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Influence of Immunogenicity on the Pharmacokinetics of BMS-191352, a *Pseudomonas* Exotoxin Immunoconjugate, in Rats and Dogs

BHARAT DAMLE, LEE TAY, CHARLES COMERESKI†, WILLIAM WARNER* AND SANJEEV KAUL

*Department of Metabolism and Pharmacokinetics, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543 and *Department of Toxicology, Bristol-Myers Squibb Pharmaceutical Research Institute, Syracuse, NY 13057, USA*

Abstract

BMS-191352 is an immunotoxin construct of modified *Pseudomonas* exotoxin conjugated to a fragment of the BR96 monoclonal antibody. We have investigated the potential for immunogenicity of BMS-191352 and its influence on the pharmacokinetics in rats and dogs.

BMS-191352 was administered intravenously at doses of 0·75, 1·5, and 3 mg m^{-2} once every two days for a total of five doses in rats, and 1·2, 2·4, and 4·8 mg m^{-2} once every three days for a total of five doses in dogs. Blood samples were collected on days 1 and 9 in rats, and on days 1, 7, and 13 in dogs to monitor pharmacokinetics and anti-BMS-191352 immune response. Plasma concentrations of BMS-191352 and serum anti-BMS-191352 antibody titre were determined using ELISA assays. Pharmacokinetics were assessed using a non-compartmental method.

Anti-BMS-191352 antibodies were not observed in rats within the drug administration interval. In all dogs, except one, markedly higher anti-BMS-191352 antibody titres were observed on day 13 compared with days 1 and 7, and its magnitude was independent of BMS-191352 dose. The single dose kinetics of BMS-191352 in rats and dogs were linear and the drug exposures were generally dose proportional. Mean half-life, total body clearance, and volume of distribution were 1·74 h, 3·35 mL min^{-1} m^{-2}, and 0·27 L m^{-2} in rats, respectively, and 4·27 h, 6·28 mL min^{-1} m^{-2}, 1·19 L m^{-2} in dogs, respectively. The multiple-dose (day 9) kinetics in rats were similar to the single-dose kinetics. In dogs, the disposition of BMS-191352 on day 7 was similar to that on day 1; however, there was a precipitous reduction in the systemic drug exposure (by 5- to 110-fold) and marked increase in drug clearance on day 13. These changes in the kinetics of BMS-191352 were attributed to the generation of anti-BMS-191352 antibodies. In the one dog that did not develop anti-BMS-191352 antibodies, the pharmacokinetics were unchanged.

The pharmacokinetics of BMS-191352 may be perturbed due to an immune response thus restricting the therapeutic utility of the immunotoxin.

BMS-191352 (BR96 sFv-PE40) is a single-chain immunotoxin construct of the anti-Lewis^Y monoclonal antibody, BR96, and a modified form of *Pseudomonas* exotoxin A [PE40] (Friedman et al 1993). Upon repeated administration, BMS-191352 has demonstrated potent antitumour activity against a variety of Lewis^Y positive human tumours such as L2987, MCF-7, and H3396. For instance, H3396 (breast carcinoma) xenografts established in athymic mice and rats completely regressed after intravenous administration of doses of 2 mg m^{-2} BMS-191352 every fourth day for a total of five doses in nude mice and 1·5 mg m^{-2} doses every fourth day for a total of four doses in nude rats (Siegall et al 1994). The use of athymic animal models, while indispensable in evaluating the antitumour activity of potential drugs, do not account for the influence of the immune system on the pharmacological properties of protein anticancer drugs such as BMS-191352 which require

Correspondence: B. Damle, Research Investigator II, Metabolism and Pharmacokinetics, Bristol-Myers Squibb Pharmaceutical Research Institute, PO Box 4000, Princeton, NJ 08543-4000, USA.

multiple doses for therapeutic benefit. This is particularly important for immunotoxins since the presence of the toxin moiety in the molecule can render them to be immunogenic (Wawrzynczak 1991). Therefore, in this investigation, the potential for antibody formation by BMS-191352 and the influence of immunogenicity on the pharmacokinetics of BMS-191352 were evaluated in rats and dogs. These pharmacokinetic studies were conducted as part of a toxicology evaluation and hence a primary criteria for species selection for these studies was the expression of Lewis^Y antigen on normal tissues.

Materials and Methods

Materials

BMS-191352 was supplied by Immunex Corp., Seattle, WA (under contract with Bristol-Myers Squibb), as a 1 mg mL^{-1} solution in 25 mM sodium phosphate and 50 mM sodium chloride buffer (pH 7·5). This solution was stored at −70°C. To prepare the dosing solution, a sample sufficient for dosing was thawed at room temperature, diluted as required with Sodium Chloride for Injection (United States Pharmacopoeia) and sterile filtered.

Animals

Sprague-Dawley rats (187–299 g) and beagle dogs (8–11 kg) were obtained from Harlan Sprague-Dawley (Frederick, MD) and Marshall Farms (North Rose, NY), respectively. The animals were housed individually and were acclimated to study conditions for at least two weeks in a temperature and humidity controlled room with alternating 12-h light–dark cycles. Rats had free access to laboratory rodent diet and dogs were offered standard amounts of laboratory canine diet. Tap water was freely available.

Pharmacokinetics in rats

BMS-191352 was administered as intravenous bolus doses of 0·75, 1·5, and 3 mg m^{-2} (0·125, 0·25, and 0·5 mg kg^{-1}, respectively; n = 8 per gender per group) once every two days for a total of five doses. Serial blood samples for pharmacokinetics were collected from a jugular vein cannula before dosing and at 3 and 30 min, and 1, 2, 4, 6, 12, and 24 h after dosing on day 1 (first four rats per gender per group) and day 9 (last four rats per gender per group). At each collection time approximately 0·2 mL blood was collected in microvette tubes containing potassium EDTA. Following collection, the EDTA tubes were inverted several times and blood samples were placed on ice before being centrifuged for 10 min at 1000 *g* and 0–5°C within 1 h. The resulting plasma samples were stored at −70°C along with quality control samples which were included to ensure drug stability until quantitation of BMS-191352. In addition, blood samples were collected before dosing on days 1 and 9 from all eight rats per gender per group and serum was obtained within 1 h of collection; these serum samples were stored at −70°C and were utilized for the determination of anti-BMS-191352 antibodies.

Pharmacokinetics in dogs

BMS-191352 was administered as a short intravenous infusion (5-min) of 1·2, 2·4, and 4·8 mg m^{-2} doses (0·06, 0·12, and 0·24 mg kg^{-1}, respectively; n = 3 per gender per group) once every three days for a total of five doses. Serial blood samples (1 mL) for pharmacokinetic analysis were collected in potassium EDTA tubes before dosing, and at 5 (end of infusion), 15, 30, and 60 min, and 3, 6, 9, 12, 16, and 24 h on days 1, 7, and 13. Additionally, blood samples for the determination of anti-BMS-191352 antibodies were collected before dosing on days 1, 7, and 13. Blood samples were processed to obtain plasma and serum as described above.

Determination of anti-BMS-191352 antibodies

The serum titre of antibodies specific for BMS-191352 was assessed using an Enzyme-Linked Immunosorbent Assay (ELISA) method (Liddell & Cryer 1991). Briefly, plates were coated with BMS-191352 overnight at 4°C. Plates were washed and blocked with 1% bovine serum albumin at 37°C for 1–2 h. The plates were washed again and a three-fold serial dilution of the test serum, starting with an initial dilution of 1 : 10, was added to the appropriate wells and the plates were incubated at 37°C for 2 h. The plates were then washed and specific antibody binding was detected using an alkaline phosphatase-conjugated affinity purified goat anti-dog or anti-rat IgG antibody (Kirkegard and Perry, Gaithersburg, MD). Subsequently, the plates were washed and substrate (1 mg mL^{-1} *p*-nitrophenyl phosphate in diethanolamine buffer) was added to each well. After 30 min at 25°C, the reactions were stopped with 3 M NaOH and the absorbance was recorded using a dual wavelength (405/550 nm) microtitre plate reader. The end-point titre was defined as the reciprocal of the highest dilution that gave an absorbance reading 2-fold greater than the mean plate background

absorbance. An individual animal was considered to have a positive anti-BMS-191352 antibody titre if its titre increased by more than two serial dilutions relative to its baseline (before dosing) value.

Quantitation of BMS-191352

The concentrations of BMS-191352 in plasma samples were determined using a published ELISA method (Damle et al 1998). Briefly, the assay employed a double antibody sandwich technique in which a monoclonal anti-PE40 antibody was used to capture BMS-191352 in plasma samples. The captured BMS-191352 was then detected using a biotinylated monoclonal BR96 anti-idiotypic antibody followed by the addition of streptavidin–horseradish peroxidase conjugate and chromogen 3,3′,5,5′-tetramethylbenzidine. The optical density was measured at 450 nm. The standard curve range in rat and dog plasma was 2–32 ng mL^{-1}. Quality control samples were included in each analytical run to verify the stability of the study samples during shipment and storage, and the accuracy and precision of the analyses. The assay utilized was specific for BMS-191352 since the likely breakdown products (chimeric BR96 and PE40) or structurally similar proteins (*Pseudomonas* exotoxin, LysPE40, and Heregulin-PE40) did not interfere with the quantitation of BMS-191352 (Damle et al 1998).

For analyses of the plasma samples from these studies, the accuracy of the assay was at least 91% and 93% for the rat and dog plasma, respectively; the precision was greater than 92% and 94%, respectively. The reproducibility of the quality control results indicated that BMS-191352 was stable until analyses, and that the assay method employed was accurate and precise.

Pharmacokinetic analysis

The plasma concentration–time data were analysed by a non-compartmental method (Gibaldi & Perrier 1982). The peak plasma concentration, C_{max}, and the time to peak concentration, T_{max}, were obtained from experimental observations. Using no weighting factor, the terminal log-linear phase of the plasma concentration–time curve was identified by least-square linear regression of at least three data points which yielded a minimum mean square error. The half-life of the terminal log-linear phase, $t_{\frac{1}{2}}$, was calculated as 0·693/K, where K is the absolute value of the slope of the terminal log-linear phase. The area under the plasma concentration–time curve from zero to infinity, AUC_∞, was determined by summing the areas from time zero to the time of last measured concentration, calculated by using conventional trapezoidal and log-trapezoidal methods, and the extrapolated area. The extrapolated area was determined by dividing the final concentration by the slope of the terminal log-linear phase. The area under the plasma concentration–time curve over a dosing interval, AUC_τ, represented an AUC over a 48- and 72-h dosing interval in rats and dogs, respectively. The total body clearance (CL_t) and the apparent steady state volume of distribution (Vd_{SS}) were calculated from the following relationships:

$$CL_t = Dose/AUC$$

$$Vd_{SS} = CL_t * MRT$$

where AUC equals AUC_∞ for the first dose and AUC_τ for subsequent doses, and MRT equals MRT_∞ for the first dose and MRT_{SS} at steady state. MRT was calculated as follows:

$$MRT_\infty = [AUMC_\infty/AUC_\infty] - [t/2]$$

$$MRT_{SS} = \{[AUMC_\tau + \tau(C_m)_{ss}/K]/AUC_\tau\} - t/2$$

where $AUMC_\infty$ is the area under the first moment curve extrapolated to infinity, $AUMC_\tau$ is the area under the first moment curve over the dosing interval, τ is the dosing interval, $(C_m)_{ss}$ is the last measurable concentration at steady state, and t is the time required for intravenous drug administration (t = 0 h for bolus administration in rats and t = 0·083 h for 5-min infusion in dogs).

Statistical analysis

The pharmacokinetic parameters were analysed using PC SAS version 6 (SAS/STAT Users Guide 1989). The effects of dose, gender, and period were evaluated by analysis of variance. Tukey's unweighted studentized range test was used to make pairwise comparisons for significant effects. All statistical tests were carried out at the 5% significance level.

Results

Anti-BMS-191352 antibodies

The geometric mean (range) anti-BMS-191352 antibody titres in rats and dogs are presented in Table 1. Anti-BMS-191352 antibody titres were evaluated in both cohorts of rats used for pharmacokinetics on days 1 and 9. In rats, the baseline (before dose on day 1) anti-BMS-191352 titre across all dose levels ranged between 10–90. On

Table 1. Geometric mean (range) anti-BMS-191352 antibody titre.

BMS-191352 dose group (mg m^{-2})	Anti-BMS-191352 antibody titre[a]		
Rat[b]	Day 1	Day 9	
0·75	11 (10, 30)	13 (10, 30)[c]	
1·5	10 (10, 10)	17 (10, 90)	
3·0	12 (10, 30)	24 (10, 90)[d]	
Dog[e]	Day 1	Day 7	Day 13
1·2	75 (30, 270)	62 (10, 810)	10514 (10, 196830)
2·4	62 (10, 810)	90 (30, 810)	54632 (21870, 196830)
4·8	43 (10, 90)	62 (10, 810)	31542 (7290, 65610)

[a]Anti-BMS-191352 antibody titre was obtained on samples before the dose on each indicated study day. Titre was defined as the reciprocal of the greatest dilution that gave an absorbance of at least two-times greater than the plate background. [b]n = 16 rats, unless otherwise noted. [c]n = 15, due to difficulty in obtaining sample in one rat. [d]n = 10, due to death of animals or difficulty in obtaining samples. [e]n = 6 dogs.

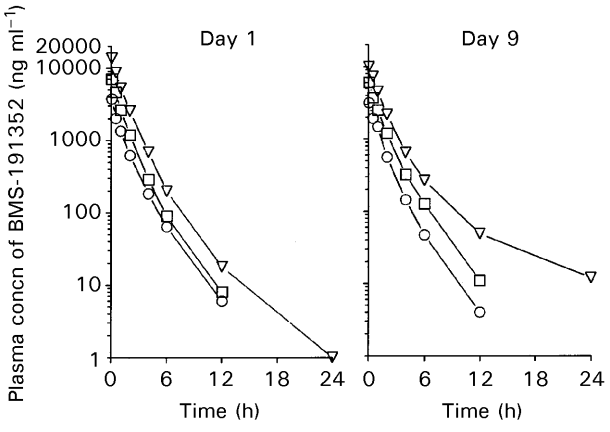

Figure 1. Mean plasma concentration–time profiles of BMS-191352 in rats after intravenous doses of 0·75 (○), 1·5 (□), and 3·0 (▽) mg m^{-2} BMS-191352 given every second day for a total of five doses. Separate cohorts of rats were used for evaluating the pharmacokinetics on days 1 and 9.

day 9, only four (from 1·5 and 3·0 mg m^{-2} dose groups) of the 48 rats had a positive anti-BMS-191352 antibody response; however, the magnitude of the antibody response was minimal since the titre value in these four rats was 90. Of the four rats that had a positive anti-BMS-191352 antibody response, three were females.

In dogs, the baseline anti-BMS-191352 titre across all dose levels ranged between 10–810. Similar titre values were also noticed on day 7. By day 13, all but one BMS-191352-treated dog had a positive anti-BMS-191352 antibody response; the range of positive antibody titre was 7290-196830. The dog (female in the low dose group) that did not develop immune response to BMS-191352 had a titre value of 10 on day 13. There were no apparent differences between gender in the magnitude of the anti-BMS-191352 immune response. Neither the onset nor the magnitude of the antibody response was dose related.

Pharmacokinetics in rats

The mean plasma concentration–time profiles after intravenous doses of 0·75, 1·5, and 3·0 mg m^{-2} BMS-191352 are depicted in Figure 1. The corresponding pharmacokinetic parameters are given in Table 2. Statistical analyses indicated no significant gender difference in the pharmacokinetic parameters. On day 1, C_{max} and AUC were dose related. Values for $t_{\frac{1}{2}}$, CL_t, and Vd_{SS} were similar across dose levels and gender; overall mean values were 1·74 h, 3·35 mL min^{-1} m^{-2}, and 0·27 L m^{-2}, respectively. After multiple doses (on day 9), C_{max} and AUC_τ were dose related and the key pharmacokinetic parameters ($t_{\frac{1}{2}}$, CL_t, and Vd_{SS}) were similar across doses. The $t_{\frac{1}{2}}$ value of BMS-191352 for the 3·0 mg m^{-2} dose appeared to be higher compared with the two lower dose levels, and was due to a long half-life in one rat at 3·0 mg m^{-2} dose. However, since all other parameters in this rat were similar to the other rats in the 3·0 mg m^{-2} group, this higher $t_{\frac{1}{2}}$ value was not considered to be dose-related. The pharmacokinetics of BMS-191352 after multiple dosing (on day 9) were generally similar to the single-dose kinetics. Statistically significant differences were noted in the C_{max} and Vd_{SS} values; the mean C_{max} values were approximately 16–23% lower on day 9 compared with day 1 while the Vd_{SS} values at the two higher doses were approximately 30 and 43% higher on day 9.

Pharmacokinetics in dogs

The mean plasma concentration–time profiles of BMS-191352 after intravenous doses of 1·2, 2·4, and 4·8 mg m^{-2} are presented in Figure 2. The pharmacokinetic parameters are given in Table 3. The data were pooled across gender since there

Table 2. Mean (s.d.) pharmacokinetic parameters of BMS-191352 in rats.

Parameter	Dose of BMS-191352 ($mg\,m^{-2}$)	Pharmacokinetic parameters[a]	
		Day 1	Day 9
C_{max} ($ng\,mL^{-1}$)	0·75	4034 (556)	3385 (501)[c]
	1·5	7604 (1176)	6096 (961)[d]
	3	13202 (1567)	10143 (1213)[e]
AUC_τ[B] ($ng\,h\,mL^{-1}$)	0·75	4739 (539)	4741 (965)[c]
	1·5	8865 (999)	7694 (641)[d]
	3	15741 (2352)	14391 (1482)[e]
$t_{\frac{1}{2}}$ (h)	0·75	1·59 (0·08)	1·72 (0·25)[c]
	1·5	1·62 (0·17)	1·63 (0·07)[d]
	3	2·00 (0·67)	3·14 (1·56)[e]
CL_t ($mL\,min^{-1}\,m^{-2}$)	0·75	3·09 (0·51)	3·20 (0·66)[c]
	1·5	3·25 (0·48)	3·82 (0·29)[d]
	3	3·71 (0·57)	3·81 (0·30)[e]
Vd_{SS} ($L\,m^{-2}$)	0·75	0·26 (0·06)	0·27 (0·05)[c]
	1·5	0·26 (0·05)	0·34 (0·05)[d]
	3	0·30 (0·03)	0·43 (0·11)[e]

[a]n = 8 (four per gender), unless otherwise noted; different cohorts of rats were used for evaluating the pharmacokinetics on days 1 and 9. [b]AUC_τ represents approximately 99% of AUC_∞ on day 1. [c]n = 7 (three males and four females) due to death of one rat. [d]n = 7 (four males and three females) due to difficulty in obtaining samples. [e]n = 4 (two per gender) due to death of two rats and difficulty in obtaining samples from two rats.

were no statistically significant ($P > 0·05$) differences in the pharmacokinetics of BMS-191352 between gender. The disposition parameters of BMS-191352 were similar on days 1 and 7. The values for $t_{\frac{1}{2}}$, CL_t, and Vd_{SS}, were not significantly different across 1·2, 2·4, and 4·8 $mg\,m^{-2}$ doses and between days 1 and 7; overall mean values were 4·27 h, 6·28 $mL\,min^{-1}\,m^{-2}$, and 1·19 $L\,m^{-2}$, respectively. On day 13, $t_{\frac{1}{2}}$ values were similar across the three dose groups but the CL_t and Vd_{SS} values for the 1·2 and 4·8 $mg\,m^{-2}$ doses were lower compared with the values for the 2·4 $mg\,m^{-2}$ dose. Compared with days 1 and 7, there was a precipitous drop in the systemic exposure on day 13. The mean C_{max} and AUC_τ values were about 5- to 55-fold and 5- to 110-fold lower on day 13 compared with day 1, respectively. Consistent with the decreased exposure, the $t_{\frac{1}{2}}$ values were significantly lower and CL_t and Vd_{SS} values were higher on day 13.

Table 3. Mean (s.d.) pharmacokinetic parameters of BMS-191352 in dogs.

Parameter	Dose of BMS-191352 ($mg\,m^{-2}$)	Pharmacokinetic parameters[a]		
		Day 1	Day 7	Day 13
C_{max} ($ng\,mL^{-1}$)	1·2	1112 (220)	1007 (139)	231 (387)
	2·4	2192 (351)	2302 (392)	40 (61)
	4·8	4419 (778)	4000 (413)	403 (350)[c]
AUC_τ[b] ($ng\,h\,mL^{-1}$)	1·2	3175 (340)	3020 (353)	703 (1365)[c]
	2·4	6502 (803)	6159 (826)	59 (102)[c]
	4·8	11743 (1610)	11944 (2309)	577 (537)[c]
$t_{\frac{1}{2}}$ (h)	1·2	4·63 (0·44)	4·63 (0·85)	2·09 (1·29)[c]
	2·4	4·31 (0·41)	4·11 (0·92)	0·97 (0·26)[c]
	4·8	3·74 (1·10)	4·18 (0·50)	1·15 (0·14)[c]
CL_t ($mL\,min^{-1}\,m^{-2}$)	1·2	6·11 (0·74)	6·36 (0·81)	605 (655)[c]
	2·4	5·99 (0·84)	6·17 (0·83)	3783 (3539)[c]
	4·8	6·64 (0·72)	6·39 (1·26)	226 (145)[c]
Vd_{SS} ($L\,m^{-2}$)	1·2	1·25 (0·26)	1·25 (0·14)	78·9 (88·3)[c]
	2·4	1·19 (0·24)	1·02 (0·41)	283 (255)[c]
	4·8	1·17 (0·13)	1·28 (0·14)	20·3 (12·3)[c]

[a]n = 6 (three per gender), unless otherwise noted. [b]AUC_τ represents approximately 99% of AUC_∞ on day 1. [c]n = 5 (two males and three females); concentrations were below the lower limit of quantification and hence certain parameters could not be determined.

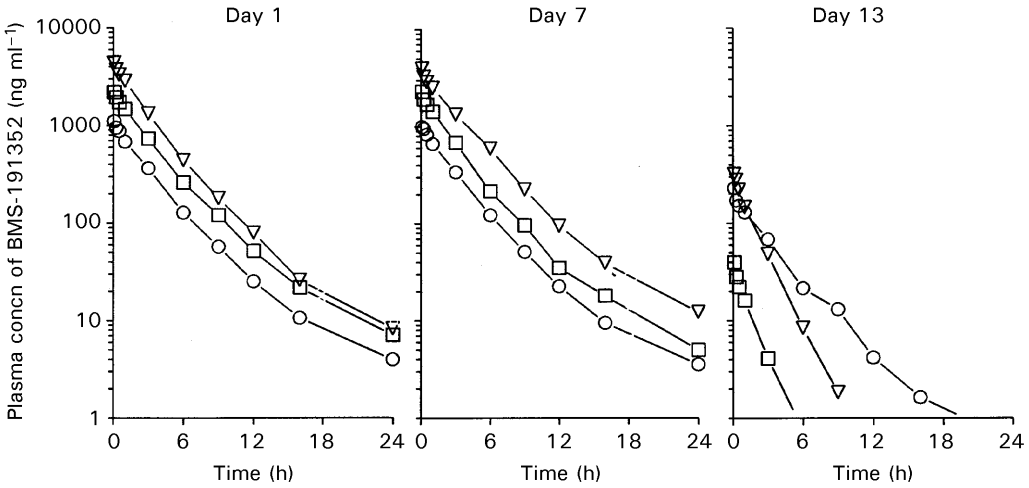

Figure 2. Mean plasma concentration–time profiles of BMS-191352 in dogs after intravenous doses of 1·2 (○), 2·4 (□), and 4·8 (▽) mg m^{-2} BMS-191352 given every third day for a total of five doses.

Correlation between anti-BMS-191352 antibody titre and AUC

Since the single and multiple dose pharmacokinetics of BMS-191352 were reasonably similar in rats, the minimal immune response seen in only a few rats after multiple dosing was considered not to influence the disposition of BMS-191352. In dogs, the plot of the ratio of the day 13/day 1 antibody titres vs the ratio of the day 13/day 1 AUC_τ values is shown in Figure 3. There appeared to be no correlation between the magnitude of the anti-BMS-191352 antibody titre and the decrease in the AUC_τ values. However, the one dog that did not develop positive anti-BMS-191352 antibody titres (not presented in Figure 3) had similar AUC_τ values on day 13 compared with day 1 (day 13/day 1 ratio of AUC_τ was 0·94).

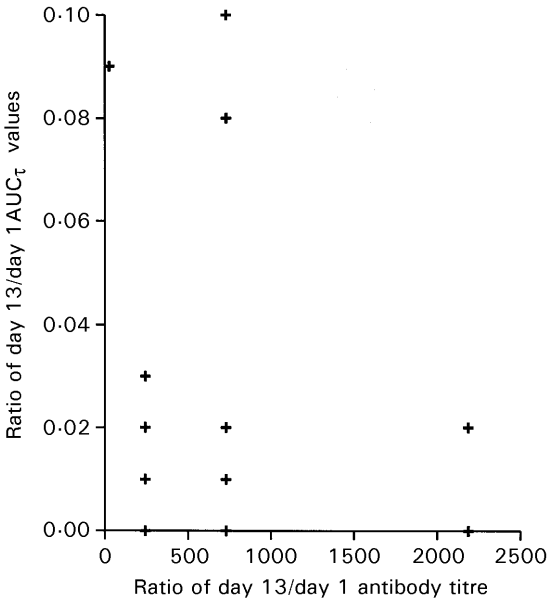

Figure 3. Ratio of day 13/day 1 anti-BMS-191352 antibody titre vs day 13/day 1 AUC_τ values in dogs. Data for one dog that did not mount a positive anti-BMS-191352 antibody response and had similar exposures across days is not shown.

Discussion

BMS-191352 was immunogenic in both rats and dogs. Although an immune response was not seen in rats used for pharmacokinetics (not evaluated for immunochemistry past day 9), the animals used for toxicology evaluation generally showed a strong immunogenic response to BMS-191352 within two to three weeks (data not presented). In both species, the magnitude of the immune response was independent of the BMS-191352 dose. The time course of the immune response (i.e. onset and peak response) was not determined since anti-BMS-191352 antibodies were analysed only on the days of pharmacokinetic sampling. However, it was evident that in the dog, an immune response developed some time between day 7 and day 13. It is unclear at the present time if anti-BMS-191352 antibodies were predominantly generated against the toxin portion (PE40) and/or the BR96 component of BMS-191352 as both BR96 and *Pseudomonas* exotoxin are known to be immunogenic (Ogata et al 1991; Sjogren et al 1997).

After intravenous administration the plasma concentrations of BMS-191352 declined rapidly in an apparent biexponential manner. The second exponential phase usually begins after 12 h by which time the concentrations were less than 1–3% of the peak values. The analytical sensitivity of the assay (2 ng mL^{-1}) afforded the ability to quantitate in some cases beyond 12 h resulting in the second

exponential phase. Therefore, for all practical purposes, the decline in BMS-191352 plasma concentrations may be considered to be mono-exponential. It should be noted that the AUC_{0-t} values were greater than 95% of AUC_{∞} which is indicative of adequate characterization of the terminal log-linear phase for non-compartmental analyses of the concentration–time data. The single-dose kinetic parameters obtained in both species indicate linear kinetics of BMS-191352, and the systemic drug exposure was generally proportional to the dose. These data are consistent with previous studies (Damle et al 1998).

The clearance of BMS-191352 in both species in the absence of an immune response was approximately 1% of the liver blood flow after single doses or after multiple doses. However, the mechanism(s) of clearance, including the contribution by the liver and kidneys, are presently unknown. Considering that the molecular weight of BMS-191352 is approximately 67 kDa, comparable with that of albumin, it seems unlikely that kidneys may contribute significantly to the clearance of intact BMS-191352 via glomerular filtration. In general, the catabolism of proteins is mediated by proteolytic enzymes ubiquitously present in the body resulting in several different breakdown products (Bocci 1987). In preliminary studies, Western Blot analyses performed under non-reducing conditions on plasma samples collected after a single dose in dogs did not indicate the presence of the PE40 and BR96 fragments of BMS-191352 (data not presented). These results suggest that PE40 and BR96 fragments may not be major metabolic products of BMS-191352 or, if formed, are rapidly metabolized further to smaller fragments. Additional studies are required to fully delineate the mechanism(s) by which BMS-191352 is eliminated.

It appeared that anti-BMS-191352 antibodies markedly enhanced the clearance of BMS-191352 as seen on day 13 in dogs. When the plasma samples, before the dose on day 13 in dogs, were mixed with BMS-191352 ($10\,\mu g\,mL^{-1}$), quantifiable levels of the immunotoxin were not obtained. It appears that BMS-191352 conjugated to these antibodies is undetectable using the ELISA method employed. This difficulty in quantifying a protein under conditions of an immune response is well recognized (Ferraiolo & Mohler 1992). Taking into consideration that products of antigen–antibody reactions may differ in molecular mass depending on the relative concentrations of two components, alternative analytical techniques such as HPLC or LC/MS were not considered for BMS-191352 analyses in the setting of an immune response. The association between BMS-191352 and the neutralizing antibodies may be considered analogous to a drug–protein binding scenario where only the unbound drug concentration is pharmacologically meaningful. The ELISA assay employed here measured free BMS-191352 and did not detect antibody-conjugated BMS-191352. It is presently unclear whether the immune complex of BMS-191352 is rapidly eliminated via the reticuloendothelial system, a mechanism that is part of the normal physiological process for removal of foreign proteins, or if the complex circulates for a certain time in the blood.

As seen in the dog study, there was no correlation between reduction in the plasma exposure of BMS-191352 (AUC values) and the increase in the anti-BMS-191352 antibody titre (Figure 3). It is important to note that the contribution of the immune complex to the plasma exposure of BMS-191352 and the titre of anti-BMS-191352 antibodies could not be assessed using the ELISA methods employed in the study. This contribution of the immune complex may become important if the complex is not rapidly eliminated from the blood. The lack of correlation between immunogenicity and pharmacokinetics may also be partly related to the polyclonal nature of an immune response. During an immune response, the foreign protein is internalized by B lymphocytes and fragmented into several antigenic epitopes. Thus a foreign protein is recognized in different antigenic forms and the antibodies generated have different affinities to the antigen (Roitt 1997). Hence the distribution of antibody affinities may also need consideration in evaluating the effect of immunogenicity on drug kinetics. However, it is clear that if positive anti-drug antibodies are not developed, as in the case of one dog, then it is likely that the disposition of BMS-191352 will be unaltered.

The volume of distribution of $0{\cdot}26–0{\cdot}30\,L\,m^{-2}$ ($0{\cdot}04–0{\cdot}05\,L\,kg^{-1}$) in rats and $1{\cdot}19–1{\cdot}25\,L\,m^{-2}$ ($0{\cdot}06–0{\cdot}07\,L\,kg^{-1}$) in dogs following single doses indicated that BMS-191352 was mainly localized in the blood since the normal blood volume in rats and dogs is reported to be $0{\cdot}05$ and $0{\cdot}09\,L\,kg^{-1}$, respectively (Davies & Morris 1993). After multiple doses and in the presence of an immune response (day 13 in dogs), the rapid binding of anti-BMS-191352 antibodies to BMS-191352 resulted in a first-pass effect between the site of drug administration and the site of pharmacokinetic sampling (Pond & Tozer 1984). The first-pass effect may be attributed to rapid clearance of the immune complex and/or to the inability of the ELISA method to recognize complexed BMS-191352. Consequently, only a fraction of the dose administered was quantifiable, leading to an over-

estimation in the volume of distribution in the dog on day 13 (Chiou 1979).

In conclusion, the development of an immune response and the difficulty in predicting its effect on the drug kinetics, and consequently on anti-tumour activity, would restrict the clinical utility of BMS-191352. Moreover, already existing and neutralizing anti-immunotoxin antibodies resulting from prior exposure to *Pseudomonas* bacteria may complicate therapy (Morgan et al 1991). Therefore, clinical therapy with BMS-191352 for an extended duration of time may only be possible by limiting or eliminating the anti-drug immune responses perhaps with the concomitant use of immuno-suppressants.

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