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Digoxin-specific Fab fragments impair renal function in the rat

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Abstract—The effect on renal function, and the plasma and urinary disposition, of digoxin-specific antibody fragments (DSFab), were studied using the rat as an experimental model. After 24 h, DSFab (2 mg kg^{-1}, i.v.) caused decreases in urine volume and creatinine clearance of 34 and 33%, respectively, when measured in the same rats. However, only the creatinine clearance was significantly changed when compared with a separate saline-treated control group. Plasma and urinary creatinine concentrations were unaffected by DSFab treatment. Since creatinine clearance approximates to glomerular filtration rate (GFR), it appears that a dose of DSFab equivalent to about one-fifth of the usual clinical dose, causes a reduction in GFR of about one-third. In patients undergoing digitalis therapy, a degree of renal impairment is common and it is possible that this may be exacerbated by treatment with DSFab. DSFab had an elimination half-life of 178 min, an apparent volume of distribution (Vd) of 106 mL kg^{-1} and a plasma clearance of 0·42 mL kg^{-1} min^{-1}. If it is assumed that the plasma volume of a rat is approximately 35 mL kg^{-1}, the measured Vd suggests appreciable penetration of DSFab into the extracellular fluid at this dose. Seventy-two hours after injection, only 7·6% of the administered dose of DSFab was found in the urine.

Digoxin-specific antibody fragments (DSFab) are used clinically in the treatment of severe cardiac glycoside toxicity (Smith 1991). After intravenous injection, DSFab attract the drug away from cardiac and other tissue receptors and sequester it in the interstitial fluid and plasma, producing a rapid reversal of the life-threatening situation. Due to its sufficiently low mol. wt (Butler et al 1977) the DSFab-drug complex can then be filtered through the kidney glomerulus before urinary excretion. Consequently, adequate renal function is a key requirement in the elimination of DSFab along with the bound cardiac glycoside.

In earlier studies with rabbits, Timsina & Hewick (1992a) showed that a dose of DSFab (2 mg kg^{-1}), equivalent to about one fifth of the clinical dose, appeared to reduce renal function. It was, therefore, decided to investigate the effect of DSFab on renal function further, using the rat, particularly as other workers (Pentel et al 1988) have reported that large doses of Fab have no adverse effect on renal function in this species. As in an earlier study (Timsina & Hewick 1992b), the disposition of DSFab in the plasma and urine was also monitored.

Materials and methods

Materials. Digoxin-specific Fab fragments, affinity purified with digoxin-sepharose, derived from anti-digoxin immunoglobulin G raised in sheep, were manufactured by Johnston et al (1988). Donkey anti-sheep immunoglobulin G (IgG) and donkey serum were supplied by the Scottish Antibody Production Unit, Carluke, Lanarkshire, UK. Donkey anti-sheep IgG-alkaline phosphatase conjugate, *p*-nitrophenyl phosphate (dicyclohexylammonium salt) and the creatinine measurement kit (Sigma Technical Bulletin No. 555) were purchased from Sigma Chemical Co., Poole, Dorset, UK. Polystyrene Maxisorp microtitre plates were bought from Gibco, Paisley, Renfrewshire, UK. All other reagents were obtained from British Drug Houses, Poole, Dorset, UK, and were of analytical grade unless otherwise stated.

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Procedures in conscious rats. Male Sprague-Dawley rats, 250–400 g, were allowed free access to food and water throughout the study. Blood was sampled (1 mL, for creatinine assay) from the tail vein on the afternoon of day 1, the rats were then placed in a metabolism cage overnight (18 h). The following morning, the urine was collected and the rats received a water load (20 mL kg^{-1}, p.o.) with either DSFab dissolved in saline (2 mg kg^{-1}, 1·09 mL kg^{-1}, i.v., n = 9), or saline alone (1·09 mL kg^{-1}, i.v., n = 6, control group). Blood was sampled (0·3 mL, for DSFab assay) from the tail vein at 15, 30, 60, 120, 240, 360 min, and urine collected at 2 and 6 h after injection of DSFab or saline. Further blood (1 mL) and urine samples were taken 24 h after DSFab or saline administration. The rats were kept in the metabolism cage and the urine collected for a further 48 h. Blood samples were centrifuged (10 000 *g*, 15 min, 21°C) and plasma and urine were stored at −20°C.

Creatinine and sodium assays. Creatinine was measured using a standard kit (Sigma). The method involved addition of alkaline picrate solution (3 mL) to samples (0·3 mL) of plasma, diluted urine (1 in 20), standards (creatinine 3 mg/100 mL) and blanks (distilled water). After 10 min, the absorbance (500 nm), using the blank as a reference, of all solutions was recorded (Initial A). Five minutes after the addition of 0·1 mL acid reagent, the absorbance was again measured (Final A). Sample creatinine concentration was calculated using the following formula:

$$\frac{\text{Initial A}_{\text{sample}} - \text{Final A}_{\text{sample}}}{\text{Initial A}_{\text{standard}} - \text{Final A}_{\text{standard}}} \times 3 \qquad (1)$$

Creatinine clearance was determined by dividing the product of urine volume and urinary creatinine by plasma creatinine.

Urinary Na^+ was determined using a flame photometer (Instrumentation Laboratory 943).

Enzyme-linked immunosorbent assay (ELISA) for DSFab. Plasma and urine DSFab concentrations were determined using a sheep protein-specific ELISA described by Timsina & Hewick (1990).

Pharmacokinetic and statistical analysis. Elimination half-life ($t_{\frac{1}{2}\beta}$) and elimination rate constant (K_{el}) for each rat were calculated from linear regression of the terminal part (30–360 min) of the DSFab elimination curve when plotted semi-logarithmically; area under the curve (AUC) was calculated by the trapezoidal rule. Apparent volume of distribution (Vd) was calculated by two methods. Vd_{extrap} was obtained by extrapolating to zero time and dividing the dose of DSFab by its zero-time concentration; Vd_{AUC} was obtained by dividing the dose of DSFab by the product of K_{el} and AUC.

Significant differences ($P < 0.05$) before and after treatment (in the same rat) were detected using Student's paired *t*-test, and those between treatments (saline vs DSFab) using Student's unpaired *t*-test. Means ± s.e.m. are given.

Results

Renal function. Table 1 shows the effect of 2 mg kg^{-1} DSFab on plasma and urinary creatinine concentrations, urine volume

Table 1. Effect of DSFab administration (2 mg kg^{-1}) on creatine in rats.

Measurement	Saline-treated		DSFab-treated	
	Before	After 24 h	Before	After 24 h
Plasma creatinine (mg/100 mL)	0·50 ± 0·05	0·55 ± 0·04	0·48 ± 0·06	0·54 ± 0·09
Urinary creatinine (mg/100 mL)	91·6 ± 9	99·9 ± 15·7	88·4 ± 13·3	92·1 ± 13·3
Urine volume (mL kg^{-1}/18 h)	32·4 ± 3·8	27·8 ± 4·4	36·1 ± 7·0	23·7 ± 2.8[a]
Creatinine clearance (mL kg^{-1} h^{-1})	300 ± 47	275 ± 54	363 ± 69	242 ± 48[ab]

[a] $P < 0·05$ compared with pre-treatment value; [b]difference between pre- and post-treatment values significant ($P < 0·05$) when compared with saline-treated group.

and creatinine clearance. In rats treated with DSFab, the creatinine clearance after 24 h was reduced by 33% ($P < 0·05$). This parameter was also lower (after 24 h) in the DSFab-treated rats than in those treated with saline ($P < 0.05$). None of the other parameters was altered significantly by the treatment when compared with the saline-treated control group, although there was a 34% ($P < 0.05$) decrease in urine volume after treatment, within the Fab-treated group. Overnight (18 h) urinary Na^+ excretion was not significantly affected by DSFab. Before and after treatment values were: control, 3·53 ± 0·56 and 3·67 ± 0·86 mmol kg^{-1}; DSFab (n = 8), 4·30 ± 0·8 and 3·49 ± 0·4 mmol kg^{-1}, respectively.

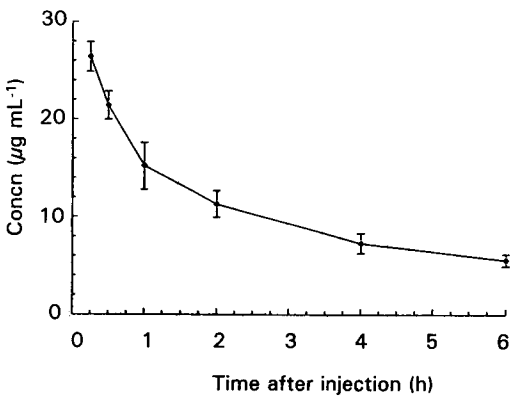

FIG. 1. Plasma DSFab concentration in rats after an intravenous dose of 2 mg kg^{-1} (n = 6). Means ± s.e.m. are shown.

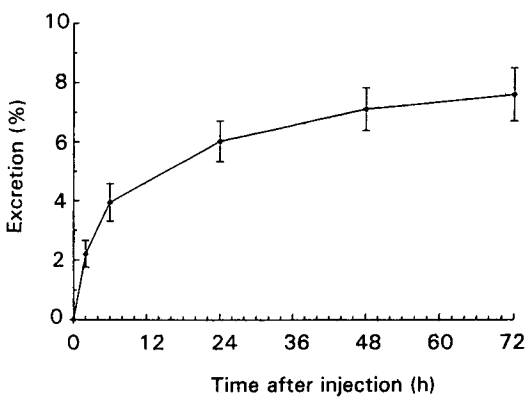

FIG. 2. Urinary elimination of DSFab in rats after an intravenous dose of 2 mg kg^{-1} (n = 6). Means ± s.e.m. are shown.

DSFab pharmacokinetics. Fig. 1 shows the plasma elimination of DSFab. The calculated pharmacokinetic parameters were: $t_{\frac{1}{2}\beta}$, 178 ± 6 min; Vd_{extrap}, 106 ± 13 mL kg^{-1}; Vd_{AUC}, 100 ± 10 mL kg^{-1}; plasma DSFab clearance, 0·42 ± 0·05 mL kg^{-1} min^{-1}. Fig. 2 shows the cumulative urinary excretion of DSFab over 72 h after administration, after which time 7·6 ± 0·9% of the injected dose had appeared in the urine as immunodetectable sheep protein.

Discussion

If it is assumed that creatinine clearance is approximately equal to glomerular filtration rate (GFR) (Brenner & Hostetter 1983), then 2 mg kg^{-1} DSFab, which is equivalent to about one-fifth of the usual clinical dose, appears to decrease GFR by about 33%. This is in agreement with the finding of Timsina & Hewick (1992a) in rabbits, where a 30% reduction in creatinine clearance was produced by the same dose of DSFab. In that study, treatment caused a decrease in urine flow as well as an increase in urinary creatinine concentration. In the present study, no such increase in urinary creatinine was observed. Within the DSFab treatment group, DSFab caused a decrease in overnight (18 h) urine volume, but this was not significantly different from the equivalent parameter in the saline-treated control group. The results show a greater reduction in creatinine clearance with DSFab treatment than with saline treatment, whereas urine volume and plasma and urine creatinine concentrations are unchanged by DSFab, when compared with saline treatment. It seems likely that the observed decrease in creatinine clearance may simply be due to a summation of non-significant trends in these other parameters, the most important of which appears to be urine volume.

Our results show some agreement with those of Keyler et al (1991), in which a transient reduction of creatinine clearance in dogs was observed after a very large dose of Fab (3·2–5·3 g kg^{-1}). An earlier paper from the same research group (Pentel et al 1988), again using very large doses of Fab (7·5 g kg^{-1}), found an increase in creatinine clearance of 73% in rats. However, in rabbits, Timsina & Hewick (1992a) recorded that, after the initial decrease, the residual effect of Fab on creatinine clearance had disappeared after five days. The extremely large doses of Fab (300–500 times the amount used to treat clinical digoxin poisoning) used in the studies of Pentel et al (1988) and Keyler et al (1991), make comparison with our results and extrapolation to the clinical situation difficult.

Urinary Na^+ concentration was unchanged by DSFab treatment in the present study. From this, a lack of change in fractional excretion of Na^+ may be inferred; however, since plasma Na^+ was not measured this cannot be stated with any confidence.

The calculated pharmacokinetic parameters for a dose of $2\,mg\,kg^{-1}$ DSFab of $t_{\frac{1}{2}\beta} = 178 \pm 6\,min$ and $Vd_{extrap} = 106 \pm 13\,mL\,kg^{-1}$ were roughly twice those of Johnston et al (1988), calculated by the same method using a dose of $1\,mg\,kg^{-1}$ in anaesthetized rats. In the present study, the more acceptable method of calculating Vd using area under the curve (Gibaldi & Perrier 1982) gave similar values to Vd_{extrap}. This dose dependency of Fab pharmacokinetics in rats is consistent with the reports of Pentel et al (1988) and McClurkan et al (1993), in which substantially larger doses of Fab than those used in the present study were associated with high values for $t_{\frac{1}{2}\beta}$ and Vd. In rabbits, increasing the dose of Digibind from 1 to $1{\cdot}9\,mg\,kg^{-1}$ increased the Vd in the same proportion but had no effect on $t_{\frac{1}{2}\beta}$ (Timsina & Hewick 1990, 1992b). As the plasma volume of a rat is approximately $35\,mL\,kg^{-1}$ (Waynforth 1980), a Vd value of $106\,mL\,kg^{-1}$ suggests appreciable penetration of DSFab into the extracellular fluid with the $2\,mg\,kg^{-1}$ dose, which could become more marked with further dose increases.

The small percentage of the injected dose of DSFab detected in the urine ($< 8\%$) is in accordance with existing data for small mammals (Johnston et al 1988; Timsina & Hewick 1992b) and man (Schaumann et al 1986), and is consistent with reports that Fab fragments may undergo extensive reabsorption and catabolism by proximal tubules after glomerular filtration (Spiegelberg & Weigle 1965; Janeway et al 1968; Arend & Silverblatt 1975; Keyler et al 1991).

In a recent post-marketing surveillance study (Smith 1991), three-quarters of patients receiving DSFab had some degree of pre-existing renal dysfunction and of these, 32% had severe renal failure. In the present study, normal renal function was, at least temporarily, impaired by DSFab and it is possible that this adverse effect may be greater where renal dysfunction already exists. Important information would be gained by measurement of renal function in man after administration of clinically-used doses of Fab. Change in creatinine clearance is equal to change in GFR only if renal tubular secretion of creatinine remains unchanged; in the present study it is assumed that this was not altered. In future investigations inulin clearance may be a more accurate determinant of GFR.

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