The Role of Liver and Kidney on the Pharmacokinetics of a Recombinant Amino Terminal Fragment of Bactericidal/Permeability-Increasing Protein in Rats

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Purpose. The pharmacokinetics of $rBPI_{23}$, a recombinant amino terminal fragment of bactericidal/permeability-increasing protein that binds to and neutralizes endotoxin, was investigated.

Methods. $rBPI_{23}$ was administered to rats at doses 0.01–10 mg/kg and plasma $rBPI_{23}$ levels were measured by ELISA. $rBPI_{23}$ was also administered to bilaterally nephrectomized rats. In addition, $rBPI_{23}$ was administered intra-hepatically via the pyloric vein to determine the first-pass effect by the liver. $rBPI_{23}$ concentrations were also simultaneously measured in the right atrium and aorta to determine the removal of $rBPI_{23}$ by the lungs.

Results. The concentration-time profile of $rBPI_{23}$ was described by a 3-compartmental model with parallel first order and Michaelis-Menten (saturable) elimination. The clearance of $rBPI_{23}$ was not altered by bilateral nephrectomy. Clearance of intra-hepatically administered $rBPI_{23}$ was 4.5 fold lower than intra-femorally administered $rBPI_{23}$. The concentration difference of $rBPI_{23}$ between aortic and right atrial blood was no greater than 11%. Clearance of $rBPI_{23}$ in rats could be reduced up to 10 fold by co-administration of heparin. Uptake by liver of intra-hepatically administered $rBPI_{23}$ was prevented by co-administration of heparin.

Conclusions. $rBPI_{23}$ is not significantly cleared by the kidneys, and no more than 11% of the $rBPI_{23}$ was removed by the lungs with each pass. The liver could remove 78% of the $rBPI_{23}$ from the hepatic circulation. Studies with heparin suggest $rBPI_{23}$ is cleared by binding to heparan sulfate sites in the liver.

KEY WORDS: bactericidal/permeability-increasing protein; pharmacokinetics; liver; kidney; heparin.

INTRODUCTION

Gram-negative bacterial sepsis is a major cause of mortality in hospitalized patients. The syndrome associated with sepsis includes hypotension, increased heart rate, and decreased systemic vascular resistance; multi-organ failure and death occur in a significant proportion of septic patients (1). Gram-negative sepsis is believed to be triggered largely by bacteria and their endotoxin (lipopolysaccharide, LPS), a component of the bacterial cell wall, which initiate a cascade of pathophysiological changes that include the release of a variety of cytokines (1–3).

Bactericidal/permeability-increasing protein (BPI) is a 55 kD neutrophil protein that kills gram-negative bacteria, and binds to and neutralizes the effects of LPS (4). A 23 kD recombinant form of BPI, $rBPI_{23}$, has been expressed in CHO cells from a construct encoding the first 199 amino acids of human BPI (4). *In vitro* studies indicate that $rBPI_{23}$ has similar LPS-binding and inhibitory properties as human holo-BPI (4–6). In addition, $rBPI_{23}$ has been reported to reverse the hemodynamic and cytokine changes induced by bacteria or LPS in rodents (7), pigs (8), and humans (9).

Preliminary pharmacokinetic studies demonstrated that $rBPI_{23}$ was cleared very rapidly from the circulation in mice, rats, rabbits and man, with a mean residence time varying from 0.5 minutes in mice to 7–13 minutes in man (10). We describe here the detailed pharmacokinetics of $rBPI_{23}$ in rats over a dose range of 0.01 to 10 mg/kg. Our results suggest that the liver is the major organ for the clearance of $rBPI_{23}$ from the circulation. Furthermore, since *in vitro* studies have shown that $rBPI_{23}$ binds to heparin with high affinity (11), we also investigated the role of heparin in the clearance of $rBPI_{23}$.

METHODS AND MATERIALS

Experimental protocols described below followed the guidelines according to *Guide for the Care and Use of Laboratory Animals* published by the U.S. Department of Health and Human Services and National Institutes of Health.

Animals

Male CD® rats (Charles River, Wilmington, MA) weighing 250–300 g were used in all experiments. Animals were received healthy, housed in conventional cages and received standard laboratory chow and water *ad libitum* in an environmentally controlled animal room with 12 hour light-dark cycles.

Reagents

$rBPI_{23}$ was cloned from a construct encoding the first 199 amino acids of human holo-BPI (12), expressed in CHO-K1 cells, and purified from culture medium as previously described (4). $rBPI_{23}$ was formulated at a concentration of 0.01 to 10 mg/ml in 20 mM sodium citrate, 150 mM sodium chloride, 0.1% polaxamer 188 and 0.002% polysorbate 80, pH 5.0.

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ABBREVIATIONS: BPI, bactericidal/permeability-increasing protein; $rBPI_{23}$, recombinant amino terminal fragment of BPI; LPS, lipopolysaccharide; I.V., intravenous; CHO-K1, chinese hamster ovary K1 cells; ELISA, enzyme-linked immunosorbent assay; Vss, steady state volume of distribution; MRT, total body mean residence time; CL, clearance; K_{12}, first order rate constant of transfer from compartment 1 to compartment 2; K_{21}, first order rate constant of transfer from compartment 2 to compartment 1; K_{13}, first order rate constant of transfer from compartment 1 to compartment 3; K_{31}, first order rate constant of transfer from compartment 3 to compartment 1; K_{10}, first order rate constant of elimination from compartment 1; V_{max}, maximal rate of elimination from compartment 1 by the saturable clearance mechanism; K_m, concentration at which the rate of saturable elimination is half of its maximal rate V_{max}; bFGF, basic fibroblast growth factor; PF4, platelet factor 4.

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Anesthesia

Prior to surgery rats were anesthetized with intramuscular administration of a mixture of Ketaset®-Rompun® solution (Ketamine HCl, 80 mg/kg, Aveco Co., Inc., Fort Dodge, Iowa, and 4 mg/kg Xylazine HCl, Miles Inc., Shawnee Mission, KS). The anesthetic level was considered adequate when flexion reflexes were absent.

Pharmacokinetic Experiments

Dose Range Studies

$rBPI_{23}$ was administered via the tail vein at doses of 0.01, 0.1, 0.3, 1, 3, or 10 mg/kg at a dose volume of 1 ml/kg. Blood samples were collected via the retro-orbital sinus in tubes containing sodium citrate (Sigma Chemical Co., St. Louis, MO) at selected times from 0.5 minutes to 8 hours after administration of $rBPI_{23}$. The plasma was extracted and stored at −70°C until assayed for $rBPI_{23}$ concentration.

Hourly and Daily I.V. Bolus Administration of $rBPI_{23}$ (QHx4)

$rBPI_{23}$ was administered to rats as an I.V. bolus hourly 4 times or daily 10 times at a dose of 1 mg/kg.

Co-administration of Sodium Heparin and $rBPI_{23}$

Mixtures of sodium heparin (10,000 units/ml, Steris Laboratories, Inc., Phoenix, AR) and $rBPI_{23}$ were prepared and administered to 3 rats at doses of 60, 600, or 6000 units/kg heparin and 0.1 mg/kg $rBPI_{23}$.

Administration of $rBPI_{23}$ via the Pyloric Vein

Pyloric vein cannulation was performed as described by Suzuki *et al.* (13). Briefly, after induction of anesthesia, the pyloric vein was exposed and cannulated such that the catheter tip was carefully placed about 2 mm into the portal vein, avoiding obstruction of blood flow through the portal vein. In addition, a catheter was placed in the jugular vein for blood sampling. $rBPI_{23}$ was administered as a ten minute infusion via the pyloric catheter. Upon completion of the study, Evans blue dye was injected into the pyloric catheter to determine if there were any leaks and to observe that the dye penetrated every lobe of the liver. In a set of control rats, the dosing catheter was inserted in the femoral vein and the pyloric vein was ligated to mimic the blood flow conditions of the test rats.

Administration of 1 mg/kg $rBPI_{23}$ in Nephrectomized Rats

Bilateral nephrectomies were performed as described by Waynforth and Flecknell (14). Additional rats designated as the sham group received incisions similar to the nephrectomized group, and incisions were sutured. The kidneys were not removed in these animals. The rats were allowed to recover and $rBPI_{23}$ was administered at a dose of 1 or 10 mg/kg three hours after surgery.

Stability Studies

$rBPI_{23}$ was diluted into plasma, serum, or whole blood at concentrations of 100,000 ng/ml, 1000 ng/ml, and 10 ng/ml and incubated at 37°C for 5 to 120 minutes. Samples were then assayed by ELISA, and the half-life of decline in the concentration was determined.

ELISA Assay

Plasma samples were assayed for $rBPI_{23}$ using affinity purified rabbit anti-$rBPI_{23}$ as the capture antibody and biotin-conjugated rabbit anti-$rBPI_{23}$ as the detection antibody as described (15).

Pharmacokinetic Analysis

Nonsaturable Pharmacokinetic Models

A two- or three-exponential disposition function was used to described the change in concentration with time when $rBPI_{23}$ was administered as an I.V. bolus for a single dose level. The data were fitted using the software program PCNONLIN (Statistical Consultants, Inc., Lexington, KY). Pharmacokinetic parameters total observed clearance (CL, ml/min/kg), steady state volume of distribution (Vss, ml/kg), and total body mean residence time (MRT, minutes) were calculated from the curve-fit parameters using standard equations (16).

Saturable Clearance Model

To describe the change in clearance of $rBPI_{23}$ with increasing dose, the data at all doses tested were fitted simultaneously to a three compartmental model with parallel first order and Michaelis-Menten (saturable) elimination from the plasma using the program ADAPT II (U. of Southern California, Los Angeles). The three differential equations describing the net transfer of $rBPI_{23}$ in this model were:

$$dX_1/dt = (-K_{12}X_1 + K_{21}X_2 - K_{13}X_1 + K_{31}X_3) - K_{10}X_1 - (V_{max}V_c)[X_1/(K_mV_c + X_1)] \quad (1)$$

$$dX_2/dt = -K_{21}X_2 + K_{12}X_1 \quad (2)$$

$$dX_3/dt = -K_{31}X_3 + K_{13}X_1 \quad (3)$$

where subscripts refer to the central (plasma) compartment (1), and two peripheral compartments (2 and 3). X_i is the amount (ng/kg) of $rBPI_{23}$ in compartment i; V_c is the volume of distribution (mL/kg) of the central compartment; K_{ij} is the first order rate constant (min^{-1}) of transfer from compartment i to compartment j; K_{10} is the first order rate constant (min^{-1}) of elimination from plasma; V_{max} is the maximal rate of elimination (ng/ml/min) of $rBPI_{23}$ from the plasma by the saturable clearance mechanism; K_m is the concentration (ng/ml) at which the rate of saturable clearance is half of its maximal rate V_{max}. The plasma concentration of $rBPI_{23}$ is calculated as $C_1 = X_1/V_c$.

RESULTS

Dose Dependent Plasma Clearance of $rBPI_{23}$

The observed clearance of $rBPI_{23}$ in rats decreased as doses were increased from 0.01 to 10 mg/kg (Figure 1A). At 0.01 to 0.3 mg/kg, $rBPI_{23}$ appeared to be cleared bi-phasically, with an initial half-life of less than 1 minute and a second half-life of less than 10 minutes. At the higher doses, the rate of decline

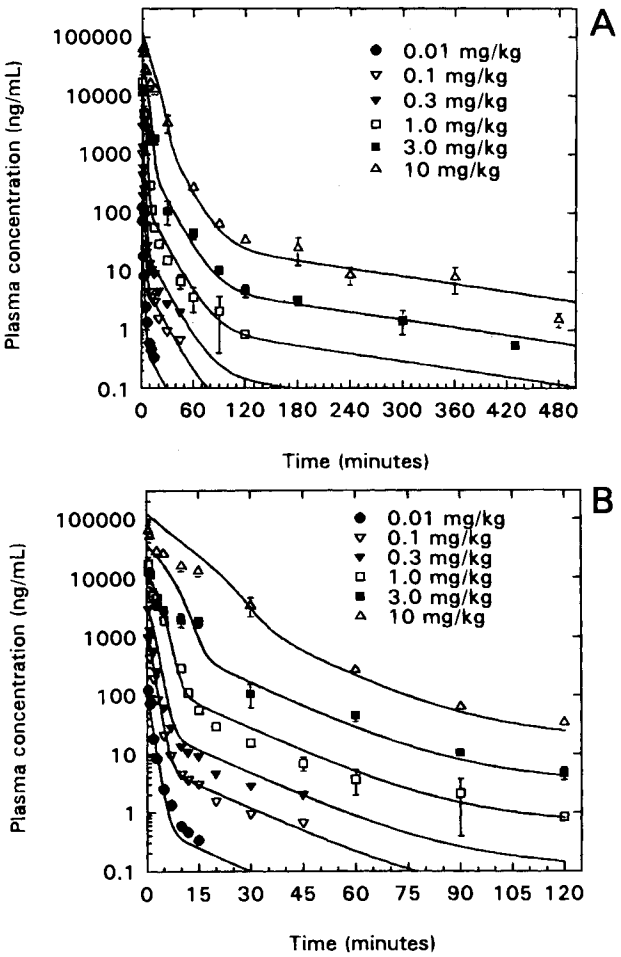

Fig. 1. Concentration-time profile of $rBPI_{23}$ administered intravenously to rats at doses from 0.01 to 10 mg/kg. A: Rats received an intravenous bolus dose of 0.01, 0.1, 0.3, 1, 3, or 10 mg/kg (data shown as mean ± SE, n = 3). The accompanying lines represent curve fits of the data. B: Plasma concentration of $rBPI_{23}$ during the first 120 minutes post-dosing is shown in detail.

of the plasma concentration of $rBPI_{23}$ became more complex and a convex component, in addition to the concave component at the lower doses, was apparent.

In order to explain the presence of a convex component and decreasing clearance with increasing dose, a pharmacokinetic model of three compartments was employed with parallel first order and Michaelis-Menten (saturable) elimination from the plasma (see METHODS section). The parameter values are listed in Table 1. At 3 and 10 mg/kg, the model overestimated the data during the first 10 minutes after administration of $rBPI_{23}$ (Figure 1B). Some of this deviation of the data from the present model could be partially corrected by including a plasma protein binding component in the model. Since the deviation was transient and no additional evidence exists for plasma protein binding, we did not pursue this possibility any further.

Using this model, the total clearance and relative contribution of the non-saturable clearance for $rBPI_{23}$ was calculated for a range of IV bolus doses (Table 2). The clearance changed by 7-fold from 0.01 to 10 mg/kg, with 93% of the administered $rBPI_{23}$ removed from the body by the saturable clearance mechanisms at the lowest doses. In addition, the model predicts that clearance should decrease by as much as 17 fold from doses of 1 μg/kg or less to doses of 100 mg/kg or greater. Evaluation of the pharmacokinetics of $rBPI_{23}$ outside the 0.01 to 10 mg/kg range was not attempted.

Table 1. Pharmacokinetic Model Parameter Values of $rBPI_{23}$ Clearance in Rats Using a 3-Compartmental Model with Parallel First Order and Michaelis-Menten Elimination (mean ± SE)

Parameter	Value
K_{12} (min^{-1})	0.0470 ± 0.0060
K_{21} (min^{-1})	0.0662 ± 0.0057
K_{13} (min^{-1})	0.00515 ± 0.00096
K_{31} (min^{-1})	0.00506 ± 0.00089
K_{10} (min^{-1})	0.0492 ± 0.0256
K_m (ng/ml)	2593 ± 479
V_{max} (ng/ml/min)	1978 ± 330
V_c (ml/kg)	80.0 ± 7.1

Note: Data from five experiments with doses of 0.01 to 10 mg/kg $rBPI_{23}$ administered to male rats were fitted simultaneously using a 3-compartmental model with parallel first order and Michaelis-Menten (saturable) elimination. The primary model parameter values from this fit are listed.

Clearance of Multiple Bolus Doses of $rBPI_{23}$

The observed clearance, Vss, and MRT of $rBPI_{23}$ following 4 hourly or 10 daily I.V. bolus injections were not statistically different ($p > 0.086$, unpaired t-test) from parameters obtained after a single dose of $rBPI_{23}$. Furthermore, the shapes of the plasma concentration-time curves were similar following single or multiple bolus injections of $rBPI_{23}$ (data not shown).

Rate of Metabolism of $rBPI_{23}$ in Whole Blood, Serum, and Plasma

The half-life of degradation of $rBPI_{23}$ in whole blood, serum, and plasma were 150 minutes or less for concentrations

Table 2. Clearance of $rBPI_{23}$ as a Function of I.V. Bolus Dose Using a 3-Compartmental Model with Parallel First Order and Michaelis-Menten Elimination

Dose mg/kg	Clearance ml/min/kg	% $rBPI_{23}$ cleared by saturable kinetics
low (<0.001) (projected values)	65.0	93.9
0.01	63.9	93.8
0.1	53.9	92.7
0.3	41.1	90.4
1.0	24.9	84.2
3.0	14.8	73.4
10.0	9.0	56.4
100.0	5.1	22.4
high (>100) (projected values)	3.9	0.0

Note: Total Clearance as defined by dose/(area under the time-concentration curve) were calculated using the model and parameter values in Table 1.

ranging 10–100,000 ng/ml. At 10 mg/kg $rBPI_{23}$ has an average *in vivo* half-life of log(2) * Vc/CL = 0.693 * 80/9 = 6.2 minutes. Thus, metabolism by the blood represents at most 4% of the observed *in vivo* clearance of $rBPI_{23}$ at this dose. At 0.01 mg/kg, metabolism by the blood represents less than 1% of total *in vivo* clearance.

Contribution of Kidneys, Liver, and Lungs to Clearance of $rBPI_{23}$

Bilateral nephrectomy was performed to evaluate the role of the kidney in the clearance of $rBPI_{23}$ (Figure 2A). The observed clearance of 1 mg/kg $rBPI_{23}$ in bi-laterally nephrectomized rats was 21 ± 4 ml/min/kg (mean ± SE, n = 6), which was not statistically different from that in sham operated rats (32 ± 6 ml/min/kg, p-value = 0.21). The clearance of 10 mg/kg $rBPI_{23}$ in bi-laterally nephrectomized rats was also not statistically different from that in sham operated rats (5.6 ± 0.8 ml/min/kg versus 7.7 ± 0.6 ml/min/kg, n = 7 in each group, p-value = 0.052).

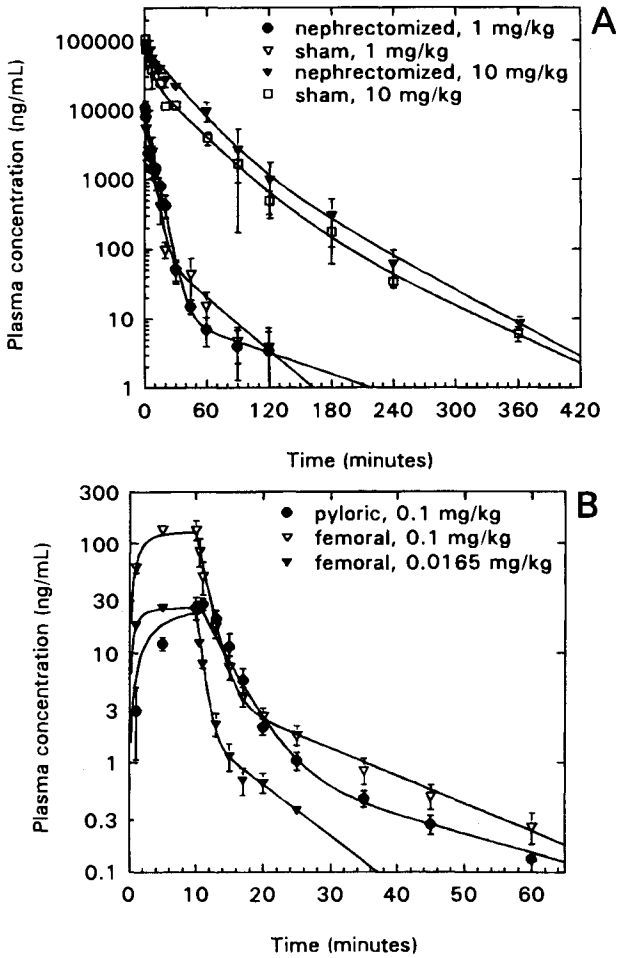

Fig. 2. Contribution of liver and kidneys in clearance of $rBPI_{23}$. A: Rats received 1 mg/kg $rBPI_{23}$ three hours after nephrectomy (data shown as mean ± SE, n = 6), or after sham surgery (n = 6), or 10 mg/kg three hours after nephrectomy (n = 7), or after sham surgery (n = 7). B: Rats received 0.1 mg/kg $rBPI_{23}$ over 10 minutes intrahepatically (filled circles, n = 5), or via the femoral vein (inverted open triangles, n = 2). To determine if non-linear clearance occurred at these low concentrations, 0.0165 mg/kg $rBPI_{23}$ was administered via the femoral vein as well (inverted filled triangles, n = 3).

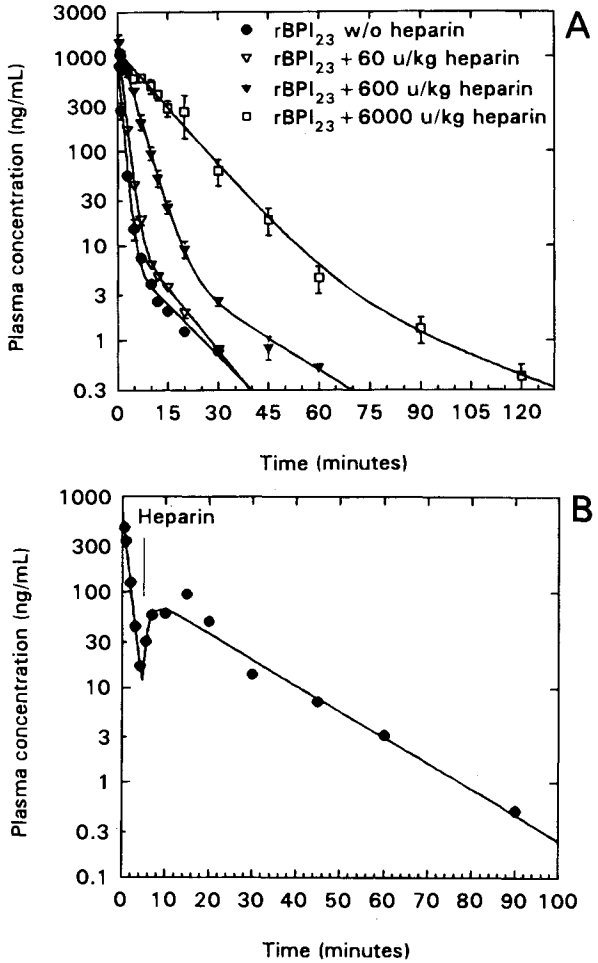

Fig. 3. Plasma clearance of 0.1 mg/kg $rBPI_{23}$ in rats with exogenously administered heparin. A: Rats received 0.1 mg/kg $rBPI_{23}$ without heparin (data shown as mean ± SE, n = 3), with 60 units/kg heparin, 600 units/kg heparin, or with 6000 units/kg heparin. B: Rats received 0.1 mg/kg $rBPI_{23}$, followed by 6000 units/kg heparin 5 minutes later.

To determine the contribution of the liver in clearing $rBPI_{23}$, rats received $rBPI_{23}$ via the pyloric vein catheter for 10 minutes. A low dose and low infusion rate were selected to ensure that $rBPI_{23}$ concentrations were sufficiently below the saturation level of any clearance mechanism and the pharmacokinetics would remain linear. The observed clearance of $rBPI_{23}$ was 295 ± 48 ml/min/kg (n = 5), which was about 6 times higher than the clearance of 0.1 mg/kg $rBPI_{23}$ administered into the femoral vein for ten minutes (66 ± 9 ml/min/kg, n = 2, Figure 2B). Thus the extraction ratio of the liver for $rBPI_{23}$ is about 0.78 ± 0.17. To obtain specific evidence that the kinetics were linear, 0.0165 mg/kg $rBPI_{23}$ was administered by the femoral vein, resulting in an observed clearance of 60 ± 3 ml/min/kg (n = 3), a value not different from the clearance obtained from 0.1 mg/kg injected into the femoral vein.

To determine the clearance of $rBPI_{23}$ from the lungs, 1 mg/kg $rBPI_{23}$ was administered over a 1 hour period into the femoral vein, and blood was sampled from the right atrium via a right jugular catheter and from the aortic arch using a right

carotid catheter. The extraction ratio was determined to be 0.11 ± 0.06 (n = 3).

Effect of Heparin on $rBPI_{23}$ Clearance

Because *in vitro* studies have shown that heparin binds to $rBPI_{23}$ (11), and the liver is known to contain a large amount of heparan sulfate (17), we investigated the effect of heparin on the clearance of $rBPI_{23}$ *in vivo*. Varying doses of heparin were co-administered with 0.1 mg/kg $rBPI_{23}$ to rats. As the dose of heparin was increased, the observed plasma clearance and Vss of 0.1 mg/kg $rBPI_{23}$ decreased. The decrease was 10 fold when $rBPI_{23}$ was co-administered with 6000 units/kg heparin (Figure 3A and Table 3).

The effect of delayed administration of heparin was studied by administering 6000 units/kg heparin 5 minutes after 0.1 mg/kg of $rBPI_{23}$ injection (Figure 3B). The plasma concentration of $rBPI_{23}$ increased immediately upon administration of heparin, and clearance slowed thereafter.

To determine if the heparin sensitive clearance mechanism resides in the liver, a mixture of 0.1 mg/kg $rBPI_{23}$-6000 units/kg was administered into the pyloric catheter for ten minutes. The observed clearance was 3.4 ± 0.4 ml/min/kg, similar to the clearance of 4.0 ± 0.7 ml/min/kg obtained by administering this mixture into the femoral vein (data not shown).

DISCUSSION

Our studies showed that the observed clearance of $rBPI_{23}$ decreased as the administered dose of $rBPI_{23}$ was increased. The complex concentration-time profile was described using a three compartmental model with parallel first order and Michaelis-Menten elimination from the central compartment. Repeated exposure of the animals to $rBPI_{23}$ did not result in a change in the clearance profile of $rBPI_{23}$, as observed in multiple dose studies. Thus, it is not likely that the change in $rBPI_{23}$ clearance with respect to dose is due to down-regulation or up-regulation of a clearance mechanism. Rather, it appears that the clearance mechanism is saturated at higher doses, as is known to occur with macrophage colony stimulating factor (18), tissue-type plasminogen activator (19), and heparin (20).

As the dose of $rBPI_{23}$ increases, the mechanism of clearance becomes saturated and a greater proportion of the administered $rBPI_{23}$ is cleared by non-saturable clearance mechanisms. Renal clearance has been shown to be an important component of non-saturable, or high-capacity, elimination for substances such as heparin (20) and macrophage colony stimulating factor (18). However, we observed little effect on the clearance of $rBPI_{23}$ in bilaterally nephrectomized rats at doses of up to 10 mg/kg $rBPI_{23}$, with only moderate change in the concentration-time profile of $rBPI_{23}$. Interestingly, the heparin binding protein platelet factor 4 (PF4) is also minimally affected by bilateral nephrectomy (21). Furthermore, the lungs appeared to remove $rBPI_{23}$ from the circulation with an extraction efficiency of only 11%. Because such a low average extraction ratio means that the arterial and venous $rBPI_{23}$ plasma concentrations differed by no more than 20%, the true extraction ratio for the lungs may be as low as 0 or as high as 0.2. Intra-hepatic administration of $rBPI_{23}$, on the other hand, resulted in 78% lower area under the curve, compared to intra-femoral administration. This result suggests that the liver is the major organ for the clearance of $rBPI_{23}$. Our results also showed that the rapid clearance of $rBPI_{23}$ is not due to enzymatic degradation by blood.

We estimated the contribution of the liver and lungs to the total clearance of $rBPI_{23}$ at low doses by combining our calculation of extraction ratios with organ flow rates. Assuming a cardiac index of 330 ml/min/kg for rats (22), the pulmonary plasma flow rate may be estimated as (1-hematocrit) * cardiac index = (1 − 0.45) * 330 = 182 ml/min/kg. Thus, the lungs may clear approximately 182 * 0.11/(1 − 0.11) = 22ml/min/kg $rBPI_{23}$ from the plasma (23). The liver receives about 25% of the total cardiac output (23), so the liver clearance of $rBPI_{23}$ is calculated to be 0.25 * 182 * 0.78 = 35 ml/min/kg. Together the lungs and liver account for 57 ml/min/kg of the 60–66 ml/min/kg total plasma clearance of $rBPI_{23}$ at low doses.

Our findings that the observed clearance of $rBPI_{23}$ was reduced by the co-administration of heparin in a dose dependent manner is consistent with the idea that the clearance of $rBPI_{23}$ may occur via binding to heparan sulfate sites, particularly in the liver. Not only is the liver an abundant source of heparan sulfate, but the heparan sulfate present in the liver is also structurally very similar to heparin (17). This effect of exogenous heparin on $rBPI_{23}$ clearance is similar to that observed with PF4 (24) and basic fibroblast growth factor (bFGF, (25)). The heparin-binding properties of these proteins are well documented (11,26,27).

The reduction in plasma clearance of PF4 and bFGF in the presence of heparin has been explained by these possible mechanisms: 1) heparin binds to the proteins and prevents their interaction with heparin sulfate sites (28); and/or 2) the heparin-protein complex is cleared by a mechanism that clears heparin (20). The present results suggest that these mechanisms may be involved in the clearance of $rBPI_{23}$. First, the immediate increase in plasma $rBPI_{23}$ observed upon injection of heparin 5 minutes after administration of $rBPI_{23}$ suggests that binding of exogenous heparin to $rBPI_{23}$ causes the displacement of $rBPI_{23}$, presumably from heparan sulfate on endothelial cells and the extracellular matrix in the liver. This hypothesis is also supported by the decrease in steady state volume of distribution of $rBPI_{23}$ that was observed with increasing dose of heparin. Presumably, heparan sulfate sites contributed to the distribution of $rBPI_{23}$, and exogenous heparin reduced this distribution by binding to $rBPI_{23}$. Second, the heparin-induced reduction of $rBPI_{23}$ clearance suggests that the heparin-$rBPI_{23}$ complex is

Table 3. Pharmacokinetic Parameter Values of Plasma Clearance of 0.1 mg/kg $rBPI_{23}$ Administered I.V. in Rats with Various Doses of Heparin (mean ± SE, n = 3 per group)

Heparin units/kg	Vss ml/kg	CL ml/min/kg	MRT min
0	165 ± 16	83.6 ± 7.4	2.0 ± 0.1
60	77 ± 15	40.7 ± 4.7	1.9 ± 0.1
600	66 ± 9	17.0 ± 1.1	3.9 ± 0.3
6000	89 ± 4	8.5 ± 1.0	10.7 ± 0.9

Note: The pharmacokinetic parameters listed were obtained from exponential curve fits of plasma $rBPI_{23}$ concentrations following I.V. bolus administration of a mixture of sodium heparin and $rBPI_{23}$ to male CD® rats. These parameters represent observed rather than actual CL, Vss, and MRT because the kinetics of $rBPI_{23}$ is non-linear with respect to dose.

not cleared by the $rBPI_{23}$ clearing mechanism. Instead, the heparin-$rBPI_{23}$ complex may be cleared by the slower heparin-metabolizing mechanisms, namely the reticuloendothelial system at low doses, and by the kidneys at higher doses (20).

The pharmacokinetics of other BPI forms have also been investigated. One variant of $rBPI_{23}$, designated $rBPI_{21}$, was produced by replacing the cysteine at amino acid position 132 with alanine (29), and revealed a similar pharmacokinetic profile to that of $rBPI_{23}$ when administered to rats at a dose of 1 mg/kg (30). The pharmacokinetics of recombinant human holo-BPI was also found to have a concentration-time profile very similar to that of $rBPI_{23}$ (30). All of these BPI proteins show similar heparin-binding properties (30).

In summary, our results show that $rBPI_{23}$ is rapidly removed from the plasma in rats by a mechanism that saturates at concentrations greater than 1000 ng/ml. The liver is the major organ clearing $rBPI_{23}$, most likely via heparan sulfate sites on the endothelial cells and in the extracellular matrix. Neither the lungs nor kidneys are as efficient as the liver in clearing $rBPI_{23}$.

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