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Digoxin-specific Fab fragments impair renal function in the rabbit

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Abstract—A 2 mg kg^{-1} intravenous bolus dose of digoxin-specific Fab fragments produced a 28% reduction in creatinine clearance in rabbits after 24 h. Urine output was reduced, while plasma and urinary creatinine concentrations were unaffected and increased, respectively. By 5 days the creatinine clearance had returned to normal. The fractional excretion of Na^+ was nearly halved, indicating that the tubular reabsorption of Na^+ increased to compensate for the reduced glomerular filtration rate, suggesting that tubular (as opposed to glomerular) function was not impaired.

Digoxin-specific antibody fragments (DSFab) have an established use in severe cardiac glycoside toxicity (Smith 1991). After intravenous injection, the DSFab distribute into the extracellular fluid and attract the drug away from the cardiac and other receptors, sequestering it in the tissue fluid and plasma to produce a rapid reversal of the life-threatening symptoms. Subsequently, the complex of DSFab and bound drug, being of relatively low molecular weight (Butler et al 1977), is filtered through the kidney glomerulus before urinary excretion. Consequently therefore, it is assumed that adequate renal function is important in the ultimate elimination of DSFab and the cardiac glycoside.

However, in preliminary studies investigating DSFab/digoxin stoichiometry using nontoxic digoxin doses in rabbits, we noted that DSFab appeared to reduce renal function. Since the amount of DSFab being given (2 mg kg^{-1}) was equivalent to about one-fifth of the suggested clinical dose, we decided to carry out the following study to provide more information on this impairment.

Materials and methods

Materials. Digoxin-specific Fab fragments (lyophilized powder, Digibind), derived from anti-digoxin immunoglobulin G raised in sheep, were received as a gift from the Wellcome Foundation Ltd. The creatinine measurement kit (Sigma Technical Bulletin No. 555) was purchased from Sigma Co. All other reagents were obtained from British Drug Houses, Dorset, UK, and were of analytical grade, unless otherwise stated.

Procedures in conscious rabbits. The same animals (female New Zealand White rabbits, 3·2–5·1 kg) were used throughout and were housed in metabolic cages for 7 days before and after DSFab, with free access to food and water.

Urine was collected for 24 h with a blood sample being taken at about 12 h. Animals were injected intravenously (infusion time 1 min) with 2 mg kg^{-1} DSFab and further 24 h urine collections and midpoint 24 h blood samples were obtained for 5 days. Blood or urine samples were centrifuged (3000 *g*, room temperature (21°C), 15 min) and stored as aliquots at −20°C.

Determination of creatinine concentrations and clearance. Creatinine was measured using a standard kit (Sigma Co.). Essentially, it involved addition of 3 mL of alkaline picrate solution to 0·3 mL samples (plasma and diluted urine (1 in 25)), or standards (creatinine 3 mg mL^{-1}) and blanks (distilled water). After 10 min, the absorbance (500 nm) of all solutions was recorded (Initial A). Five minutes after the addition of 0·1 mL of acid reagent, the absorbances of the above solutions were measured (Final A). Blank absorbances were subtracted from standard or sample absorbance and the following formula was used to calculate sample creatinine concentration:

$$\frac{\text{Initial A}_{\text{sample}} - \text{Final A}_{\text{sample}}}{\text{Initial A}_{\text{standard}} - \text{Final A}_{\text{standard}}} \times 3$$

Creatinine clearance (mL min^{-1}) was determined by dividing the product of urine volume (mL min^{-1}) and urinary creatinine (mg mL^{-1}) by plasma creatinine (mg mL^{-1}).

Determination of plasma and urine sodium and fractional excretion of sodium. Plasma and urine Na^+ was analysed using a flame photometer (Instrumentation Laboratory 943). The fractional excretion of Na^+, which is sodium clearance expressed as a percentage of glomerular filtration rate (GFR), was calculated by dividing the product of urinary Na^+ and plasma creatinine concentration by the product of plasma Na^+ and urine creatinine concentration and multiplying by 100.

Statistical analysis. To test for significant differences ($P < 0.05$), the Wilcoxon Signed Rank test (paired, two-tailed) was used.

Results

Fig. 1 shows the effect after 24 h of 2 mg kg^{-1} DSFab on plasma and urinary creatinine, urine volume and creatinine clearance. Two out of three parameters determining the creatinine clearance are significantly ($P < 0.05$) altered after DSFab resulting in a net 28% reduction in creatinine clearance. The reduction in urine volume was associated with an increase in urinary creatinine concentration (Fig. 1). The daily creatinine excretion values before and after DSFab were 192.3 ± 54.3 and 195.9 ± 83.9 mg, respectively. Renal function recovered to control levels 5 days after DSFab injection.

Fig. 2 illustrates plasma Na^+, urinary Na^+ and fractional excretion of Na^+ in individual rabbits pre- and post-treatment. Plasma concentration of Na^+ was entirely within the normal mammalian range and DSFab injection did not produce any significant changes (0.141 ± 0.001 vs 0.137 ± 0.004 mmol mL^{-1}). Urinary Na^+ concentrations were significantly reduced after DSFab in all rabbits used (0.127 ± 0.011 vs 0.065 ± 0.020 mmol mL^{-1}). This resulted in clear reductions in the fractional excretion of Na^+ in 4 out of 6 rabbits, with a net reduction of 48% ($P < 0.05$).

Discussion

Since it is well established that creatinine clearance closely approximates the GFR (Brenner & Hostetter 1983), it follows that the DSFab treatment reduced GFR by about 30%.

Our findings are in contrast with those of Pentel et al (1988) using rats. Those workers found that very large doses of human Fab (7·5 g kg^{-1}) increased creatinine clearance by 73%. However, those measurements were made one week after Fab

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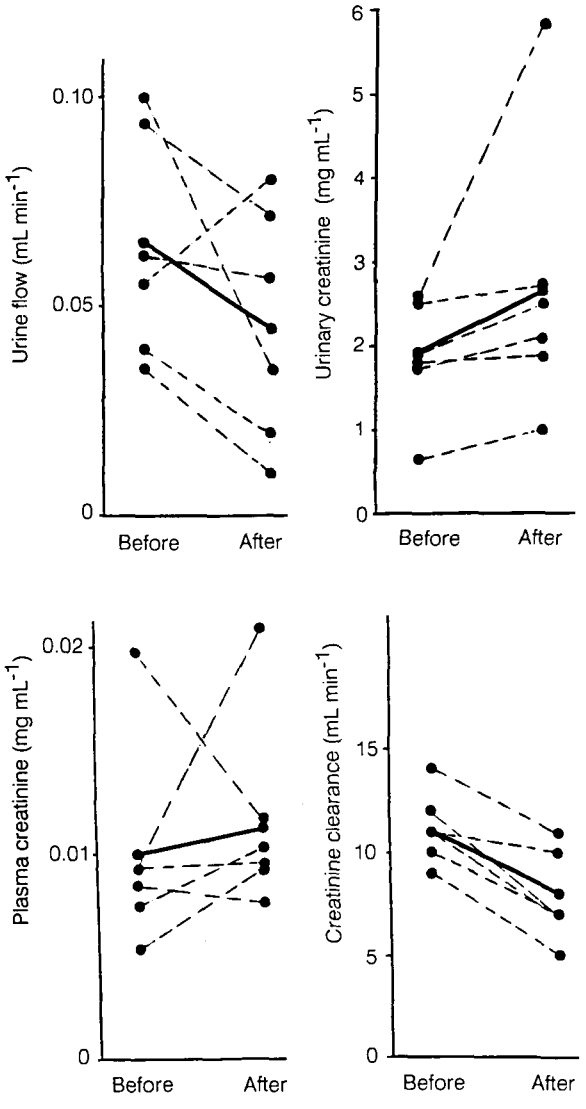

FIG. 1. The effect of DSFab on urine volume, urine creatinine, plasma creatinine and creatinine clearance in rabbits 24 h following a 2 mg kg^{-1} i.v. dose. The observation in individual rabbits is shown by broken lines while the solid lines represent the mean (n = 6).

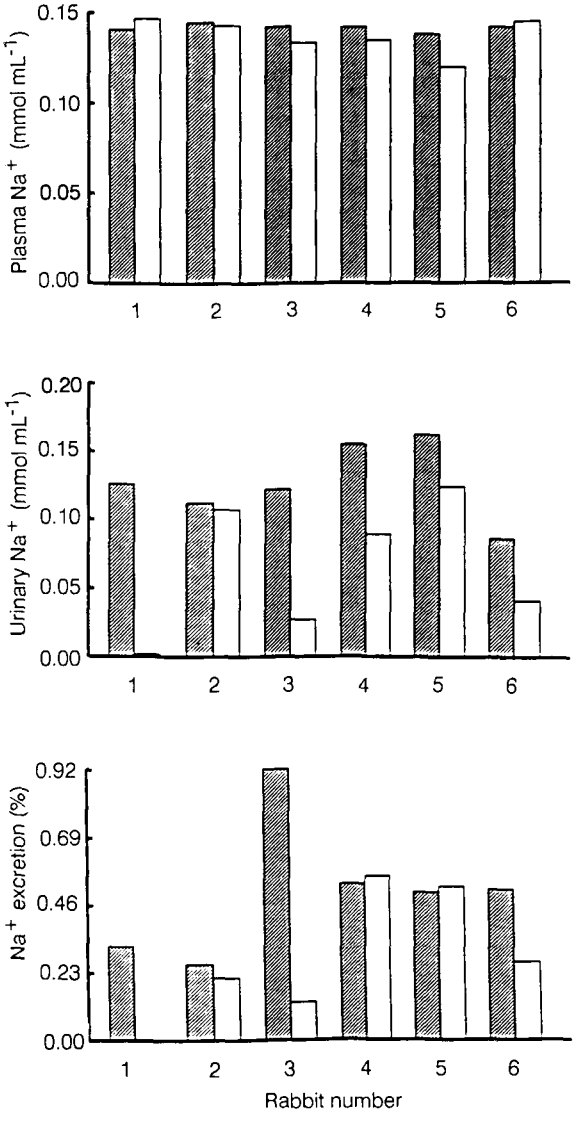

FIG. 2. Plasma and urinary sodium concentrations and fractional excretion of sodium measured before (■) and 24 h after (□) 2 mg kg^{-1} DSFab (i.v.) in individual rabbits.

administration. In our experiments creatinine clearance had normalized after 5 days. After the initial submission of the present communication, we became aware of a further paper by the same research group (Keyler et al 1991), which this time described a Fab-induced reduction in creatinine clearance. This study in dogs, while broadly agreeing with our findings, involved considerable differences in experimental design. Apart from the different animal species used, the dose of Fab injected was again very large (3·2–5·3 g kg^{-1}) and equivalent to about 300 times the amount used to treat clinical digoxin poisoning. There is some similarity between the findings of Keyler et al (1991) and Schifferli et al (1991) who showed that patients with renal dysfunction receiving high-dose (0·4 g kg^{-1}) intravenous immunoglobulin G treatment showed a transient rise in plasma creatinine.

Recently, using rats, Darling & Morris (1991) suggested that when the standard procedures for assaying creatinine (which also detect non-creatinine chromogens) are used, subsequently calculated clearance values reflect both the glomerular filtration and tubular secretion of creatinine. The consequence of non-specific creatinine assays in determining creatinine clearance in other species remains to be evaluated and in future more specific methods may have to be employed (Meyer et al 1985). However, these considerations do not alter the impact of the present findings suggesting that small doses of DSFab adversely affect renal function.

The present finding that the fractional excretion of sodium was decreased, implied that sodium reabsorption by the tubules compensated for the reduced GFR, suggesting that tubular function is not impaired. The high-Fab dose study of Keyler et al (1991) indicated that fractional sodium excretion was unchanged although (as in the present study) urinary sodium concentration was reduced.

For DSFab in rabbits, the filtration fraction (renal clearance divided by GFR multiplied by 100) is only 2% (using the renal clearance value of Timsina & Hewick (1992)) suggesting a poor degree of filtration. However, extensive degradation of Fab (only 3% of an administered dose of DSFab was detected in the

urine (Timsina & Hewick 1992)) may have a considerable influence. The degradation will lower the detectable concentration of DSFab in the urine, which will decrease the calculated clearance and hence the filtration fraction. However, whatever proportion of renally delivered DSFab is filtered by the glomerulus, it seems that they impair glomerular function and may interfere with their own elimination.

In our study, normal renal function was affected and the possibility exists that where impairment is already present, the adverse effect may be more marked. This is relevant in view of the finding in a recent postmarketing surveillance survey (Smith 1991) that three-quarters of patients receiving DSFab had some degree of renal dysfunction and 32% of these patients had severe renal failure. It is therefore important that renal function is thoroughly investigated after the administration of clinically-used doses of DSFab. These investigations may include the measurement of inulin clearance for the more accurate determination of GFR and the inclusion of suitable control animals injected with normal saline (vehicle in which DSFab are normally dissolved).

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Effects of selective histamine receptor antagonists on skin responses to intradermal bradykinin in healthy volunteers

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Abstract—The effects of chlorpheniramine and cimetidine on the cutaneous responses to intradermal injections of bradykinin were investigated in a randomized, double-blind, placebo-controlled, cross-over study. Chlorpheniramine significantly attenuated the increase in cutaneous blood flow and erythema induced by bradykinin but not the weal response. Cimetidine was without influence on these parameters and the effects of the combined therapy of chlorpheniramine and cimetidine were not significantly different from those due to chlorpheniramine alone. These results suggest that the cutaneous vasodilator effect of bradykinin is in part due to histamine release acting on histamine H_1-receptors,

Intradermal injection of bradykinin produces erythema and wealing in human skin (Greaves & Shuster 1967). The erythema reflects an increase in local blood flow in keeping with the vasodilator effect of bradykinin and the weal formation indicates an increase in vascular permeability. These actions of bradykinin may result from a direct action on receptors in the cutaneous microvasculature or may be mediated via the secondary release of vasoactive chemical substances.

Histamine is a potential candidate mediator of these effects of bradykinin. Intradermal injection of histamine elicits the 'triple response' (Lewis 1927). This consists of an initial local erythema due to histamine-directed vasodilatation, a circumferential erythema due to vasodilatation mediated via an axon reflex, and finally a central oedematous weal due to a histamine-directed increase in permeability, and represents a combined histamine H_1- and H_2-receptor response (Marks & Greaves 1977; Greaves et al 1977; Robertson & Greaves 1978). Bradykinin and histamine are released simultaneously when there is tissue damage (Rocha e Silva & Rosenthal 1961), and any tissue damage produced by bradykinin may trigger a release of histamine and vice versa. Bradykinin stimulates histamine release when it is injected into granuloma pouches in rats. This effect and the inflammatory reaction to bradykinin were considerably reduced in histamine-depleted skin following pretreatment of the animals with the histamine liberator, compound 48/80, suggesting that the effects of bradykinin are in part mediated by histamine release (Stern et al 1962). Antihistamines reduce the exudation produced by injections of bradykinin in the rat hindpaw (Maling et al 1974) and rabbit skin (Marceau et al 1981) indicating possible participation of endogenous histamine in the increased vascular permeability evoked by bradykinin. Bradykinin has also been reported to liberate histamine from rat peritoneal mast cells in-vitro (Johnson & Erdös 1973; Ishizaka et al 1985), but similar findings were not observed with human skin mast cells or basophils (Lawrence et al 1989). However, Zachariae et al (1969) previously demonstrated that the mean histamine content of the human skin is reduced after local injections of bradykinin into the subcutaneous tissue, support-

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