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Fab-bound Colchicine Appears to Adopt Fab Fragment Disposition in Rats

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Abstract—The disposition of colchicine-specific Fab fragments and the effect of Fab fragment administration on the disposition of colchicine were studied in anaesthetized bile duct-cannulated rats. One group of rats (n = 6) received a ^{125}I-Fab dose of 38 mg kg^{-1} i.v. The plasma disposition was characterized by a volume of distribution of 179 ± 48 mL kg^{-1}, total body clearance of 1·02 ± 0·07 mL min^{-1} kg^{-1}, $t_{\frac{1}{2}\alpha}$ of 0·17 ± 0·03 h and $t_{\frac{1}{2}\beta}$ of 1·3 ± 0·3 h. Fab fragments were in part excreted by the renal route (15·6 ± 6% of the Fab dose), while biliary excretion was a minor route (< 2% of the Fab dose). Two other groups of rats received 15 μg kg^{-1} colchicine (n = 6) or 15 μg kg^{-1} colchicine plus 38 mg kg^{-1} colchicine-specific Fab fragments (n = 6) by intravenous infusion. Pharmacokinetics of colchicine was markedly altered in the Fab-colchicine-treated rats. In this group, distribution volume and total body clearance of colchicine were decreased by factors of 22 and 10, respectively, compared with the values in the colchicine-treated group and were very similar to those of Fab fragments. An 80% reduction of cumulative biliary excretion of colchicine was observed in Fab-colchicine-treated rats ($P < 0·01$). The fraction of colchicine dose excreted by the urinary route was 38 ± 6·9 and 9 ± 0·7% respectively in Fab-colchicine- and colchicine-treated groups ($P < 0·01$). These data show that during Fab treatment, colchicine followed the elimination kinetics of Fab fragments. This study supports the view that Fab fragments could be of benefit in acute colchicine poisoning by neutralizing colchicine in the vascular compartment and imposing its elimination kinetics on colchicine.

Digoxin-specific Fab fragments were first demonstrated to reverse digoxin toxicity in man in 1976 (Smith et al 1976). Today, polyclonal digoxin-specific Fab fragments are currently used in the treatment of cardiac glycoside poisoning (Hickey et al 1991). Despite its efficacy, clinical use has not yet been extended to other drugs. Recently, colchicine-specific IgG and Fab fragments have been demonstrated to reverse acute colchicine intoxication in mice (Terrien et al 1990; Sabouraud et al 1991). The antidote (IgG or Fab fragment) acts by sequestrating the toxin in the vascular compartment and by removing it from the target organs (Colburn 1980). To evaluate their potential in the reversal of acute intoxication, there have been studies on the effect of specific IgG or Fab fragments on the pharmacokinetics of digoxin (Butler et al 1977; Griffiths et al 1984; Johnston et al 1987, 1988a, b), digitoxin (Ochs & Smith 1977), colchicine (Terrien et al 1989), desipramine (Pentel et al 1987, 1991; Hursting et al 1989), phencyclidine (Owens & Mayersohn 1986) and paraquat (Nagao et al 1989). These studies have shown that IgG or Fab fragment infusion resulted in redistribution of drug into plasma and decrease of drug concentrations in most tissues (Terrien et al 1989; Griffiths et al 1985; Pentel et al 1987). Few studies have investigated the effect of Fab fragments on the biliary excretion of drugs. Specific IgGs have been demonstrated to inhibit biliary and urinary excretion of digoxin and sulphanilic acid in actively immunized rats (Johnston et al 1988a, b; Yamamoto et al 1991). However, Fab fragments have been shown to reduce only slightly biliary excretion of digoxin (Johnston et al 1987). Urinary excretion of drugs was slightly increased by the Fab administration (Butler et al 1977; Owens & Mayersohn 1986); this can be related to the ability of Fab fragments to be excreted by the renal route. Colchicine is known to be excreted by the biliary (52% of the dose) and the urinary (14% of the dose) routes (Hunter & Klaassen 1975). The study of the effects of colchicine-specific Fab fragments on colchicine biliary and urinary excretion was considered to be of interest before clinical use. It can be assumed that pharmacokinetics of the hapten depends on pharmacokinetics of the antibody or its Fab fragment. The present work was undertaken to study the pharmacokinetics of colchicine-specific Fab fragments and to evaluate their influence on colchicine plasma disposition and excretion routes in anaesthetized bile duct-cannulated rats.

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Materials and Methods

Materials

[^{3}H]Colchicine (Ring C, ^{3}H-methoxy, 20 Ci mmol^{-1}, New England Nuclear, Paris, France) was used as a tracer. Its purity was checked before the experiments by TLC (> 97%). Colchicine (Fluka, Paris, France) was dissolved in physiological saline and then mixed with the tracer. Pentobarbitone sodium (60 mg kg^{-1}) was from Clin-Midy (St Jean de la Ruelle, France). Na^{125}I was obtained from New England Nuclear, Paris, France), Iodogen from Pierce (Interchim, France). Pico-Fluor 40 scintillation liquid was from Packard (Rungis, France). Prepacked columns PD-10 containing Sephadex G-25 M were from Pharmacia (Les Ulis, France). Radioactivity was measured in a Tri-Carb model 4530 liquid scintillation spectrophotometer for tritium and an Autogamma 5000 series gamma counter for iodine (Packard, Les Ulis, France). GF/A glass microfibre filters (2·5 cm) were from Whatman (UK). After purification of polyclonal goat

IgG, specific to colchicine by the method of Cohn, Fab fragments were prepared by papain enzymatic cleavage and purified by ion-exchange chromatography as previously described (Sabouraud et al 1991). The Fab fragment solution was 92% pure. Previous binding experiments showed that polyclonal colchicine-specific antibodies cross-reacted with 2- and 3-demethylcolchicine respectively at 13·5 and 43·9% and exhibited an affinity constant of 2×10^{10} M^{-1} for colchicine.

Radioiodination of colchicine-specific Fab fragment

Purified colchicine-specific Fab fragments were labelled with ^{125}I according to the method of Fraker & Speck (1978) for use in the study of Fab fragment pharmacokinetics. Tubes were coated with 10 μg of Iodogen previously solubilized in chloroform and evaporated to dryness under nitrogen. One hundred microgrammes of colchicine-specific Fab fragments (0·4 mg mL^{-1} in 0·05 M phosphate buffer, pH 7·4) were placed in Iodogen-coated tubes followed by the addition of 4 μCi of Na^{125}I. Iodination was carried out for 5 min. After radioiodination, final volume was completed up to 250 μL and the reaction mixture was passed over a 5 cm column of Sephadex G-25 and the ^{125}I-Fab fragment fraction was isolated after gamma scintillation counting of serial fractions. Ninety-six percent of ^{125}I radioactivity in the Fab fragment preparation was precipitable by trichloroacetic acid.

Experimental

Male Sprague-Dawley rats, 290 ± 10 g (Iffa Credo, Lyon, France), with access to food and water before the experiments, were anaesthetized with pentobarbitone sodium (60 mg mL^{-1}, 60 mg kg^{-1}, i.p.) and an additional dose (5 mg kg^{-1}) was given when necessary. Rectal temperature was continuously monitored by a rectal probe (Ellab Thermometer model TE 3, Copenhagen, Denmark) and kept at 37 ± 0·5°C throughout the experiments by a heating lamp to prevent hypothermic alteration in biliary excretion (Roberts et al 1967). Femoral vein and artery were cannulated with PE-10 tubing (Biotrol, Paris, France) for drug administration and blood sampling, respectively. Total blood was infused via the femoral vein to compensate for the blood sampling. After median laparotomy, the common bile duct was cannulated with PE-50 tubing. The abdomen was then closed. The drug was infused after a control period of 10 min after cannulation.

Drug administration

Animals were divided into three groups of six animals; the first received a single intravenous bolus dose of 15 μg kg^{-1} (2·5 μCi kg^{-1}) [^{3}H]colchicine, the second received 38 mg kg^{-1} ^{125}I-Fab fragments (5 μCi kg^{-1}) and the third received colchicine plus unlabelled Fab fragments at the same doses allowing the formation of stoichiometric complexes (Fab/colchicine molar ratios 1:1) via the same route. The Fab dose was calculated assuming 5% of colchicine-specific active binding sites in the polyclonal reagent as previously calculated (Cano et al 1992), i.e. 1·9 mg kg^{-1} of active Fab fragments corresponding to 38 mg kg^{-1} of total Fab fragments. Drug solutions were prepared in physiological saline and a 2·5 mg kg^{-1} volume injected.

Collection of samples

Blood samples (0·4 mL) were collected at the times indicated below. Bile samples were collected in preweighed vials for 150 min at the intervals 0–5, 5–10, 10–15, 15–20, 20–30, 30–45, 45–60, 60–90, 90–120 and 120–150 min. Before drug injection, the penis was ligated to prevent loss of urine. At the end of plasma and bile sampling (180 min), the urine was withdrawn through the bladder wall with a syringe. The bladder was then rinsed with 1 mL of physiological saline. Bile volume was determined gravimetrically.

Analytical methods

Quantitation of [^{3}H]colchicine. Twenty-five microlitre aliquots of plasma, bile or urine samples (in triplicate) were mixed with 3 mL Pico-Fluor 40 scintillation liquid in a minivial and radioactivity was measured by liquid scintillation counting using automatic external standardization for the quench correction. Total radioactivity which may have included unchanged colchicine plus metabolites was expressed as colchicine equivalent.

Quantitation of ^{125}I-Fab fragments. One hundred microlitres of plasma, urine and bile were applied to glass microfibre filters (in triplicate). The filters were washed twice with 2 mL 10% trichloroacetic acid (TCA) and then once with 2 mL ethanol. TCA-precipitable ^{125}I was measured by gamma scintillation counting.

Pharmacokinetic analysis

Estimates of α and β (distribution and elimination rate constants) were obtained by fitting the concentration time data with a biexponential equation using the computer program MK MODEL (Biosoft, UK). Distribution and elimination half-lives ($t_{\frac{1}{2}\alpha}$, $t_{\frac{1}{2}\beta}$) were calculated as 0·693/α or β, respectively. Plasma concentration–time data from individual animals were analysed using a model-independent method. The parameters were calculated as follows: the area under the concentration–time curve (AUC) was calculated from 0 to 150 min by the trapezoidal method and from 150 min to infinity by extrapolation using β. The values of total body clearance (CL_T), distribution volume (V_β), fraction of urinary excreted dose (f_U), renal clearance (CL_R), fraction of biliary excreted dose (f_B) and biliary clearance (CL_B) were determined as follows:

$$CL_T = \text{dose}/AUC_{0-\infty} \quad (1)$$

$$V_\beta = \text{dose}/AUC_{0-\infty}.\beta \quad (2)$$

$$f_U = \text{urinary excretion (\% dose)} \quad (3)$$

$$CL_R = \text{urinary excretion to 2·5 h}/AUC \text{ to 2·5 h} \quad (4)$$

$$f_B = \text{biliary excretion (\% dose)} \quad (5)$$

$$CL_B = \text{biliary excretion to 2·5 h}/AUC \text{ to 2·5 h} \quad (6)$$

Statistical analysis

Pharmacokinetic parameters are expressed as mean ± s.e.m. (n = 6). Statistical analysis was performed using Student's *t*-test. Significance was set at $P < 0·05$.

Results

Fig. 1 presents the concentration–time profiles after intravenous administration of ^{125}I-Fab fragments specific to

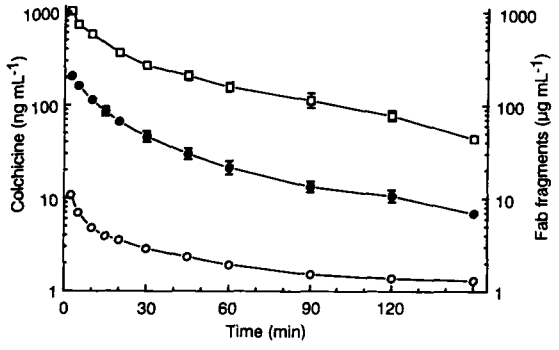

FIG. 1. Plasma concentration–time curves of [^{3}H]colchicine and ^{125}I-Fab fragments. ○ [^{3}H]Colchicine in colchicine-treated rats, ● [^{3}H]colchicine in Fab-colchicine-treated rats, □ ^{125}I-Fab in Fab fragment-treated rats. Results are expressed as the mean ± s.e.m. (n = 6).

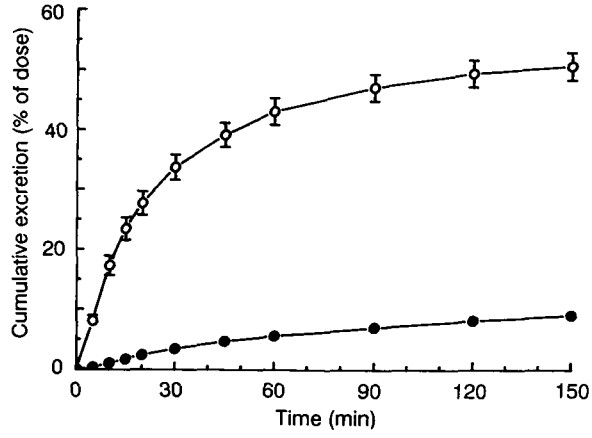

FIG. 2. Cumulative biliary excretion of [^{3}H]colchicine in colchicine- (○) and Fab-colchicine- (●) treated rats. Results are expressed as the mean ± s.e.m. (n = 6).

Table 1. Pharmacokinetic parameters for colchicine and Fab fragments in rats.

Parameter		Fab fragment	Colchicine	
			Alone	Complexed to Fab
$t_{\frac{1}{2}\alpha}$	(min)	10·1 ± 2	6 ± 1·04	8·9 ± 0·6
$t_{\frac{1}{2}\beta}$	(min)	76 ± 16	125 ± 9·2	71 ± 6·9
AUC	(ng mL^{-1} min)	38·5 ± 2·9 × 10^6	578 ± 17·6	5786 ± 389
V_β	(mL kg^{-1})	179 ± 48	4586 ± 201	205 ± 33
CL_T	(mL min^{-1} kg)	1·02 ± 0·07	27·3 ± 0·9	2·64 ± 0·18
f_U	(%)	13 ± 3	9·0 ± 0·7	38·0 ± 6·9
f_B	(%)	—	50·7 ± 2·3	9·1 ± 0·4
CL_R	(mL min^{-1} kg)	0·17 ± 0·05	2·3 ± 0·3	1·0 ± 0·2
CL_B	(mL min^{-1} kg)	—	22·1 ± 0·7	0·26 ± 0·02

Results are expressed as the mean ± s.e.m. of six animals.

colchicine, [^{3}H]colchicine and Fab-[^{3}H]colchicine. The corresponding mean pharmacokinetic parameters (± s.e.m.) are presented in Table 1. Plasma disposition was adequately described by a biexponential decay for Fab fragments, colchicine and Fab-bound colchicine. Plasma disposition of colchicine was markedly altered when colchicine was given in a complex form with specific Fab fragments (Fig. 1). Plasma concentrations at 5 min were 20-fold higher in the Fab-colchicine-treated group than in the colchicine-treated group. Colchicine plasma concentrations decreased with a mean elimination half-life respectively of 125 and 71 min in the colchicine- and Fab-bound colchicine-treated groups ($P < 0·01$). The elimination half-life values of Fab fragments and Fab-colchicine were very similar. Similarly, V_β and CL_T of colchicine when administered bound to Fab fragments (205 ± 33 mL kg^{-1} and 2·64 ± 0·18 mL min^{-1} kg, respectively) were similar to those of Fab fragments ($P < 0·1$).

Biliary excretion of colchicine was studied over a 150 min period. The bile flow rate was constant and not statistically different in colchicine- (7·08 ± 1·59 μL min^{-1}/100 g), Fab- (6·47 ± 1·4 μL min^{-1}/100 g) and Fab-bound colchicine- (6·1 ± 1·1 μL min^{-1}/100 g) treated rats. Fig. 2 represents the cumulative biliary excretion of colchicine: 50·7 ± 2·3% of the dose administered was excreted in the bile in colchicine-treated rats. The biliary excretion of colchicine was significantly lower in Fab-treated rats (9·1 ± 0·4%, $P < 0·01$). The biliary excretion of ^{125}I-Fab fragments was < 2% of total radioactivity injected.

The urinary excretion of colchicine was increased 4-fold in Fab-colchicine-treated rats compared with colchicine-treated rats (Table 1). In contrast, renal clearance in Fab-colchicine was one-half that in colchicine-treated rats.

Discussion

Drug-specific IgG or Fab fragments are effective in reversing drug toxicity by sequestering and redistributing the drug into the IgG or Fab distribution volume (Scherrmann et al 1989). This results in an increase of drug plasma concentrations, extensively bound to IgG or Fab fragments as a pharmacologically inactive complex (Butler et al 1977; Owens & Mayersohn 1986; Hursting et al 1989). Thus, IgG or Fab fragments constitute a new circulating compartment for the drug, resulting in a decrease of distribution volume and of total body clearance (Owens & Mayersohn 1986; Yamamoto et al 1991). The molecular weight of the complexes does not permit their glomerular filtration and so the urinary clearance of the drug is inhibited (Colburn 1980; Scherrmann et al 1989) and elimination of the drug in the body depends on the catabolism of IgG. Surprisingly, a marked decrease of urinary clearance of digoxin (Butler et al 1977) and phencyclidine (Owens & Mayersohn 1986) has been described after injection of specific Fab fragments, although Fab-drug complexes are able to be excreted by the renal route. Despite the large amount of drug in the vascular compartment, the urinary excretion of Fab-drug complexes seems to be capacity-limited. Moreover, Fab fragments could modify the extrarenal clearance of drugs. Metabolism of phencyclidine and desipramine has been reported to be decreased because of its binding to specific Fab (Owens & Mayersohn 1986; Hursting et al 1989), and digoxin-specific IgG and Fab fragments have been found to alter the biliary excretion of digoxin (Johnston et al 1987, 1988a). The present study focused on the alteration of colchicine disposition when colchicine is bound to Fab. Thus, administration of preformed Fab-colchicine complexes was preferred to the

sequential administration schedule currently used in experimental models. A sub-toxic dose of colchicine (15 $\mu g\ kg^{-1}$) was used to avoid a large dose of Fab fragments. Fab fragments were infused in a dose equimolar with the colchicine dose. No adverse effects have been observed in Fab-treated rats. Pentel et al (1988) reported that rats tolerated high Fab doses of 7·5 $g\ kg^{-1}$. The distribution volume of Fab fragments ($179·3 \pm 48\ mL\ kg^{-1}$) is between the plasma volume ($35\ mL\ kg^{-1}$, Waynforth 1980) and the extracellular fluid volume ($305\ mL\ kg^{-1}$ Johnston et al 1988b). The low diffusion of Fab fragments into the interstitial space is in accordance with previous studies for digoxin-specific Fab fragments determined using an enzyme-linked immunosorbent assay for Fab plasma concentration measurement (Johnston et al 1988b). The amount of ^{125}I-Fab fragments recovered in bile was negligible. Similarly, Johnston et al (1987) did not observe digoxin-specific binding in bile of Fab-infused rats. Urinary excretion of Fab fragments represented only 17% of total body clearance, which is greater than that described in a previous study in rats (2%) (Johnston et al 1988b) but near to those recently described in dogs (10% (Keyler et al 1991)).

Plasma colchicine disposition was markedly altered when colchicine was administered with Fab fragments. The lower distribution half-life and the 20-fold higher plasma concentrations in the Fab-colchicine group compared with the colchicine group are in accordance with the sequestration of colchicine by Fab fragments in the vascular space. As previously described for digoxin (Griffiths et al 1984; Schaumann et al 1986), Fab fragments reduced the plasma elimination half-life of colchicine. Distribution volume and total body clearance of colchicine were decreased when colchicine was injected with Fab and were very similar to values for Fab fragments. These data suggest that Fab fragments imposed their disposition kinetics on colchicine. Colchicine has been reported to be extensively excreted by the biliary route (Hunter & Klaasen 1975). Fifty-two percent of the administered tritiated colchicine dose was recovered in bile of rats within 2·5 h. In this study, a similar percentage (51%) was recovered in bile of colchicine-treated rats. Hunter & Klaassen (1975) showed that 53% of the bile-excreted radioactivity was unchanged colchicine, the other fractions were presumed to be desmethylated colchicine and colchicine-glucuronide. A dramatic decrease of biliary excretion of colchicine was observed in Fab-treated rats (80%). Similarly, Johnston et al (1987) reported a 90% reduction in biliary digoxin elimination after injection of 10 $\mu g\ kg^{-1}$ [^{3}H]digoxin in digoxin-actively immunized rats. These results confirm that homologous or heterologous IgG is little eliminated by the biliary route and there is little contribution to the elimination of their hapten by this route. Similarly, Fab-colchicine complexes are not extensively excreted by this route. This observation was supported by the absence of biliary excretion of ^{125}I-Fab fragments in the rat. The inhibition of colchicine biliary clearance by Fab fragments has to be considered a contributory factor in the extensive decrease of colchicine total body clearance in Fab-treated rats. In colchicine-treated rats, renal excretion was found to be a minor route of elimination as previously described by Hunter & Klaassen (1975). A 4-fold increase of urinary excretion of colchicine was observed in Fab-colchicine-treated rats. The increase in renal excretion might partly compensate for the absence of biliary excretion. Johnston et al (1987) have described a 3-fold increase in digoxin urinary excretion in Fab-infused rats while other studies have shown a slight increase in urinary elimination of digoxin and phencyclidine in control and Fab-infused dogs (Butler et al 1977; Owens & Mayersohn 1986). In both rat groups, 90–95% of the urinary radioactivity was extractable into dichloromethane (data not shown). The proportion of polar metabolites or nonspecific radioactivity in urine seemed to be low. Ninety percent of the urinary radioactivity was bound to Fab in the Fab-colchicine treated group (data not shown). In relation to the low renal clearance of Fab fragments, a decrease in the renal clearance of colchicine was observed when it was administered bound to Fab.

In conclusion, this parmacokinetic study demonstrates that colchicine bound to Fab fragments follows the disposition of Fab fragments. Fab-colchicine complexes are stable in-vivo as suggested by the high affinity constant of $2 \times 10^{10}\ M^{-1}$ determined in-vitro. These data support the detoxification effect of Fab fragments already reported in acute murine intoxication (Sabouraud et al 1991) and are encouraging for the future clinical use of this antidote in acute poisoning.

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