two-thirds of the drug would have been expected to be bound to DS-Fab. It is possible that this discrepancy can be partly explained on the basis that some of the filtered DS-Fab was catabolised in the proximal tubule [24, 25] resulting in a release of previously-bound digoxin into the urine. However, for a detailed assessment of this aspect of DS-Fab/digoxin disposition, improved methodology would have to be employed, including catheterization of the bladder/ureters and infusion of fluid to increase urine flow.

Although in the present experiments DS-Fab had no significant effect on the biliary excretion of digoxin-derived radioactivity, there was a trend for this to be reduced with antibody treatment. The trend would probably have become significant if DS-Fab had been given in higher doses with a more prolonged infusion time. A relative lack of effect of DS-Fab on biliary digoxin elimination has also been shown in preliminary experiments using guinea pigs, in contrast to the marked reduction demonstrated after active immunisation with a digoxin-protein conjugate [19]. Unlike with urine, it seems that heterologous DS-Fab prepared from immunoglobulin G are not filtered/transported into the bile, since digoxin-specific binding in bile samples was similar in those from control and experimental rats. The preponderance of unchanged digoxin with a relative lack of metabolites in the bile of young adult female rats has similarly been reported by Kitani *et al.* [26]. The present work indicates that the proportion of metabolites found in the urine is also low.

The highest tissue concentration of digoxin found, both in control and experimental rats, was in the adrenal glands. This high concentration has been previously noted in rats for both digoxin [27, 28] and digitoxin [29, 30] as well as for digoxin in mice [15]. However, similarly high concentrations do not occur with ouabain [28] and nor are digoxin and digitoxin concentrated in the adrenal glands of dogs [27] or guinea pigs [28]. The roughly similar concentrations of drug in the adrenal cortex and adrenal medulla argue against a postulated [15, 29] specific steroid uptake mechanism based on the similarity in structure between digoxin/digitoxin and adrenocorticosteroids, since this would presumably favour higher concentrations in the adrenal cortex. The very high lymph node concentrations of digoxin-derived radioactivity reported for the mouse [15] do not appear to occur in the rat.

Apart from in the kidneys, DS-Fab treatment appeared to have little effect on tissue concentrations of digoxin-derived radioactivity. However, in the rats killed 2 min after administration of DS-Fab (Table 2), if the tissue concentrations are corrected for plasma drug content (which may be relatively high in the case of DS-Fab treated rats), some DS-Fab-induced reductions in tissue radioactivity can be detected. For instance, significant reductions are obtained in the heart (63%, 10.7 ± 0.8 vs 3.9 ±

0.7 ng/g), liver (58%, 39.0 ± 6.1 vs 16.4 ± 1.5 ng/g) and small intestine (48%, 17.9 ± 2.9 vs 9.6 ± 1.4 ng/g). On the other hand, where tissue concentrations were measured at rather later times after DS-Fab, applying corrections for the presence of plasma digoxin do not reveal similar significant differences between control and experimental rats. For instance, where the DS-Fab were given at 60 min and the rats were killed at 120 min (Table 1) the "corrected" cardiac concentrations are 7.7 ± 1.2 and 6.1 ± 0.5 ng/g for control and DS-Fab-treated rats respectively. It should be remembered that although DS-Fab-induced reduction in tissue drug concentration can be detected shortly after DS-Fab administration, the full extent of digoxin redistribution is not seen, since a key component in the toxicity reversal effect of DS-Fab is the binding of the cardiac glycoside to the antibody fragments in the interstitial fluid. Such intra-tissue redistribution would not have been detectable with the methods used in this study.

In contrast with the other tissues, in the kidney (the main excretory organ for DS-Fab), high drug concentrations were found in the antibody-treated animals. This feature, which has also been observed in guinea pigs [19], is presumed to be due to the presence of large amounts of DS-Fab-bound digoxin in the glomerular filtrate. A marked increase (3-fold after "correction" for plasma digoxin) in kidney digoxin-derived radioactivity was observed in rats within a few minutes of DS-Fab injection, indicating that as well as the prompt reduction in many tissue drug levels, there is a rapid enhancement of renal drug elimination. Unlike the kidney, the liver was not associated with high concentrations of digoxin-derived radioactivity after DS-Fab treatment, which further indicates, along with the finding that there was no significant digoxin-specific binding activity in the bile, that the liver is not involved in the elimination of the hapten-Fab complex.

Although rapid drug-specific Fab-induced redistribution is a crucial feature of toxicity reversal, obviously the subsequent removal of the drug from the body is of great importance. The speed at which this occurs depends on the rate at which the Fab-bound drug is excreted by the kidney. Whether or not the elimination of a drug hapten is enhanced presumably depends on the relative Fab/drug elimination rates in the particular animal species studied, assuming that the Fab–drug complex is filtered by the glomerulus at the same rate as the antibody fragments alone. It is assumed that the filtration of the monovalent antibody fragments is unaffected by the bound hapten because digoxin has a molecular weight only one-eightieth that of DS-Fab. In the bile duct-cannulated rat model used in the present study, DS-Fab seemed capable of increasing the net elimination of digoxin via enhanced urinary excretion, even though excretion by non-renal routes may have been reduced. In man knowledge on the clearance of Fab fragments is limited, and in view of the likelihood of their increasing use in treating poisoning due to cardiac glycosides and possibly other toxins, more detailed information in this area would be of value.

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