J. Pharm. Pharmacol. 1997, 49: 277–281
Received August 13, 1996
Accepted October 16, 1996

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Pharmacokinetics of Heterologous and Homologous Immunoglobulin G, $F(ab')_2$ and Fab after Intravenous Administration in the Rat

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Abstract

Because few pharmacokinetic studies of antibodies and their fragments have compared the influence of species origin and antibody size, the plasma pharmacokinetics of a single intravenous dose (0.7 mg kg^{-1}) of ^{125}I-labelled mouse, rat and human immunoglobulin G (IgG), and mouse $F(ab')_2$ and Fab were investigated in the rat.

IgG reached equilibrium after six distribution half-lives, i.e. only 36–50 h post-dosing, and the distribution volume was about four times the rat plasma volume. IgG elimination half-lives ranged from 5·33 to 8·10 days. Fragmentation of IgG into smaller fragments, $F(ab')_2$ and Fab, resulted in pharmacokinetics that were molecular-weight-dependent with volume of distribution and systemic clearance values inversely related to antibody size.

We conclude that antibody variability in terms of species origin and size influences antibody pharmacokinetics and should be carefully studied before selection of the best antibody for a clinical application.

Polyclonal and monoclonal antibodies are frequently used for diagnosis and in therapeutics (Knapp & Colburn 1990; Kojima et al 1993; Baud et al 1995). The pharmacokinetic properties of antibodies can, however, limit their medical uses. In man, intact IgG has a volume of distribution only twice the plasma volume because of its high molecular weight (150 kDa). As a consequence, the immunoactivity of IgG is restricted to interaction with the antigen molecules in a relatively small space (Smith et al 1979). Intact IgG does not cross the renal barrier, moreover, and different catabolic processes are responsible for the total body clearance (Henderson et al 1982; Covell et al 1986). With the aim of increasing antibody distribution and improving biological efficacy, several types of sub-structure have been developed: $F(ab')_2$ (100 kDa), Fab (50 kDa) and sFv (27 kDa) (Yokota et al 1993). Although the reduction in molecular weight is associated with improved body distribution, increased body clearance reduces antibody residence time, and antibody diversity in terms of origin, recipient species-related factors and the development of new types of antibodies such as chimeric antibodies (Brown et al 1987; Buist et al 1993) multiplies the risk of variability in the pharmacokinetic parameters, especially for systemic clearance. Although many independent pharmacokinetic studies of antibodies and their fragments have been published, few have compared the influence of species origin and antibody size or fragments in one study. In order to evaluate the influence of these different factors, the plasma pharmacokinetics of a single intravenous dose of mouse, rat and human IgG, and mouse $F(ab')_2$ and Fab were investigated in the rat using ^{125}I-labelled proteins.

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Materials and Methods

Immunoglobulins and fragments

Mouse monoclonal digoxin-specific IgG_1 was prepared by somatic cell fusion and raised in ascites fluid from balbC mice, as described by Wahyono et al (1990). Corresponding $F(ab')_2$ were obtained by pepsin-hydrolysis of purified monoclonal IgG. Briefly, purified IgG was dialysed against sodium formate buffer (10 mM, pH 2·8) and then against sodium acetate buffer (0·2 M, pH 4). IgG were hydrolysed by pepsin digestion (3085 units (mg protein)$^{-1}$) for 15 min at 37°C. The reaction was stopped by adding Tris buffer (2 M, pH 8) and the digestion mixture was then dialysed against phosphate buffer (0·1 M, pH 7·4). Fab were obtained by digestion of IgG as described by Andrew & Titus (1991). Purified IgG was cleaved by papain (15 μg (mg protein)$^{-1}$) for 3 h at 37°C in a buffer containing 20 mM EDTA and 5 mM cysteine. The mixture containing undigested IgG, Fc and Fab was dialysed against phosphate buffer (0·1 M, pH 8·2).

$F(ab')_2$ and Fab were purified by chromatography on a protein A-Sepharose CL-4B column (Pharmacia, St Quentin en Yvelines, France). The purity of the products was assessed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on a PhastSystem (Pharmacia, St Quentin en Yvelines, France).

Rat monoclonal IgG_1 directed against mouse IgG_{2b} and polyclonal human IgG_1 were from Jackson ImmunoResearch Laboratories (Interchim, Montluçon, France).

Radiolabelling of proteins

Antibodies and fragments were labelled with [^{125}I]-NaI using the Iodogen method (Fraker & Speck 1978). Protein (100 μg) was incubated with [^{125}I]-NaI (0·5 mCi) in Eppendorf tubes coated with Iodogent reagent (10 μg) for 5 min at room temperature. Free iodine was removed by chromatography on a

Sephadex G-25 PD-10 column (Pharmacia, Uppsala, Sweden) equilibrated with phosphate-buffered saline (PBS). Precipitation of the iodinated proteins by trichloroacetic acid gave more than 95% of bound iodine. The specific activity was in the range of 2-3 μCi μg^{-1} for IgG and 1·5–2 μCi μg^{-1} for $F(ab')_2$ and Fab. Purity of antibody preparations was also analysed by SDS-PAGE.

Intravenous administration of immunoglobulins and fragments

Male Sprague-Dawley rats, 250–300 g (Iffa Credo, Lyon, France), with free access to food and water before the experiments, were anaesthetized with pentobarbital sodium (60 mg mL^{-1}, 60 mg kg^{-1}, i.p.). Temperature was continuously monitored by means of a rectal probe (Ellab Thermometer model TE 3, Copenhagen, Denmark). The femoral vein was cannulated with PE-10 tubing (Biotrol, Paris, France). Groups of six rats received a single bolus of unlabelled antibody and about 10 μCi of labelled antibody dissolved in 0·9% NaCl (total dose 0·7 mg kg^{-1}, 2·5 mL kg^{-1}) via the cannulated femoral vein. Blood samples (0·3 mL) were collected in heparinized tubes via the tail vein before injection and at 1, 15, 30, 60, 120, 240 and 480 min, and 0·5 mL was collected every other day up to day six for rats that received $F(ab')_2$ and day 20 for those that received IgG. Blood samples from rats that received Fab were collected at 1, 10, 20 and 30 min and at 1, 2, 4, 6, 8, 24, 28, 32, 48 and 72 h. Replacement blood from a rat donor (0·3 mL) was injected via the cannula immediately after the first seven (IgG, $F(ab')_2$) and nine (Fab) blood samples only. Blood samples were then centrifuged for 5 min at 4000 rev min^{-1}. Plasma was isolated and an sample (0·1 mL) was applied to glass microfibre filters and was counted for radioactivity in a gamma counter (Minaxi gamma 5000, Packard Instruments, Rungis, France). Total radioactivity was then assayed for trichloroacetic acid (TCA) precipitable radioactivity. The filters were washed with 10% TCA (2 × 2 mL) and then once with ethanol (2 mL). TCA-precipitable ^{125}I was counted and corrected for radioisotope decay. For blood samples collected after 8 h only, plasma measurements were run in duplicate.

SDS-polyacrylamide gel electrophoresis

Plasma samples (0·1 mL) containing ^{125}I-antibody or fragments were analysed by SDS-PAGE. The gels were run according to Laemmli (1970) using a 12% or 8% polyacrylamide gel with a stacking gel of 3% acrylamide. Radiolabelled proteins and metabolites were autoradiographed using X-ray film (Amersham, France) and intensifying screens for several weeks. The migration zone of native ^{125}I-antibodies or fragments was compared with protein markers of known molecular weights (myosin, 205 kDa; β-galactosidase, 116·5 kDa; bovine serum albumin, 80 kDa; ovalbumin, 49·5 kDa; soybean trypsin inhibitor, 27·5 kDa and lysozyme, 14·3 kDa) and visualized with Coomassie brilliant blue.

Pharmacokinetic analysis

Estimates of α and β (distribution and elimination rate constants) were obtained by fitting the curve to the TCA-precipitable plasma concentration-time data of each experiment with a biexponential equation using the non-linear least squares SIPHAR program (Simed, Creteil, France). The constants of the exponential equation (A_1, A_2, α, β) were calculated with the reciprocal of the (concentration)^2 as the weighting factor. Residual analysis (an examination of the standard deviation) was performed to assess the 'goodness of fit'. In addition to the likelihood test, Akaike and Schwarz criteria were used to select the most appropriate model. Distribution and elimination half-lives ($t_{\frac{1}{2}}\alpha$, $t_{\frac{1}{2}}\beta$) were calculated as 0·693/α or 0·693/β, respectively. The area under the curve from time zero to infinity ($AUC_{0-\infty}$) and the mean residence time (MRT) were calculated by use of the equations:

$$AUC_{0-\infty} = A_1/\alpha + A_2/\beta \quad (1)$$

$$MRT = (A_1/\alpha^2 + A_2/\beta^2)/AUC_{0-\infty} \quad (2)$$

Distribution volume (Vd_β), distribution volume at steady-state (Vd_{ss}) and total body clearance (CL_t) were determined as follows:

$$Vd_\beta = dose/(AUC_{0-\infty}.\beta) \quad (3)$$

$$Vd_{ss} = dose.AUMC_{0-\infty}/(AUC_{0-\infty})^2 \quad (4)$$

$$CL_t = dose/(AUG_{0-\infty}) \quad (5)$$

Statistical analysis

Mean values for pharmacokinetic parameters were compared by use of one-way analysis of variance followed by Student's *t*-test with the Bonferroni adjustment. Significance was set at $P < 0.05$.

Results

The plasma disposition for each experiment was described by a biexponential decay for all IgG_1. Fig. 1 shows the mean disposition curve for IgG_1 from mouse, rat and man. The mean pharmacokinetic parameters are presented in Table 1. Over the 20 days of the experiment all the circulating radioiodine activity was protein-bound, as assessed by TCA precipitation of plasma samples, and was higher than 95%. Plasma disposition of mouse $^{125}[I]$-$F(ab')_2$ and Fab in the rat was also characterized by a biexponential decay (Fig. 2). The terminal half-lives of $F(ab')_2$ and Fab were considerably shorter than those of IgG (Table 1). The pharmacokinetic parameters of $F(ab')_2$ and Fab are also given in Table 1. The TCA precipitable fraction remained over 90% during the whole $F(ab')_2$

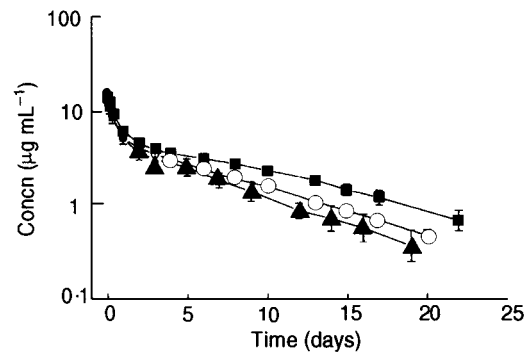

FIG. 1. Semi-logarithmic plasma concentration–time profiles after intravenous administration (bolus) of ^{125}I-labelled IgG (0·7 mg kg^{-1}) to the rat. Results are expressed as mean ± s.d. from six rats. ▲ Human IgG_1, ■ mouse IgG_1, ○ rat IgG_1.

Table 1. Pharmacokinetic parameters for human, rat and mouse IgG and $F(ab')_2$ and Fab in the rat.

	Rat IgG_1	Human IgG_1	Murine IgG_1	Murine $F(ab')_2$	Murine Fab
Distribution half-life (h)	6·11 ± 0·96	6·76 ± 1·92	8·4 ± 1·17	3·33† ± 0·81	0·89†† ± 0·048
Elimination half-life (days)	6·08 ± 0·14	5·33 ± 0·58	8·10* ± 0·80	0·699† ± 0·035	0·41†† ± 0·031
Area under the plasma concentration–time curve (μg mL^{-1} day)	43·7 ± 2·99	37·62 ± 8·14	61·06* ± 6	5·67† ± 0·30	1·09†† ± 0·016
Mean residence time (days)	7·96 ± 0·22	6·85 ± 0·79	10·54* ± 1·23	0·615† ± 0·031	0·267†† ± 0·034
Total body clearance (mL kg^{-1} day^{-1})	16·06 ± 1·11	19·13 ± 3·71	11·55 ± 1·14	123·5† ± 6·75	640†† ± 9·6
Distribution volume (mL kg^{-1})	141·04 ± 8·75	145·31 ± 14·75	133·9 ± 4·48	124·34 ± 5·16	382·33†† ± 29·62
Distribution volume at steady-state (mL kg^{-1})	131·63 ± 7·65	133·59 ± 12·78	125·02 ± 3·93	90·71† ± 8·54	186·7†† ± 23·29

In all experiments the dose was 0.7 mg kg^{-1}. *$P < 0.05$, significantly different from rat IgG_1; †$P < 0.05$, significantly different from mouse IgG_1; ††$P < 0.05$, significantly different from mouse $F(ab')_2$.

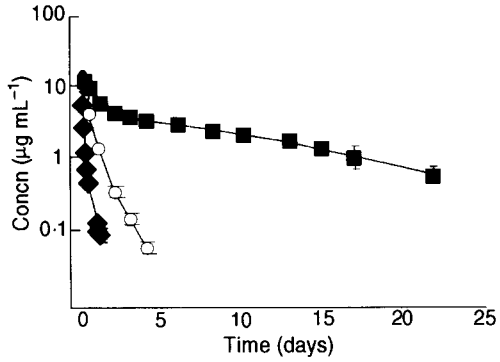

FIG. 2. Semi-logarithmic plasma concentration–time profiles after intravenous administration (bolus) of mouse ^{125}I-labeled IgG (■), $F(ab')_2$ (○) and Fab (◆) (0·7 mg kg^{-1}) to the rat. Results are expressed as mean ± s.d. from six rats.

sampling period but declined to 60% 8 h after Fab administration.

Plasma samples collected at various times (1, 3, 6, and 14 days for IgGs; 15 min, and 8, 48, 72 h for $F(ab')_2$; 1 min, and 1, 2, 6, 8 and 24 h for Fab fragments) were studied by SDS-PAGE and visualized by autoradiography to characterize plasma radioactivity. A time-dependent decrease in the intensity of the protein bands was observed and these became undetectable after 14 days, 3 days and 1 day for IgG_1, $F(ab')_2$ and Fab, respectively. No lower or higher molecular-weight products were observed under our experimental conditions. Migration of plasma radiolabelled-protein samples was identical with that of native molecules.

Discussion

Therapeutic and diagnostic antibodies are mainly used for treatment of cancer but also in the treatment of septic shock and sepsis, modulation of immune response, organ transplant therapy and as toxin antidotes (Pentel et al 1988; Timsina & Hewick 1992; Baud et al 1995). The choice of the best antibody depends on the intended use. Some improvements have been made as a result of the production of antibody fragments, although the use of these has both advantages and disadvantages. For example, their use optimizes the neutralization of small toxins in immunotoxicotherapy (Pentel et al 1988) or tumour localization in immunoscintigraphy by firstly preventing non-specific interactions between the Fc present on the IgG immunoglobulin and biological effectors and, secondly, increasing their diffusion into the body (Yokota et al 1993). Their increased systemic clearance compared with whole IgG can, therefore, limit the duration of their clinical efficacy. The administration of antibodies of heterologous origin could, moreover, be an advantage if rapid clearance is desired for accelerating toxin elimination in immunotoxicotherapy, or a disadvantage in the case of multiple antibody dosing, owing to increased immunogenicity and the risk of adverse immunological reactions. In fact, the medical use of antibodies requires knowledge of their pharmacokinetics in order to select the most appropriate antibody for optimum efficacy.

The pharmacokinetics of antibodies or fragments have frequently been described but few studies have gathered the main factors that must be considered in terms of variability such as: the origin and nature of the antibodies studied; the recipient species-related factors; and the influence of the analytical assay. For this reason we performed this study using one recipient species, i.e., the rat, and the same analytical assay using radiolabelled proteins. In contrast with several previous studies, moreover, the experiment was performed over a relatively long period (20 days for IgGs) enabling accurate determination of the disposition slopes of the native IgG, and the $F(ab')_2$ and Fab fragments.

We first compared the pharmacokinetics of two heterologous IgG_1, with the homologous IgG_1 in rats at the same dose (0·7 mg kg^{-1}). All IgG_1, were cleared from the circulation of the rat with biphasic kinetics consisting of distribution and elimination phases whose half-lives ranged from 6·1 to 8·4 h and 5·33 to 8·10 days, respectively.

The distribution properties of antibodies and fragments are limited by diffusion through biological membranes. Molecular weight, electric charge and glycosylation are known to be factors that control the diffusion of proteins across barriers (Triguero et al 1991; Yokota et al 1993). Our study shows that the extent and rate of IgG distribution are limited. In parallel, on the basis of the distribution half-life values, the rate of distribution was low because complete distribution required about 36–50 h for all IgG_1 ($6 \times t_{1/2}\alpha$). The distribution volume was about four times the rat plasma volume (35 mL kg^{-1}) for all IgG_1. These volumes of distribution are consistent with previous reported values in the rat. For example, Arizono et al (1994) reported a mean volume of distribution of 119·6 (103·4–151·2) mL kg^{-1} for a human monoclonal IgG. Zhu et al (1994) reported that the volume of distribution for mouse

IgG1 and IgG_{2a} was about three times the mouse plasma volume. In man the volume of distribution of homologous and heterologous IgG corresponds to about twice the plasma volume (Azuma et al 1991). Distribution properties appear similar for all the IgG_1 and are independent of the homologous or heterologous origin. We found a longer elimination half-life for rat IgG_1 than that reported by Henderson et al (1982) ($t_{\frac{1}{2}}\beta = 3{\cdot}4$ days) and Peppard & Orlans (1980) ($t_{\frac{1}{2}}\beta = 2{\cdot}2$ days), but these were estimated after shorter experimental periods (6 and 4 days, respectively) compared with our protocol. For heterologous IgG_1 in our study the elimination half-life of IgG_1 from man was in the same range as that from the rat. Ookubo et al (1989) reported slightly longer elimination half-lives for human ^{125}I–IgG in the rat (8 to 10 days) which was similar to the terminal $t_{\frac{1}{2}}$ they found for rat IgG. For mouse IgG_1 the elimination times were significantly longer. Our mouse IgG_1 data are comparable with those reported by Wawrzynczak et al (1992) who found an elimination half-life of 7·4 days for mouse IgG_{2b} in the rat. Weigle (1957) found a longer half-life for rabbit γ-globulin (6·7 days) compared with rat γ-globulin (5·5 days) when administered to the rat. In our study total body clearance (the main parameter characterizing elimination) of IgG_1 from man was similar to that from the rat and greater than that from the mouse. These data show that there are minor differences between distribution and elimination parameters for IgG_1 whether or not it is similar to that of the species of origin.

The effect of antibody molecular weight was the second factor studied. For this we selected mouse IgG, $F(ab')_2$ and Fab molecules derived from the same hybridoma. The distribution half-lives of mouse $F(ab')_2$ and Fab were shorter than that of IgG, the time to reach equilibrium being about 15 and 4·5 h, respectively. The $F(ab')_2$ distribution volume was in the same range as that of the corresponding IgG but the Fab distribution volume was 3-fold greater, showing the ability of Fab to diffuse into the rat extracellular water volume. The elimination properties were also dependent on antibody molecular weight because the elimination half-life and the mean residence time became considerably shorter as the molecular weight decreased (Table 1). In parallel, the total body clearance was 11- and 56-fold more rapid for $F(ab')_2$ and Fab, respectively, than for IgG. No data are yet available for $F(ab')_2$ pharmacokinetics in the rat.

In this study, we first describe the plasma kinetics of $F(ab')_2$ of mouse origin. Distribution half-life and volume values that are similar to ours have been reported by McClurkan et al (1993) and Pentel et al (1988) after administration of 0·12 g kg^{-1} and 7·5 g kg^{-1} of mouse and human Fab, respectively. The main differences concern the considerably higher systemic clearance (3888 mL kg^{-1} day^{-1}) in the study of McClurkan et al (1993) whereas Pentel et al (1988) and Moran et al (1994) in a recent study found values similar to ours, 648 and 604 mL kg^{-1} day^{-1}. Some of the differences could be accounted for by differences in the analytical techniques used to measure Fab concentrations and in the Fab origin, i.e. monoclonal murine Fab, or in the dose-dependent property of Fab clearance. The increased distribution and clearance we observed when antibody molecular weight was reduced was also observed by Mehvar & Shepard (1992) after administration of dextran with molecular weights ranging from 4 to 150 kDa. The more rapid clearance of $F(ab')_2$ and Fab can be explained by the absence of the Fc portion which reduces the interactions of $F(ab')_2$ and Fab with cells. Several studies have, moreover, reported that the kidney is the major route for the catabolism of Fab and elimination of unchanged Fab (Covell et al 1986; McClurkan et al 1993), in contrast with IgG and $F(ab')_2$ which have higher molecular weights (150 kDa and 100 kDa, respectively) and are not filtered at the glomerulus. The progressive appearance of free ^{125}I after administration of Fab, could be a consequence of renal catabolism and subsequent release of free iodine in the blood. Plasma analysis by SDS-PAGE did not reveal the presence of radiolabelled proteins smaller than Fab under our experimental conditions using a fixed percentage of acrylamide. The use of a gradient gel would have shown lower molecular weight products and would have enabled the separation of Fc from light chains. Similar observations were made by Demignot et al (1990) who recovered 60% of free radioiodine from blood 6 h after the injection of homologous Fab in balb C mice. More recently, the urinary excretion of the intact form of a murine monoclonal ^{3}H-Fab administered to rats was reported to account for $21 \pm 15\%$ of the total dose whereas the total radioactivity appearing in urine was $51 \pm 11\%$ (McClurkan et al 1993). The renal pathways of Fab catabolism and elimination remain to be clarified.

In conclusion, this study underlines the considerable variability in the pharmacokinetic parameters of intact antibody and corresponding $F(ab')_2$ or Fab. These significant differences in distribution and elimination should be taken into account in order to select the best antibody for a clinical application.

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