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The plasma disposition of sheep antibody (Fab) Fragments in the guinea-pig and rabbit

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Abstract—The plasma elimination of sheep digoxin-specific Fab (fragment antigen-binding) antibody fragments has been studied after intravenous injection (1 mg kg^{-1}) in guinea-pigs and rabbits using an enzyme-linked immunosorbent assay. The log concentration versus time profiles were best described by biexponential and triexponential functions for the response in the guinea-pig and rabbit, respectively. However, the elimination half-lives and apparent volumes of distribution were similar in both species (about 140 min and 120 mL kg^{-1}, respectively). The value for the Fab distribution volume suggests that the antibody fragments distribute out of the vascular compartment but do not fully occupy the extracellular space. Our estimates of the latter, using thiocyanate as a marker, ranged from 220 to 327 mL kg^{-1} (rabbits and guinea-pigs, respectively). The distribution of Fab fragments in these two species differs significantly from that in the rat, where our earlier studies have shown that these antibody fragments are confined to the intravascular compartment with a distribution volume approximately equivalent to that of plasma (about 40 mL kg^{-1}).

Digoxin-specific Fab (fragment antigen-binding) fragments, derived from corresponding sheep immunoglobulin G, are used to treat severe cardiac glycoside toxicity (Wenger et al 1985). It is possible that drug-specific antibody fragments could be used in an analogous fashion to treat poisoning by certain other drugs and poisons such as phencyclidine (Owens & Mayersohn 1986), tricyclic antidepressants and amanita toxins (Henry 1986). The extension of this type of treatment would be aided by the availability of suitable animal models for disposition studies. However, it has been recently found that, in rats (Johnston et al 1988), the apparent volume of distribution of sheep Fab fragments may be an order of magnitude less than that in man (Schaumann et al 1986; Sinclair et al 1989) and baboons (Smith et al 1979). In view of the consequent possible lack of suitability of the rat as a model for studies on sheep Fab fragments, we have examined their plasma disposition in two other common laboratory animal species.

Materials and methods

The sheep Fab fragments (Digibind) were a gift from the Wellcome Foundation Ltd.

Four female Duncan-Hartley guinea-pigs (500–600 g) and four male New Zealand-White rabbits (3·6–4·6 kg) were used. The guinea-pigs were anaesthetized (pentobarbitone, 35 mg kg^{-1}, 60 mg mL^{-1}) before Fab fragment injection (1 mg kg^{-1}, 1 mg mL^{-1} in saline via a jugular vein) and blood collection (carotid artery). The rabbits were not anaesthetized and Fab fragments (dose as above) were injected into the marginal vein of the right ear of each animal. Blood was collected via a cannula from the marginal vein of the left ear.

Sheep Fab fragments were measured in plasma using an enzyme-linked immunosorbent assay basically as described by Johnston et al (1988). Briefly, the assay comprised coating the wells of a microtitre plate with donkey anti-sheep serum, which quantitatively bound the sheep Fab in the test samples. The bound sheep Fab was then detected by the yellow coloration (405 nm) obtained with anti-sheep immunoglobulin G alkaline phosphatase/*p*-nitrophenyl phosphate reagents.

The thiocyanate space was used as an index of extracellular fluid volume, using the procedure and assay essentially as described by Bianchi et al (1981) for rats. NaSCN was injected i.v. (50 mg for guinea-pigs, 100 mg for rabbits) and plasma samples taken for assay (40 min for guinea-pigs or 180 min for rabbits).

The Fab plasma concentration versus time data were analysed as follows. The elimination rate constant (Kel) and elimination half-life were obtained from the terminal part (80–240 min for guinea-pigs, 210–360 min for rabbits) of the log concentration versus time plot using least-squares regression analysis. Also using this plot, the theoretical Fab concentration at zero time was obtained by extrapolating the elimination regression line to zero time. The area (AUC) under the concentration versus time curve was obtained from 0–80 min (guinea-pigs) or 0–210 min (rabbits) by the linear trapezoidal rule and from 80 min (guinea-pigs) or 210 min (rabbits) to infinity by extrapolation using Kel. Using the parameters obtained, the apparent volume of distribution was calculated by three methods (a) extrapolation (V_{extrap}), (b) area under the curve (Vd_{AUC}), (c) moments (Vd_{ss}), (Gibaldi & Perrier 1982). Clearance was calculated by dividing the dose by AUC. To obtain information on the distribution phase, an exponential stripping program (Brown & Manno 1978) was used.

Results

The plasma elimination of sheep Fab fragments in guinea-pigs and rabbits is shown in Fig. 1A, B). The corresponding pharmacokinetic parameters are given in Table 1. The plasma concentration data from the rabbits fitted best to a triexponential function, so that an additional ('intermediate') half-life was generated. In the case of guinea-pigs, a bi-exponential function was adequate. Apart from these apparent initial differences in rates of distribution, other parameters (elimination half-life, clearance, total apparent volume of distribution) were similar in the guinea-pig and rabbit. In both species, the three different methods of calculating volume of distribution gave similar results, although the highest and lowest values were always obtained for Vd_{extrap} and Vd_{ss} respectively.

The value obtained for the extracellular fluid volume (by measuring the 'thiocyanate space') was about 50% greater in the guinea-pig (Table 1).

Discussion

Table 2 compares the terminal elimination half-life and apparent volume of distribution (Vd_{AUC}) of heterologous Fab fragments reported in man and various species of laboratory animal. The elimination half-life varies from a few hours in small animals to a value of 96 h obtained in an elderly female patient. For the rabbit, there is a large discrepancy between the value reported by Yasmeen et al (1976) and those obtained by Butler et al (1974) and in the present work. However, the investigation by Yasmeen et al, in which radio-iodine-labelled human Fab fragments were

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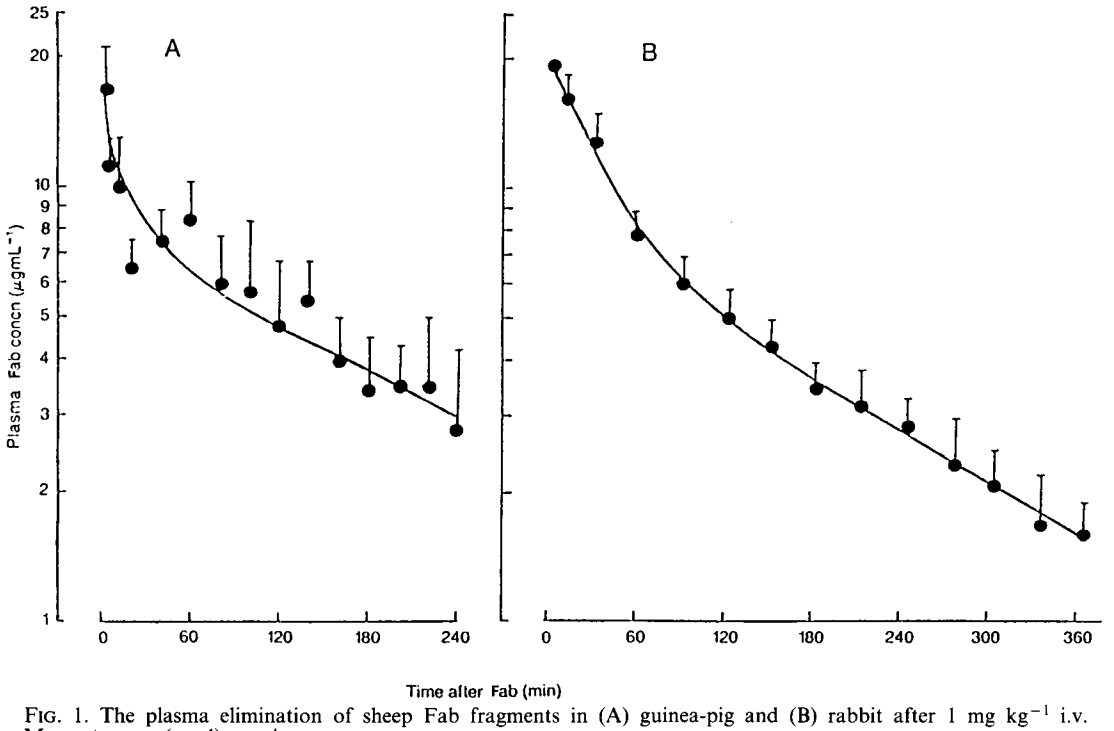

FIG. 1. The plasma elimination of sheep Fab fragments in (A) guinea-pig and (B) rabbit after 1 mg kg^{-1} i.v. Means ± s.e.m. (n = 4) are given.

Table 1. Pharmacokinetic parameters for Fab, and extracellular fluid volumes in the guinea-pig and rabbit.

Parameter		Guinea-pig	Rabbit
Fab plasma half-life (min):	distribution	4·4 ± 1·4	8·2 ± 3·5
	intermediate	—	45 ± 10
	elimination	133 ± 26	159 ± 25
Fab apparent volume of distribution (mL kg^{-1}):	Vd_{extrap}	125 ± 17	131 ± 20
	Vd_{AUC}	120 ± 24	120 ± 16
	Vd_{ss}	109 ± 20	115 ± 16
Fab clearance (mL kg^{-1} min^{-1})		0·72 ± 0·19	0·54 ± 0·09
Extracellular fluid volume (mL kg^{-1})		327 ± 17	220 ± 15

Means ± s.e.m. (n = 4) are given. Values for Vd_{extrap}, Vd_{AUC} and Vd_{ss} are obtained from the different methods of calculating apparent volume of distribution. The measure of extracellular fluid volume was obtained using thiocyanate as a marker.

Table 2. Elimination and distribution of heterologous Fab fragments in various animal species.

Species		Reference	Terminal elimination half-life (h)	Apparent volume of distribution (Vd_{AUC} mL kg^{-1})
Rat (anaesthetized)		Johnston et al (1988)[a]	1·8	46
Guinea-pig (anaesthetized)		Current paper[a]	2·2	120
Rabbit	1	Current paper[a]	2·7	120
(conscious)	2	Butler et al (1974)[b]	4·3	—
	3	Yasmeen et al (1976)[c]	16	—
Dog (conscious)		Lloyd & Smith (1978)	16·4	—
Baboon (conscious)		Smith et al (1979)	9–14	280–461
Human	1	Schaumann et al (1986)	25[e]	870
(conscious)	2	Sinclair et al (1989)[a, d]	96	322

[a] In these studies the sheep Fab fragments were analysed using the same method by the same laboratory. [b, c] Rabbit and human Fab fragments were used respectively. [d]An 82 year old female patient was studied. [e]Calculated from data in Table 6, Schaumann et al (1986). The dashes indicate that the information was not available.

used, was not concerned primarily with a detailed investigation of the plasma disposition of Fab fragments and only three or four serum samples were assayed over a three-day period. In our experiments, we found that by 24 h, Fab fragments were not detectable ($<0{\cdot}05$ $\mu g\ mL^{-1}$) in rabbit plasma.

When different methods of calculating apparent volume of distribution are used, the values obtained for this parameter, decrease in the order $Vd_{extrap} > Vd_{AUC} > Vd_{ss}$ (Gibaldi & Perrier 1982). Where it is possible to compare apparent volumes of distribution for Fab calculated using the same method, it is seen (Table 2) that the values for the different species decrease in the following order: human > baboon > guinea-pig = rabbit > rat. The value for the rat approximates to the plasma volume (Johnston et al 1988), while that for man exceeds the extracellular fluid volume (the thiocyanate space is about 220 mL kg^{-1}, Diem & Lentner 1970). Thus it seems that for heterologous Fab, with a molecular weight of about 50 kDaltons, marked interspecies differences in distribution can occur, and that with larger animals there is an increasing ability of the molecule to penetrate into the extravascular compartment.

It is not clear why the distribution volume of the thiocyanate ion, used as an approximate index of extracellular volume, appears significantly larger in the guinea-pig than rabbit.

If it is confirmed that in man the apparent volume of distribution of sheep Fab is markedly greater than the extracellular fluid volume, it could indicate that the Fab fragments are extensively bound to extracellular sites or that they are penetrating cells. In terms of small laboratory animal models for studying the disposition of sheep Fab fragments, it would appear that compared with the rat, the use of guinea-pigs and rabbits would more closely resemble the situation in man.

As a final comment, it should be noted that only in the human studies (Table 2) was the disposition of Fab fragments examined in the presence of a hapten. It is unlikely that digoxin (with a molecular weight about one-fiftieth that of Fab) would alter the disposition of the antibody, but the possibility should be checked.

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