Pharmacokinetics and Tissue Distribution of SB-251353, a Novel Human CXC Chemokine, after Intravenous Administration to Mice

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ABSTRACT

The pharmacokinetics and tissue distribution of SB-251353, a novel truncated form of the human CXC chemokine growth-related gene product beta, were studied after intravenous administration to the mouse (0.1–250 mg/kg). At the lowest dose, the clearance exceeded blood flow to the kidney. As the dose increased, clearance approached the glomerular filtration rate in the mouse. Clearance of this chemokine may be mediated by its pharmacologic receptor, CXCR2, via endocytosis with subsequent lysosomal degradation, as has been observed for several growth and hematopoietic factors. Apparent distribution volumes were high (±1 l/kg). Moderate binding to the Duffy antigen/receptor for chemokines on erythrocytes was observed. Consistent with the pharmacokinetic analysis, microscopic autoradiography showed uptake into renal proximal tubule epithelial cells. Limited excretion of SB-251353 in the urine (<2%) was consistent with catabolism of the chemokine in the tubules. Binding to hepatic sinusoids and connective tissue in the dermis was observed. This possibly reflected interaction of SB-251353 with heparin sulfate proteoglycan and may explain the large distribution volumes. This first study of the disposition of a chemokine provides insight into mechanism of action and physiological factors that may influence chemokine pharmacodynamics.

Chemokines are potent chemotactic cytokines classified structurally by the invariant position of cysteine residues near the amino terminus of the protein (CC, CXC, C, and CX3C, where C is the standard letter designation for cysteine and X represents an unspecific amino acid residue). Chemokines attract host defense effector cells along concentration gradients and are involved in numerous physiological processes, including leukocyte trafficking, inflammation, angiogenesis, tumor growth, and human immunodeficiency virus suppression (Rollins, 1997; Bagnoli, 1998; Schwarz and Wells, 1999).

SB-251353 is a novel truncated form of the human CXC chemokine growth-related gene product beta (GROβ, residues 5–73), with potent anti-infective and hematopoietic activities (King et al., 2000). SB-251353 is a basic, heparin-binding protein with a molecular mass of ~7500 Da. Studies in mice have shown that SB-251353 increases blood neutrophil counts with a single administration of 0.1 mg/kg. With higher single doses (≥2.5 mg/kg), SB-251353 mobilizes hematopoietic stem cells into the peripheral blood. Although the details of the molecular transduction pathway initiated by SB-251353 have not been elucidated, it has been shown that stem cell mobilization by GROβ-T is mediated by matrix metalloproteinase-9 (King et al., 2001).

Although there exists significant potential for the therapeutic use of this class of protein agents, extremely limited information exists regarding the pharmacokinetics of chemokines in animals (Laterveer et al., 1996; Vetillard et al., 1999), and no studies of the tissue distribution of a chemokine have been reported. To begin to develop an understanding of the mechanism and time course of pharmacologic action of SB-251353, we studied its pharmacokinetics following single and repeated i.v. administration in the mouse (0.1–250 mg/kg). Tissue distribution following i.v. administration of radioiodinated chemokine was studied using both macroscopic and light microscopic autoradiographic techniques (0.1 and 2.5 mg/kg).

These data provide information regarding the likely mech-

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ABBREVIATIONS: GROβ, growth-related gene product beta; DARC, Duffy antigen/receptor for chemokines; RANTES, regulated on activation, normal T cell expressed and secreted; PAGE, polyacrylamide gel electrophoresis; LLQ, lower limit of quantification; CL, clearance; Vss, steady-state volume of distribution; MRT, mean residence time; WBA, whole body autoradiography; GCSF, granulocyte colony-stimulating factor; %CV, mean Cmax values.
anisms of elimination of the chemokine, including the dose range over which different mechanisms may be influential. The potential influence of receptors, including CXCR2, the Duffy antigen/receptor for chemokines (DARC), as well as tissue glycosaminoglycan, on the fate of SB-251353, is considered. Comparisons are made between the disposition of this chemokine and previously studied growth and hematopoietic factors.

Materials and Methods

Chemicals. Recombinant SB-251353 and RANTES (regulated on activation, normal T cell expressed and secreted) were expressed in *Escherichia coli* at GlaxoSmithKline (King of Prussia, PA). Goat anti-mouse IgG (Fc-specific polyclonal antibody) and horseradish peroxidase-conjugated streptavidin were purchased from Pierce Chemical Co. (Rockford, IL). Mouse anti-human GRO monoclonal antibody and goat anti-human GROα polyclonal antibody were from R & D Systems (Minneapolis, MN). Na<sup>125</sup>I was from Amersham Pharmacia Biotech (Arlington Heights, IL). Iodobeads were from Bio-Rad (Hercules, CA). All other chemicals were of reagent grade or better.

Preparation of [125I]SB-251353. SB-251353 was radio-iodinated using a modified solid-phase chloramine T method (Tsumida et al., 1991). SB-251353 has no tyrosine residues, and therefore the method was designed to incorporate the radiolabel into histidine. The reaction mixture contained 250 μg of SB-251353 and 1 mCi of Na<sup>125</sup>I in 100 μl of 0.2 M sodium borate, 0.05% (v/v) Tween 20, pH 8.7, and a single IODO-BEAD (Pierce Chemical Co.). After a 10-min incubation at ambient temperature, protein-associated radioactivity was separated from free radiolabel by size exclusion chromatography in 10 mM HEPES buffer, 0.05% Tween 20, 150 mM sodium chloride, pH 7.4. Approximately 20% of the radioactivity in the final preparation was not protein-associated and could be separated from the product by diafiltration. For the in vitro blood cell binding studies (below), the preparation was dialyzed before use so that >95% of the total radioactivity was associated with the protein. SDS-polyacrylamide gel electrophoresis (PAGE) was used to assess radiochemical purity of the product (>90%). Specific radioactivity was 0.4 μCi/μg.

Blood Cell Association and Stability in Vitro. Fifty male BALB/c mice were sacrificed by carbon dioxide asphyxiation, and whole blood was collected from the vena cava. [125I]SB-251353 was added to ice-cold EDTA whole blood at nominal concentrations of 50 ng/ml and 25 μg/ml. Aliquots were removed for analysis, and the remaining blood was placed in a shaking water bath at approximately 37°C for 10 or 60 min. Following incubation, plasma was separated by centrifugation, and blood cells were washed sequentially (three times) with phosphate-buffered saline. The percentage of trichloroacetic acid soluble radioactivity in plasma was determined as described previously (Davis et al., 1992). Radioactivity in blood, plasma, washes, and blood cells was determined by scintillation counting. In a separate experiment, the effect of RANTES (25 μg/ml) on the blood cell association of [125I]SB-251353 (50 ng/ml, on wet ice) was assessed.

Pharmacokinetics. Mice were housed individually in stainless steel cages in a controlled environment (68–76°F; 40–70% relative humidity) with a 12-h light/dark cycle. The mice were offered Certified Rodent Diet (PMI Feeds, Inc., St. Louis, MO) or similar food ad libitum; filtered tap water was available from an automatic watering system. Thirty-six male (30–40 g) and 36 female (20–30 g) CD-1 mice (Charles River Laboratories, Raleigh, NC) were given 14 daily i.v. bolus doses of 2.5, 25, or 250 mg/kg SB-251353 by tail vein injection. The dosing volume was 10 ml/kg in 20 mM succinic acid, 6% (w/v) sucrose, 0.02% (w/v) Tween 80, pH 4. A terminal blood sample was collected from the vena cava of each mouse (following carbon dioxide asphyxiation) such that three mice/time point were sampled for each dose group at nominal times of 5 and 30 min and 1, 3, 6, and 24 h after dosing on days 1 and 14. Single dose pharmacokinetic studies were also performed in CD-1 and BALB/c mice (Charles River Laboratories) at an i.v. bolus dose of 0.1 mg/kg (n = 20–30 per group). In these studies, the dose volume was 4 ml/kg in sterile saline. Terminal blood samples were obtained from three to five mice/time point at nominal times of 2, 10, 20, 40, 60, 80, 120, and 160 min post dose.

Tissue Distribution. Four groups of male BALB/c mice (n = 7 per group) received a single i.v. dose of [125I]SB-251353 by bolus injection via a tail vein. Animal husbandry was as described above except that 20 mM sodium iodide was added to the drinking water beginning 72 h before dose administration. Doses were 0.1 mg/kg to groups 1 and 2 and 2.5 mg/kg to groups 3 and 4 (~1 μCi/mouse). The vehicle was phosphate-buffered saline, and the dose volume was 10 ml/kg. Each group was divided into three subgroups [A (n = 3), B (n = 2), and C (n = 2)]. At either 10 min (groups 1 and 3) or 120 min (groups 2 and 4), the mice were sacrificed and samples collected.

From subgroup A mice, blood was collected via cardiac puncture under halothane anesthesia, and radioactivity in blood and plasma was determined by direct radioanalysis of weighed aliquots. After exsanguination, tissues (listed in Fig. 3) were excised, rinsed with saline, blotted dry, and the weights recorded. The gastrointestinal tract contents, including small and large intestines, were collected as one sample for each mouse and homogenized. Carcasses were frozen in liquid nitrogen and then homogenized in a prechilled food grinder containing solid carbon dioxide. All samples (including weighed aliquots of homogenates) were counted directly in a gamma counter.

Subgroup B mice were sacrificed by halothane overexposure. Each carcass was frozen in a hexane/solid carbon dioxide bath for 2 min and placed on solid carbon dioxide for 2 h. Each carcass was embedded, along with radioactive reference standards, in chilled carboxymethylcellulose and frozen into a block. The embedded carcasses were stored at −20°C prior to sectioning for whole-body autoradiography. Forty-micrometer sections of each mouse were prepared using a cryomicrotome (Leica CM 3600, Leica Microsystems, Inc., Deerfield, IL) maintained at −20°C. Sections were collected on adhesive tape, dehydrated, then exposed to PhosphorImaging screens (Molecular Dynamics, Sunnyvale, CA). Exposed screens were scanned using a Molecular Dynamics 445 SI.

From subgroup C mice, blood was collected via cardiac puncture under halothane anesthesia, and radioactivity in blood and plasma was determined by direct radioanalysis of weighed aliquots. The following tissues were collected for light microscopic autoradiographic analysis: bone (femur with marrow), cerebellum, cerebrum, heart, kidneys, colon, liver, lungs, lymph nodes (superficial cervical, superior mesenteric), skin, small intestines (duodenum, jejunum, ileum), spleen, and thymus. Tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin, and 5-μm sections were cut. Slides were deparaffinized, dipped in Kodak NTB3 emulsion (diluted 1:1 with distilled water at 44°C; Eastman Kodak, Rochester, NY), dried, and stored refrigerated in light-tight boxes. Slides were developed at various times up to 4 weeks (Kodak D19), fixed, counterstained with hematoxalin, dehydrated, and cover-slipped. Slides were evaluated microscopically and the distribution of silver grains over the various tissues recorded as background, equivocal (+/−), minimal (+), mild (+++), moderate (++++) or intense (+++++).

Urinary Excretion in the Mouse. Eight BALB/c mice (Charles River Laboratories) were divided into two groups of four animals and acclimated to metabolism cages (Nalgene, Rochester NY). Following i.v. bolus administration of unlabeled chemokine (0.1 or 25 mg/kg; 10 ml/kg phosphate-buffered saline), urine was collected over a 0- to 24-h interval into polycarbonate containers surrounded by dry ice. Urine collection volume was determined gravimetrically. The amount of SB-251353 excreted in urine was calculated from the urine drug concentration (by immunoassay) and the urine collection volume.

Immunoassays for SB-251353. Plasma samples were analyzed for SB-251353 using an enzyme-linked immunosorbent assay. In the
assay, microtiter plates were coated with orienting goat anti-mouse IgG (Fc-specific polyclonal antibody), and then the plates were coated with mouse anti-human GRO monoclonal antibody. Neat plasma samples were diluted to within the assay calibration range of 25 to 1600 pg/ml in 50% (v/v) EDTA mouse plasma, 50% (v/v) PBS-Tween-bovine serum albumin buffer (10 mM sodium phosphate, 150 mM sodium chloride, 0.05% Tween 20, 0.1% bovine serum albumin, pH 7.4). SB-251353 was subsequently captured on the plate, and bound SB-251353 was probed with biotin-conjugated goat anti-human GROα polyclonal antibody. The sandwich was detected with horseradish peroxidase-conjugated streptavidin. The urine assay was performed similarly except that the assay calibration range was 125 to 8000 pg/ml in 10% (v/v) urine in PBS-Tween-BSA buffer.

Assay performance (precision and accuracy) was assessed by assaying spiked plasma and urine samples against a calibration curve prepared independently. For the plasma assay, within-run assay precision was ≤2.2% while bias was ≤3.2% across the calibration range. The lower limit of quantification (LLQ) of the plasma assay was 50 pg/ml (50 μl of neat mouse plasma). For the urine assay, within-run assay precision was ≤12.8% while bias was ≤5.3% across the calibration range. The LLQ of the urine assay was 250 pg/ml (10 μl of neat mouse urine).

Pharmacokinetic Analysis. For the pharmacokinetic studies, plasma clearance (CL), steady-state volume of distribution (Vss), and mean residence time (MRT) were estimated by noncompartmental methods (Gibaldi and Perrier, 1982) using WinNonlin Professional version 1.1 (Apex, NC). Mean concentrations and blood collection times for each group were used in the analysis. The initial distribution volume was estimated by dividing the dose by the maximum observed plasma concentration after bolus i.v. administration.

Results

Pharmacokinetics. The single and multiple dose pharmacokinetics of SB-251353 were studied over a broad dose range in the mouse. Plasma concentration time profiles for these studies are depicted in Fig. 1. Results of noncompartmental analyses of these data are summarized in Table 1. Single and multiple dose pharmacokinetic data were similar, indicating no accumulation or time dependence of the chemokine disposition. Similarly, there was no difference in the pharmacokinetics between male and female CD-1 mice over the dose range of 2.5 to 250 mg/kg. Therefore, summary statistics of pharmacokinetic parameters were calculated using pooled data from both males and females from days 1 and 14.

Noncompartmental analysis indicated that CL was ~25 ml/min/kg (mean CL ranged from 21–28 ml/min/kg) over the dose range of 2.5 to 250 mg/kg in CD-1 mice. Apparent Vss increased 3.6-fold from 900 to 3200 ml/kg (MRT increased 4-fold from 30–120 min) as the dose increased from 2.5 to 250 mg/kg.

The pharmacokinetics of SB-251353 at 0.1 mg/kg were characterized in separate single dose studies in male CD-1 and male BALB/c mice. At this dose level, the concentration-time profiles were qualitatively very different than for the higher dose groups (Fig. 1). The earliest post dose concentrations were consistent between the BALB/c and CD-1 mice, and they increased in proportion to the dose from 0.1 to 2.5 to 25 mg/kg. However, the concentrations in BALB/c and CD-1 strains, for the remainder of the profile, were considerably lower than would be predicted from the data obtained at 2.5 mg/kg in the CD-1 mouse assuming pharmacokinetic linearity. At 0.1 mg/kg, CL was at least 4-fold higher than at 2.5 mg/kg, and MRT was ~10 min (both strains). Although initial plasma concentrations increased in proportion to the dose from 0.1 to 25 mg/kg, the maximum observed concentration for the 250-mg/kg dose group was lower (and more variable) than expected based on the data obtained at lower doses. The mean Cmax (%CV) values following administrations of 0.1, 2.5, 25, and 250 mg/kg were 0.088 (6.5%), 2.37 (21.9%), 24.9 (22.1%), and 120 (40.3%), respectively. Specifically, observed Cmax increased only 5-fold from 25 to 250 mg/kg.

Blood cell association and stability of SB-251353 in blood were assessed by incubating radiolabeled SB-251353 in whole mouse blood on ice and for up to 1 h at 37°C over a concentration range of 50 ng/ml to 25 μg/ml. The results of these studies are summarized in Table 2. The blood to plasma ratio was concentration-dependent. At 50 ng/ml in ice-cold blood, the blood to plasma ratio was ~0.9 while at 25 μg/ml on ice, the blood to plasma ratio was ~0.6 (no cellular association). Furthermore, the binding to blood cells was entirely blocked (blood to plasma ratio reduced to 0.6) by adding 25 μg/ml RANTES. Trichloroacetic acid precipitation of the plasma from incubation of whole blood at 50 ng/ml indicated no change in the integrity of the molecule for up to 1 h at 37°C.

Tissue Distribution. The mean tissue to plasma radioactivity concentration ratios as determined by direct radioanalysis, at 10 min and 120 min after the intravenous administration of [125I]SB-251353 (0.1 and 2.5 mg/kg), are summarized in Fig. 2. Following intravenous administration of radiolabeled chemokine, radioactivity was rapidly and widely distributed into the tissues with the highest concentration found in the kidney. The overall pattern of distribution was similar for both dose levels, and as assessed by the tissue concentration ratios, most of the tissue radioactivity concentrations were dose-proportional at 10 and 120 min post dose. Although the bone marrow appeared to be an exception, variability between animals was high for this tissue (70–100% coefficient of variation). Furthermore, whole body autoradiographic analysis showed no difference in dose-normalized marrow concentration, and these were comparable with blood concentrations. For both dose levels, the concentrations of radioactivity in most tissues were higher at 10
min relative to 120 min; the exceptions that occurred in both dose groups were the cerebellum, cerebrum, eyes, pancreas, skeletal muscle, stomach, submandibular gland, testes, and thyroid/parathyroid.

In both the low- and high-dose groups, the mean concentrations of total radioactivity (µg Eq/g) in plasma at 10 min (0.155 and 4.36) and 120 min (0.072 and 2.11) were generally higher than the corresponding tissue concentrations. The following exceptions occurred for both dose levels: at 10 min, kidneys (1.06 and 27.8), thyroid/parathyroid (0.411 and 6.31), and liver (0.371 and 8.63); and at 120 min, thyroid/parathyroid (1.17 and 20.0), kidneys (0.533 and 14.6), pancreas (0.160 and 5.19), submandibular gland (0.179 and 4.79), stomach (0.162 and 4.74), bone marrow (0.111 and 2.43), and urinary bladder (0.103 and 2.20).

The plasma concentrations of radioactive activity at 10 min were similar to those measured for SB-251353 using immunoassay methodology when unlabeled SB-251353 alone was administered. However, using immunoassay, by 120 min after a 0.1-mg/kg dose, SB-251353 concentrations were at least 100-fold lower than the 10-min value, inconsistent with the relatively high plasma radioactivity concentrations observed in the tissue distribution study. Subsequent analysis of plasma radioactivity by SDS-PAGE demonstrated that the majority of the radioactivity was not retained on an 8% running gel and hence was not associated with protein.

At 10 min, essentially all of the administered radioactivity was accounted for in the analyzed tissues (means, 66.0 and 54.2% of the dose, respectively) and residual carcass (means, 46.3 and 38.3% of the dose, respectively). The lower overall total mean recoveries observed at 120 min (82.6 and 69.3%) of the dose for the low- and high-dose groups, respectively) was probably due to excretion of the administered radioactivity.

Whole body autoradiography (WBA) results were qualitatively similar to the results from direct scintillation counting of the tissues, with the exception of the bone marrow as discussed above. WBA showed the highest concentrations of radioactive activity in the kidneys for both doses (Fig. 3). The distribution of radioactivity in the kidneys was heterogeneous. At 10 min, levels of total radioactivity were higher in the renal cortex than in the renal medulla, and at 120 min, the relationship was reversed.

Microscopic autoradiography revealed the principal site of silver grain distribution to be the cortex of the kidney at 10 min post dose (Fig. 4, A and B). For both dose levels, the cortex had moderate numbers of silver grains localized over the epithelial cells at the urinary pole of the glomerulus and continuing down into the proximal convoluted tubule for approximately 10 to 20 cell lengths. The silver grains were principally localized over apical portions of the epithelium, indicating that SB-251353 had been filtered by the glomerulus and reabsorbed by the epithelium. In the high dose group, silver grains were present in a greater number of proximal tubule cross-sectional profiles, which indicates progression further down the nephron and suggested saturation of uptake. At 120 min, we observed a minimal number of silver grains in the kidney for both dose levels. This finding differed from the WBA analyses at 120 min. Given the results from
SDS-PAGE of the 120-min plasma, it is likely that \(^{[125\text{I}]}\)SB-251353 had been metabolized by 120 min, and that the regions visualized on the whole body autoradiolumigrams were due to free \(^{125\text{I}}\)I or smaller-molecular-weight breakdown products. The processing procedures used to prepare the tissues for microscopic autoradiography would not preserve nonprotein-associated \(^{125\text{I}}\)I in tissues.

After both the low and high dose of \(^{[125\text{I}]}\)SB-251353, the liver and skin had silver grains above background (Fig. 4, C–F). In the liver at 10 and 120 min post dose, we observed a minimal number of silver grains, specifically in the sinusoids, and localization appeared to be over the Space of Disse, the region between the epithelial cells lining the sinusoids and the hepatocytes. In the skin at 10 and 120 min post dose, we observed a smaller (mild) number of silver grains bordering the dense irregular connective tissue of the dermis. In both the liver and skin, the distribution of these silver grains appeared orderly, as if bound to elements of the connective tissue. In these tissues, distribution was similar for both dose groups.

In a number of the other tissues examined, equivocal (slightly above background) numbers of silver grains were observed in the lumens of sinusoids and vessels (blood or lymphatic) at both 10 and 120 min. This pattern of staining was evident in the bone marrow (Fig. 4, G–I), where no specific localization was apparent. This distribution is consistent with the localization of \(^{[125\text{I}]}\)SB-251353 (at 10 min) or other \(^{125\text{I}}\)I labeled breakdown products (at 120 min) in the blood. Minimal numbers of silver grains were also observed in the lumens of the small and large intestines at both 10 and 120 min, but there was inconsistency between samples.

**Urinary Excretion.** To quantify the extent of urinary excretion of SB-251353, groups of male BALB/c mice (n = 4/group) received single i.v. doses of 0.1 or 25 mg/kg unlabeled chemokine, urine was collected over 24 h, and drug in urine was quantified by sensitive immunoassay. Concentrations of SB-251353 were not quantifiable in urine for all four animals in the 0.1-mg/kg group. Using the assay LLQ and the urine collection volume, the extent of excretion of peptide was estimated to be <0.03% of the administered dose for the 0.1-mg/kg group. In the 25-mg/kg group, SB-251353 was quantifiable in the urine for all four animals. Mean urinary excretion was 0.4% (range of 0.005–1.63%) of the administered dose of 25 mg/kg.

**Discussion**

The disposition of SB-251353, a novel truncated form of the human CXC chemokine GRO\(_\alpha\), has been characterized in the mouse following intravenous administration. The integration of pharmacokinetic and tissue distribution data provides insight regarding physiological factors that may potentially influence the pharmacokinetics, and thereby the pharmacodynamics of this chemokine, in animals and humans. Furthermore, these studies provide insight into the disposition of endogenous chemokines generally and potentially their mechanism of action. Extremely limited information currently exists regarding the disposition of any chemokine in vivo.

SB-251353 has nonlinear pharmacokinetics in the mouse. At the highest doses studied, clearance was comparable with the glomerular filtration rate in the mouse (Davies and Morris, 1993). At the lowest dose, clearance was at least 4-fold greater and exceeded the rate of blood flow to the kidney (Davies and Morris, 1993). It is plausible that at the lower dose, clearance of this chemokine occurs through its pharmacologic receptor, CXCR2, via endocytosis with subsequent lysosomal degradation. Granulocyte colony-stimulating factor (GCSF) receptor has similarly been hypothesized to be responsible for the saturable clearance of GCSF (Kuwabara et al., 1994; Houston et al., 1999), and similar phenomena have been reported for hepatocyte growth factor (Liu et al., 1992; Liu et al., 1995).

In the present investigation, saturable binding of SB-251353 to blood cells was observed in vitro. The fact that
this was blocked by excess RANTES (a CC chemokine) suggests that binding of SB-251353 was due to DARC on red blood cells (Darbone et al., 1991; Chaudhuri et al., 1993; Neote et al., 1994). It has been postulated that erythrocyte DARC acts as a sink for circulating chemokines (Darbone et al., 1991). Erythrocyte DARC is not expected to internalize or degrade chemokine; however, the possibility exists that chemokine clearance may be mediated in part through DARC in tissues (Darbone et al., 1991; Chaudhuri et al., 1997; Luo et al., 1997).

We did not directly observe binding of $^{125}$I-SB-251353 to tissues expected to express the CXCR2 receptor or DARC (other than erythrocytes). This may be because the specific radioactivity of the radiolabel was low (<0.5 μCi/μg), as incorporation of $^{125}$I into histidine was inefficient. The diffuse tissue distribution of CXCR2 may also limit our ability to detect binding to the receptor (Sozzani et al., 1997; Asagoe et al., 1998; Tani et al., 1998). On the other hand, it is plausible that the uptake in the bone marrow detected by direct scintillation counting at 0.1 mg/kg (10 min), which was diminished at the higher dose (Fig. 3), may be an indication of specific, saturable binding to the CXCR2 receptor. Saturable binding of GCSF to the GCSF receptor in bone marrow has been reported previously (Kuwabara et al., 1995).
Macroscopic and microscopic distribution of radioactivity after i.v. administration of SB-251353 to the mouse supports the hypothesis that this chemokine undergoes glomerular filtration. Microscopic distribution suggested uptake into renal proximal tubule epithelial cells. Limited excretion of SB-251353 in the urine at doses up to 25 mg/kg is consistent with catabolism of the chemokine in the tubules. Similar phenomena have been observed for other filtered proteins (for example, Hepburn et al., 1995).

Uptake clearance (CL\text{uptake}) by the kidney can be estimated using the chemokine concentration in the kidney at 10 min (Fig. 2) from the distribution data, and from the area under the plasma concentration time curve from time 0 to 10 min from the pharmacokinetic study (as in Kuwabara et al., 1995). With this approach, mean kidney CL\text{uptake} was 21 ml/min/kg for the 2.5-mg/kg group. CL\text{uptake} was very similar to total CL estimated from the pharmacokinetic studies at the higher doses (2.5–250 mg/kg), and this value is similar to the glomerular filtration rate in the mouse (as discussed above).

As the dose increased from 2.5 to 250 mg/kg, the apparent steady-state distribution volume estimated by noncompartmental analyses increased dramatically (~3.6-fold). Also, initial plasma concentrations increased only 5-fold between 25 and 250 mg/kg. Estimates for the initial distribution volume from the observed C\text{max} data (~1/l/kg) are unusually large for a protein and approach total body water in the mouse (Davies and Morris, 1993). These observations cannot be explained by the limited binding of SB-251353 to erythrocyte DARC.

Increasing volume without a change in clearance may be an indication of increased binding to tissues as the dose was increased. Microscopic autoradiographic data are consistent with this hypothesis, as binding to connective tissue in the liver and dermis was evident at both 0.1 and 2.5 mg/kg (independent of dose over this lower dose range). Because the connective tissue elements contain large amounts of heparin sulfate proteoglycans, it is possible that distribution of the highly basic SB-251353 (pI ~ 10) to these sites reflects a charge interaction. As there is a large amount of heparin sulfate proteoglycan distributed throughout the extracellular matrix, the capacity for binding SB-251353 would be large. This may explain the large, relatively constant distribution volume that was observed from 0.1 to 25 mg/kg. The V\text{ss} increases over the dose range of 2.5 to 250 mg/kg may reflect more complete titration of the lower-affinity binding sites at the highest concentrations studied.

Localization of exogenously administered heparin-binding proteins, particularly growth factors, to heparin sulfate proteoglycans has been reported previously (Liu et al., 1995, 1998). The biological importance of chemokine-glycosaminoglycan complexation has not been fully elucidated, although it may be important for presentation to the pharmacologic receptor or for sequestration (Ruoslahti and Yamaguchi, 1991; Kuschert et al., 1998). Proteolytic release of heparin-bound chemokines may regulate their activity in vivo as suggested for growth factors (Saksela and Rifkin, 1990).

In summary, the pharmacokinetics and tissue distribution of a novel human CXC chemokine have been characterized in the mouse over a broad intravenous dose range. It is proposed that saturable binding and clearance may occur through the pharmacologic receptor, and this leads to nonlinearity in the pharmacokinetics. At low concentrations, the saturable component(s) of the clearance are more significant than clearance due to renal filtration, while at higher concentrations, renal filtration (with subsequent uptake and degradation in the proximal tubule) is the predominant route of elimination. Glycosaminoglycan complexation may be responsible for the large distribution volume of this chemokine and the apparent increase in distribution volume with increasing dose.

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