Sites of Elimination and Pharmacokinetics of Recombinant [^{131}I]Lepirudin in Baboons

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Abstract □ Lepirudin has a short half-life, and only 50–60% of the intravenously administered dose is excreted by the kidneys. The fate of the remainder is unknown. We designed a study to determine the fate of this lepirudin. In each of six baboons, [^{131}I]lepirudin was given intravenously as a bolus or infused over 30 min, 24 h apart. The in vivo redistribution of [^{131}I]lepirudin was determined and quantified by scintillation camera imaging. In all studies, the half-life of [^{131}I]lepirudin, as determined from the disappearance of radioactivity, was 21 ± 3 min. The half-life determined from the disappearance of lepirudin, measured by the Ecarin Clotting Time (ECT) method, was similar at 23 ± 8 min. Results obtained with the labeled lepirudin are therefore comparable with those obtained using the plasma concentration of lepirudin. When lepirudin was administered as a bolus, the half-life was 18 ± 4 min, and lepirudin was cleared from the plasma at a rate of 42 ± 12 mL/min and by the kidneys at 23 ± 2 mL/min. Following infusion over 30 min, the half-life and total and renal clearances were not significantly different. In both studies, between 50 and 60% of the administered lepirudin was excreted by the kidney. Studies on sacrificed baboons showed that appreciable amounts of lepirudin were present in the bile, indicating the liver as a contributor to the elimination of lepirudin.

Introduction

Hirudin is regarded as the most potent direct inhibitor of thrombin and its recombinant form, lepirudin ([leu^1, Thr^2]-63-disulfatohirudin), has the same physiochemical characteristics and biochemical properties as the native protein.[1,2] Lepirudin is a 65 amino acid polypeptide (7 kDa) produced by transfected yeast cells.[1] The interaction between hirudin and thrombin results in the formation of a stable, noncovalent stoichiometric 1:1 complex that inhibits all functions of thrombin.[1,2] Apart from its inhibition of coagulation, it also inhibits platelet-dependent arterial-type thrombosis when given in high enough dosages.[3−5] Although the pharmacokinetics of lepirudin is well-known, there are still some unresolved issues. It is generally accepted that lepirudin is mainly excreted in an unchanged form by the kidneys.[1,6] However, approximately 40 to 50% of injected protein cannot be accounted for in the urine. The sites of metabolism and/or uptake of this lepirudin are unknown. We designed this study to investigate the metabolism and fate of lepirudin in baboons.

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Subjects and Methods

Experimental Animals—Eight male baboons (Papio ursinus) supporting permanent femoral arteriovenous shunts (A-V shunts) of silicone rubber tubing[7,8] were used. The animals were sedated with intramuscular ketamine hydrochloride (10 mg/kg body mass, Centaur Laboratories, South Africa) to enable handling. Anesthesia was maintained with subsequent administrations when necessary. One hundred milliliters of saline were given intravenously (iv) to each baboon 30 min before the start of a study to ensure that they were normovolaemic.

Experimental Protocol—Six baboons received [^{131}I]lepirudin as an iv bolus of 0.3 mg/kg as well as an infusion at a rate of 0.01 mg/kg/min for 30 min on separate days. The treatment requirements were such that an equal number of baboons received the bolus or infusion on each day. The lepirudin (HBW 023, Hoechst AG, Frankfurt and Behringwerke AG, Marburg, Germany) was labeled with 131-iodine (^{131}I; Radiochemical Centre, Amersham, UK) using the IODO-GEN method.[9] Labeling efficiency was 98 ± 1%. It is important to note that only labeled lepirudin was infused and that we determined the plasma concentration of the labeled lepirudin (see later). Therefore, when reference is made to lepirudin, it refers to the results obtained from the plasma concentration as determined using the ECT method.[10] Similarly, reference to labeled lepirudin refers to results obtained from radioactive count rates of [^{131}I]lepirudin.

Scintillation Camera Imaging—Imaging of the in vivo distribution of labeled lepirudin was done with a Large Field of View Scintillation Camera fitted with a high-energy collimator. Image analysis was done with an A^3-MDS data processing system that was interfaced with the camera. Imaging was done in two phases; they are, a dynamic image acquisition phase and a static acquisition phase.

Dynamic Image Acquisition—The baboons were positioned in front of the detector of the camera so as to include the kidneys, bladder, liver, and spleen in the field of view. Dynamic image acquisition, consisting of 3-min images (64 × 64 word mode) for 120 min (infusion study) and 90 min (bolus study), started simultaneously with the infusion of [^{131}I]lepirudin. For analysis, a region of interest was selected for the kidneys, the bladder, the spleen, and the liver, and the radioactivity in each region was expressed as a percentage of injected [^{131}I]labeled lepirudin, which was estimated by the geometrical mean method of quantification (see Static Image Acquisition). The radioactive count rates of the left and right kidneys were summed to obtain total kidney radioactivity.

Static Image Acquisition and Quantification of In Vivo Distribution of [^{131}I]Lepirudin—After the dynamic image acquisition, static anterior and posterior images of 3 min (64 × 64 word mode) were acquired of the head, thorax and abdomen, and legs. A background image was also acquired to correct whole body and region of interest (organ) radioactivity for background radioactivity. Before treatment on the second day, anterior and posterior images of the thorax and abdomen were acquired to determine and correct for residual ^{131}I radioactivity as a result of infusion of [^{131}I]lepirudin on the previous day.

To obtain anterior and posterior whole body radioactivity, the radioactivity in the head, thorax and abdomen, and legs was

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corrected for background radioactivity and/or residual radioactivity and summed. Regions of interest for the kidneys, bladder, spleen, and liver were selected to determine organ radioactivity. On the first day of the experiment, these radioactivities were corrected for background radioactivity. On the second day, organ radioactivity was corrected for background radioactivity and residual radioactivity, measured before the infusion of [^{131}I]lepirudin. The in vivo organ distribution of [^{131}I]lepirudin was quantified by the geometric mean method.[11]

Blood Collection and Analysis—Blood, 4.5 mL in 0.5 mL of a 3.2% sodiumcitrate solution, was collected before a study was started and then every 15 min thereafter. The radioactivity in 1 mL of plasma was determined in a gamma counter. Plasma levels of lepirudin were determined by the ECT method, which was specifically developed to measure hirudin levels in plasma and body fluids.[10] The activated partial thromboplastin time (aPTT) was measured with a fibrinometer (Clotex II, Hyland Division, Travenol Laboratories, Costa Mesa, CA), and reagents were supplied by the same company. The aPTT was measured to give an indication of the level of anticoagulation achieved and to show that the labeled lepirudin was functional.

To determine if the labeling procedure affected the function of [^{131}I]lepirudin, unlabeled and labeled lepirudin were added to plasma obtained from 4 baboons. Plasma concentrations were 2.5 and 5.0 μg/mL of lepirudin. In addition, a 50/50 mixture of labeled and unlabeled protein was also used. The aPTT was determined on all samples.

Urine Collection and Analysis—Urine was collected into a urine-collecting bag with a Teflon-coated latex Foley catheter (GRS Medicals, Kelvin, SA) for up to 24 h and analyzed for lepirudin by the ECT method.[10] The total amount of lepirudin was calculated from the total urine volume and the concentration in urine.

Urine was also analyzed by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). Samples analyzed were [^{131}I]lepirudin, urine without [^{131}I]lepirudin (native urine), [^{131}I]-lepirudin added to native urine, and urine collected from the treated baboons. The results strongly suggest that [^{131}I]lepirudin was excreted in an unchanged form. The results are not given.

Calculations—*Plasma Half-Lives of Lepirudin and [^{131}I]-Lepirudin*—The elimination half-lives of lepirudin and [^{131}I]-lepirudin were calculated by adjusting a one-phase exponential function to the appropriate phases of the log−linear plasma concentration−time and radioactive count rate−time profiles by the method of least-squares analysis. The value k, in the function $C_{max} \times e^{-kt}$, was thus found, where C_{max} is the maximum lepirudin concentration or maximum radioactive count rate in plasma. The half-lives were calculated as $t_{1/2} = 0.693/k$.

Total Clearance of Lepirudin and [^{131}I]Lepirudin—For the constant *infusion*, total clearance (CL_{total}) was calculated by dividing the rate of infusion (mg/min or radioactive count rate/min) by the maximum concentration of lepirudin or radioactive count rate in the plasma. To estimate the maximum plasma concentration or plasma radioactive count rate, an exponential association function was fitted to the data points obtained during the infusion period and extrapolated to maximum (i.e., an estimate of steady-state levels).[12]

For the *bolus* injection, CL_{total} was calculated by dividing the total dose, or the total counts injected, by the area under the curve ($AUC_{0-\infty}$) of the plasma lepirudin concentration (μg/mL)−time or plasma radioactivity (radioactive count rate/ml)−time profiles. The area under the plasma concentration−time and plasma radioactive counts rate−time curves up to 90 min (AUC_{0-90}) were calculated using the trapezoid rule. The curves were extrapolated to ∞ using the formula $AUC_{0-\infty} = AUC_{0-90} + C_p^*/k_e$, where C_p^* is the plasma lepirudin concentration or plasma radioactive count rate at the last sampling time (90 min). The terminal rate constant (k_e) was determined from the slope of the terminal portion of the log-linear C_p versus time curve.

Renal Clearance of [^{131}I]Lepirudin—For the constant *infusion*, renal clearance (CL_{renal}) was calculated by dividing the rate of appearance of [^{131}I]lepirudin in the bladder (radioactive count rate/min) by the maximum counts in the plasma. The latter was estimated by fitting an exponential association function to the available radioactive count rate/mL−time profiles and extrapolated to estimate the maximum value where a steady state was reached.

For the *bolus* injection, CL_{renal} was calculated by dividing the maximum radioactive count rate in the bladder by $AUC_{0-\infty}$ of the plasma radioactivity (radioactive count rate/mL)−time profiles.

Extravascular Clearance—The plasma clearance of lepirudin by the extravascular compartment was estimated by calculating the difference between the total clearance and the renal clearance.

Determination of Sites of Lepirudin Accumulation in Sacrificed Baboons—Two baboons were used. The one baboon was sacrificed after 30 min of infusion of [^{131}I]lepirudin. The other one received no lepirudin and was sacrificed to obtain organs and body fluids that served as controls for immunohistochemistry and lepirudin determinations, respectively. The baboons were deeply anesthetized. The test baboon was exsanguinated through the arterial side of the A-V shunt. About 2 L of saline at 37 °C were simultaneously infused under pressure (140 mmHg) into the venous side of the shunt to replace the blood that was lost.

Ex Vivo Quantification of the Distribution of [^{131}I]Lepirudin—The liver, spleen, kidneys, and bladder of the sacrificed baboons were immediately excised by careful dissection. Residual radioactivity in the body and that present in the liver, spleen, kidneys, and bladder were determined by scintillation camera imaging.[11] Images of 3 min (64 × 64 word mode) were acquired. Organ radioactivity was then expressed as a percentage of total body radioactivity, obtained by summation of residual and organ radioactivities.

Organ Biopsies—Biopsies of the kidneys (cortex and medulla), spleen, and liver were collected to establish the location of the lepirudin by using immunohistochemistry techniques. A primary antibody, rabbit anti-hirudin antibody (diluted 1:100 in PBS; Celsus Laboratories, Cincinnati, OH) was added to thin processed sections of the tissues and incubated for 60 min. A secondary goat anti-rabbit antibody (Signet kit, Johnson & Johnson, Johannesburg, Republic of South Africa) was added to bind to the primary antibody and a third antibody complex, a peroxidase−antiperoxidase immune complex, was used as the marker complex. These antibody complexes become visible when the tissues are exposed to the substrate diaminobenzidine. By adding hydrogen peroxide to the diaminobenzidine solution, a brown precipitate forms where lepirudin is present. Light microscopy was used to interpret the stains. Lightmicrographs of all tissues were enlarged 200 times and examined for the presence of lepirudin in cells.

Biopsies of the kidneys, liver, and spleen were also homogenized in saline and centrifuged at 8000 g for 24 h. The ECT method[10] was used to test for the presence of lepirudin in the supernatant of the homogenates.

Collection and Analysis of Duodenal and Gastric Juices and Bile—Duodenal and gastric juices and bile were collected from the sacrificed baboons to test for the presence of lepirudin by the ECT method.[10]

Statistics—Statistical comparison was done using Student's t test for paired data. Values of $p < 0.05$ were considered significant. The results are expressed as mean ± 1 standard deviation (SD).

Results

Changes in aPTT and Pharmacokinetics of Lepirudin—In the in vitro studies, concentrations of 2.5 and 5.0 μg/mL lepirudin lengthened the aPTT to 87 ± 4 and 174 ± 5 s. [^{131}I]Lepirudin, at the same concentrations, lengthened the aPTT to 92 ± 4 and 180 ± 6 s. When labeled and unlabeled lepirudin were mixed, the aPTT lengthened to 90 ± 3 and 178 ± 4 s. The difference in measurements was not significant. The labeling process therefore does not affect the function of lepirudin.

The in vivo changes in aPTT are summarized in Table 1. The labeled hirudin was biologically active because it caused a 2−3-fold lengthening in the aPTT. When lepirudin infusion was stopped and following the bolus injection, the aPTT rapidly shortened to reach near pre-infusion values after 90 min.

The changes in circulating lepirudin and labeled lepirudin are summarized in Figure 1. Following infusion or the bolus injection, the plasma levels of lepirudin and [^{131}I]-lepirudin decreased rapidly. In all studies, the plasma half-life of lepirudin was 24 ± 9 min. When calculated from the

Table 1—Changes in APTT[a]

time (min)	infusion	bolus
–30	40 ± 4	–
–10	–	–
0	131± 20	42 ± 6
30	79 ± 10	98 ± 16
60	64 ± 9	74 ± 13
90	56 ± 8	64 ± 12

[a] Results are given in seconds and are expressed as mean ± 1 SD.

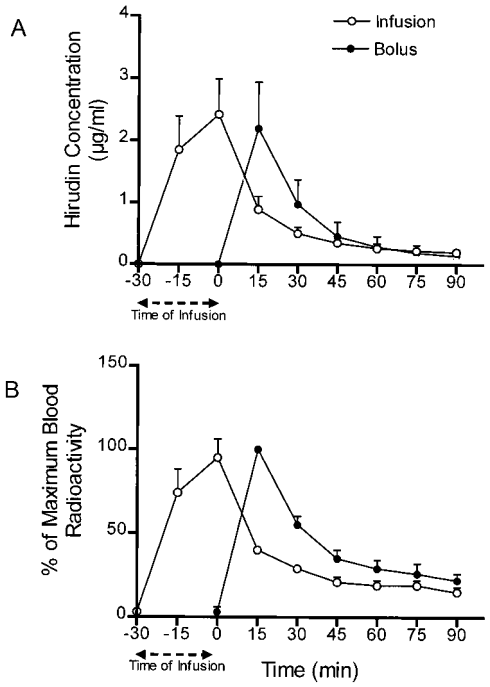

Figure 1—Dynamic changes of (A) plasma lepirudin and (B) [^{131}I]lepirudin following infusion (open circles) or bolus administration (black circles). Values are given as a mean ± 1 SD.

Table 2—Total, Renal, and Extravascular Clearance of Lepirudin and [^{131}I]Lepirudin

route	clearance	r-Lepirudin	[^{131}I]Lepirudin
infusion	total	49 ± 16	52 ± 10
	renal	–	32 ± 10
	extravascular	–	20 ± 8
bolus	total	42 ± 12	45 ± 12
	renal	–	23 ± 2[b]
	extravascular	–	22 ± 12

[a] Values are given in mL/min and are expressed as mean ± 1 SD. [b] $p < 0.05$, infusion versus bolus (Student's *t* test for paired data).

disappearance of radioactivity from the blood, it was 21 ± 6 min. The difference was not statistically significant ($p > 0.05$). After infusion of [^{131}I]lepirudin was stopped, lepirudin was eliminated from the plasma with a half-life of 23 ± 8 min ([^{131}I]lepirudin = 21 ± 3 min). When the same amount of [^{131}I]lepirudin was given as a bolus, the half-life was 18 ± 4 min ([^{131}I]lepirudin = 19 ± 8 min). The mean difference in the half-lives between infused and bolus injected lepirudin was 5 ± 10 min. The 95% confidence interval of between −5 and 16 min indicates that the difference was not statistically significant.

Total, renal, and extravascular clearance of lepirudin is summarized in Table 2. Total clearance was ≈45 mL/min, whether it was calculated from the data obtained from the concentration of lepirudin or from labeled lepirudin. Between 51 and 61% of this lepirudin was cleared by the kidneys.

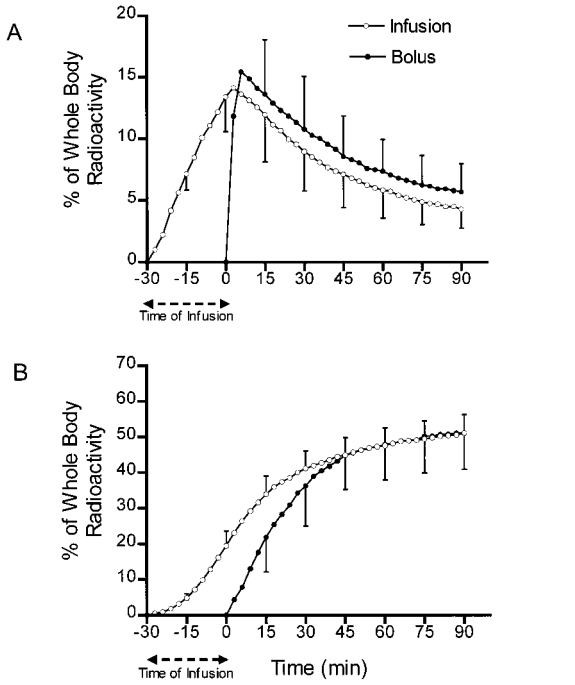

Figure 2—Dynamic changes of [^{131}I]lepirudin in the (A) kidneys and (B) bladder following infusion (open circles) and bolus administration (black circles). The radioactivity is expressed as a mean percentage ± 1 SD of whole body radioactivity at the end of each study.

Table 3—Organ Distribution of [^{131}I]Lepirudin at the End of Treatment

organ	infusion	bolus
kidneys	4.3 ± 1.5	5.7 ± 2.3
bladder	51.2 ± 5.2	51.2 ± 10.3
liver	1.7 ± 0.8	1.8 ± 0.9
spleen	3.6 ± 2.4	3.0 ± 2.7

[a] Percent of whole body radioactivity at the end of each study. Values are given as mean ± 1 SD.

Table 4—ECT of Bile and Duodenal and Gastric Juices, and of the Supernatant of Homogenized Kidney, Liver, and Spleen Tissue[a]

sample	ECT, s
bile	229
gastric juice	49
duodenal juice	39
kidney	77
liver	44
spleen	49

[a] Control values for the ECT method are between 38 and 40 s.

In Vivo Distribution of [^{131}I]Lepirudin—The changes in radioactivity in the kidneys and bladder are summarized in Figure 2, and the in vivo organ distribution at the end of the study in Table 3. Only ≈5% of total injected [^{131}I]-lepirudin was quantified in the liver and the spleen. The results obtained in the sacrificed baboon, 0.1% of the injected radioactivity in the spleen and 1.5% in the liver, also showed that the liver and spleen contained little [^{131}I]-lepirudin. Between 50 and 60% of the injected labeled lepirudin could be accounted for in the bladder. At the end of infusion or shortly after the bolus was injected, the kidneys contained ≈15% of the injected radioactivity (Figure 2A).

The presence of lepirudin in the bile and gastric and duodenal juices of the sacrificed baboon was measured by the ECT method, and the results are shown in Table 4. Of these, only the bile contained appreciable amounts as indicated by the markedly longer than normal ECT results.

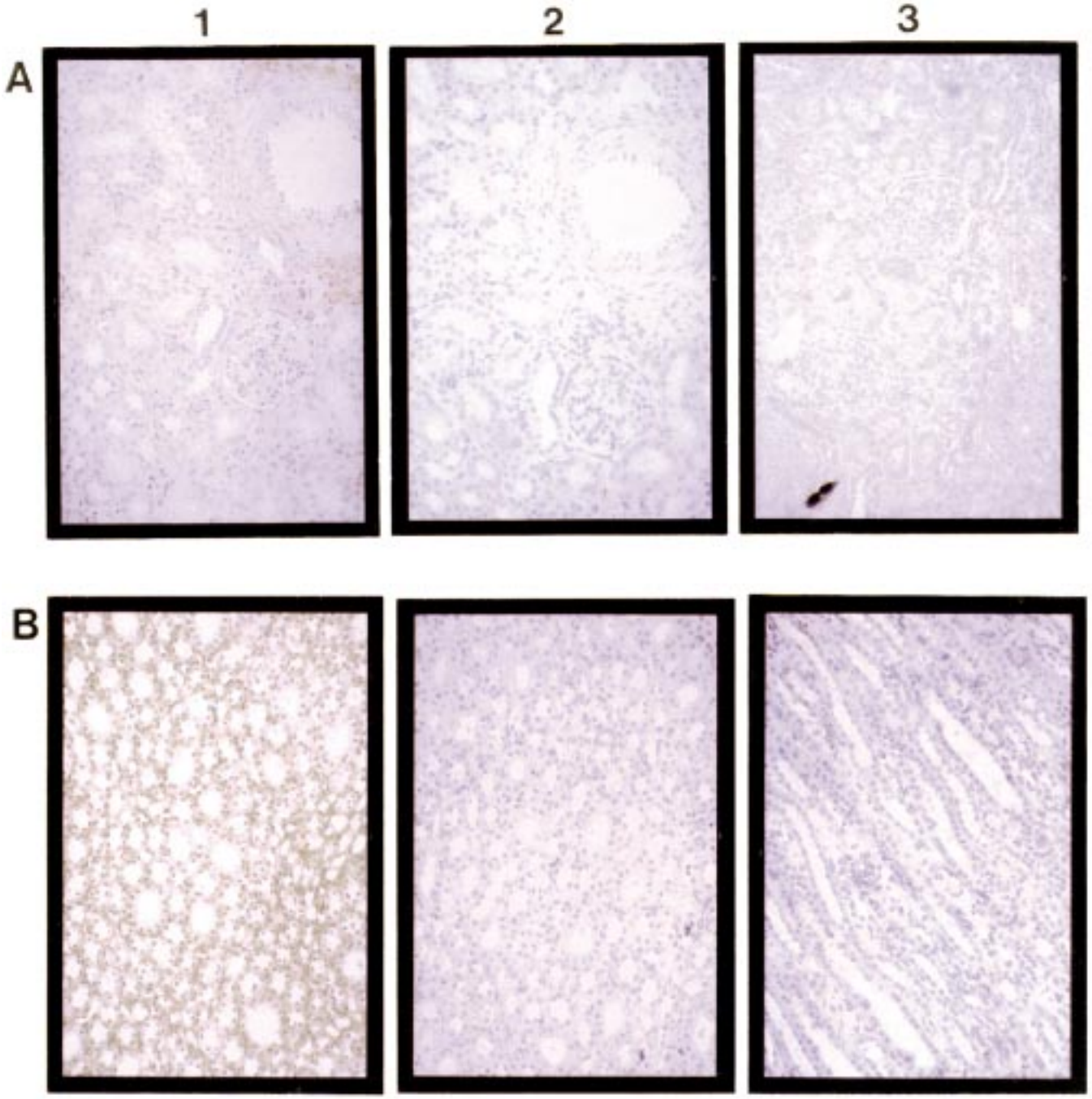

Figure 3—Light micrographs (enlarged 200X) of the (A) renal cortex and (B) medulla. A1 and B1 are from baboons that received lepirudin. A2 and B2 are negative controls where no anti-hirudin antibody was used. A3 and B3 show the renal cortex and medulla of the control baboon that did not receive lepirudin but was treated with anti-hirudin antibody.

The amount of lepirudin excreted by the kidneys was calculated from the total urine volume and the concentration of r-hirudin in urine. In two cases (1 × bolus study and 1 × infusion study), very little urine could be collected. The results are nevertheless given. After infusion of labeled lepirudin, 2.2 ± 1.9 mg of lepirudin was excreted, after the bolus 2.2 ± 1.5 mg was excreted. This amount was ≈54% of the total dose of lepirudin given.

Immunohistochemisty—The results are presented in Figures 3 and 4. The kidney, liver, and spleen of the baboon that was not treated with lepirudin stained negative when the tissues were incubated with the anti-hirudin antibody (Figure 3, A3 and B3, and Figure 4, A3 and B3). This result indicates that these tissues did not contain proteins that interact with the antibody. Kidney, spleen, and liver tissue from baboons treated with lepirudin also stained negative when the tissues were not incubated with the antibody (Figure 3, A2 and B2, and Figure 4, A2 and B2), indicating that the positive staining obtained in the test samples was not a result of the staining procedure.

In the renal cortex, the cytoplasm of the epithelial cells of the convoluted tubules, the cytoplasm of the endothelial cells of the blood vessels, and the connective tissue stained for lepirudin. The glomeruli stained negative (Figure 3, A1). The renal medulla stained intensely. It is likely that lepirudin was present in the epithelium of the thick and thin segments of the loop of Henly and the collecting tubes. The cytoplasm of the endothelial cells of the vas recta and the connective tissues between the cells stained strongly (Figure 3, B1).

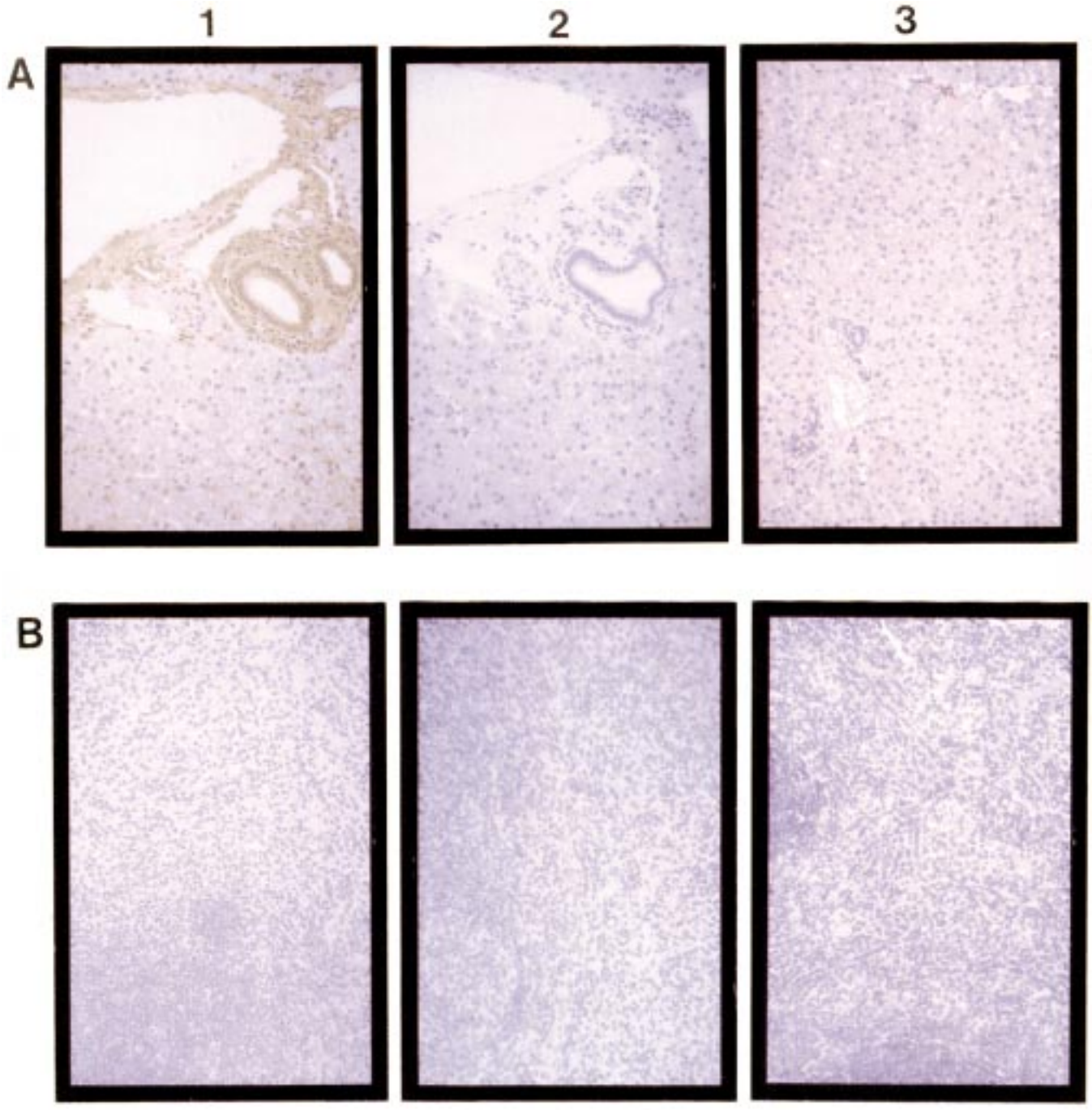

Figure 4—Light micrographs (enlarged 200X) of the (A) liver and (B) spleen. A1 and B1 are from baboons that received lepirudin. A2 and B2 are negative controls where no anti-hirudin antibody was used. A3 and B3 show the liver and spleen of the control baboon that did not receive lepirudin but was treated with anti-hirudin antibody.

The spleen did not stain for lepirudin (Figure 4, A1). On the other hand, staining of liver is ambiguous (Figure 4, B1). The cytoplasm of the hepatocytes did not stain strongly, but the cytoplasm of the endothelial cells of the hepatic artery and portal vein and that of the epithelial cells of the bile duct stained strongly.

Discussion

In a study of this nature, where the pharmacokinetics and mechanism of clearance of a compound labeled with a radioisotope is studied, it is vital that the labeled compound reacts in the same way as the native compound. There are several lines of evidence to show that the behavior of lepirudin, when labeled with ^{131}I, was not markedly affected by the labeling procedure. First, treatment with the labeled lepirudin lengthened the aPTT 3−4-fold (Table 1). In addition, when unlabeled and labeled hirudin, either alone or as a 50/50 mixture, were added to plasma in vitro, the lengthening in aPTT was equivalent. This result indicated that the labeled lepirudin was functional. Second, when the half-life was measured from the radioactive count rate in blood, it was 21 ± 6 min, which was not significantly different from that estimated for lepirudin (24 ± 9 min). These estimates were also not significantly different from that obtained in another study.[5] Third, after 90 min of treatment with labeled lepirudin, no accumulation of ^{131}I was imaged in the thyroid or spleen, both of which rapidly

remove free iodine in plasma.[13] It is therefore reasonable to conclude that labeling of lepirudin with ^{131}I did not adversely affect the function and plasma clearance of lepirudin and that the ^{131}I remained bound to the lepirudin. The results obtained with the labeled lepirudin can therefore be compared with those of lepirudin, and conclusions relating to the pharmacokinetics of lepirudin can be made from the results obtained with labeled lepirudin.

It is evident that lepirudin was mainly excreted by the kidneys (Figures 2A and B). Between 50 and 60% of the injected lepirudin was detected in the bladder 90 min after it was injected (Table 3), which agrees with results obtained in humans and rhesus monkeys.[6,14,15] Because the clearance of lepirudin approximates that of creatinine in humans, it was proposed that excretion be by glomerular filtration.[14,15] We used immunohistochemical techniques in an attempt to verify this proposal (Figure 3). The results were rather confusing and do not confirm glomerular filtration. Glomeruli in the renal cortex did not stain for lepirudin. Tissue surrounding the glomerulus and the blood vessel did stain. Even more surprising was the fact that the tubuli in the medulla, and especially the epithelial cells lining the tubules, stained intensely, which suggests tubular reabsorption and secretion. A well-planned study to investigate glomerular filtration and possible tubular reabsorption and secretion is called for. Such a study will provide definitive answers about the mechanisms by which the kidneys excrete lepirudin.

It is generally accepted that the fraction of lepirudin that is not excreted by the kidneys is distributed in the extravascular compartment.[14] The in vitro results in this study however showed that the liver plays a part in the catabolism of lepirudin and that lepirudin was excreted in the bile. Although we could not quantify appreciable quantities of [^{131}I]labeled lepirudin in the liver (Table 3), immunohistochemical studies showed that lepirudin was present in the hepatocytes and the tissues surrounding the blood vessels and bile ducts. The finding that lepirudin was present in the bile means that it has to be excreted into the digestive system. We explain the absence of lepirudin in the duodenal juices (Table 4) and the finding that lepirudin was not present in the feces of normal humans 24 h after it was administered[15] by digestion and absorption of lepirudin by the enzymes present in the duodenum. It is reasonable to assume that the 40% of administered lepirudin that was not excreted by the kidneys, could be excreted by the liver into the bile. However, a well-planned study is needed to quantify the contribution of the liver to the clearance of lepirudin from plasma. It is further evident that the spleen plays no part in the catabolism of lepirudin (Table 3, Figure 4).

The plasma elimination half-life of lepirudin was 18 ± 4 min when it was injected as a bolus. When the same quantity was infused over 30 min, the estimated half-life was not significantly different (23 ± 8 min). This result is similar to the estimated half-life that we determined in baboons where different dosages of lepirudin were infused over 30 min.[5] The half-lives estimated in the baboons are shorter than the 55−70 min measured in humans, rhesus monkeys, rats, rabbits, and dogs.[6,15−17] We have no easy explanation for this discrepancy.

The total clearance of lepirudin from plasma was ≈45 mL/min, whether the same amount was infused or given as a bolus. Similar results were obtained when clearance was calculated from the radioactive radioactive count rates (Table 2). This value is not very different from that determined in rhesus monkeys,[18] but differs greatly from the results reported for humans, rats, rabbits, and dogs.[6,15] Labeled lepirudin, infused over 30 min, was cleared by the kidneys at a significantly higher rate than that administered as a bolus (Table 2). However, when renal clearance was expressed as a percentage of plasma clearance, 62 ± 16% of infused lepirudin was cleared by the kidneys. The corresponding value for bolus administrated lepirudin, 55 ± 15%, was not significantly different ($p > 0.05$, Student's *t* test for paired data). Thus, although the rate of clearance was different, the relative contribution of the kidneys to plasma clearance was the same. It is important to note that in rhesus monkeys that received a bolus of lepirudin, renal clearance also contributed ≈50% to plasma clearance.[18] These results are similar to those obtained in this study. The method of administration of the labeled lepirudin did not affect the extravascular clearance (Table 2).

In summary, whether 0.3 mg/kg of labeled lepirudin was infused over 30 min or given as a bolus, it did not influence its plasma half-life and clearance. This result suggests that the study was performed with plasma concentrations below the maximum threshold levels of excretion of lepirudin by the kidneys. The mode of administration did not affect the clearance of lepirudin from plasma, nor that by the kidneys or the extravascular compartment. The kidneys were the main sites of excretion of lepirudin, where between 50 and 60% was excreted, probably by tubular secretion. It has been speculated that the remainder may be catabolized by the kidneys and that the methods used to determine lepirudin are unable to recognize the degradation products.[6,14,18,19] The quantification of the percentage labeled lepirudin found in the bladder argues against renal catabolisation. If the labeled lepirudin was catabolized, much more than 50 to 60% of administered labeled lepirudin would have been detected in the bladder at the end of the study. In addition, SDS-PAGE suggested excretion in an unchanged form. A surprising finding was that the bile contained appreciable amounts of lepirudin.

References and Notes

1. Adkins, J. C.; Wilde, M. I. Lepirudin: A review of its potential place in the management of thrombotic disorders. *Biodrugs* **1998**, *10*, 227−255.
2. Harvey, R. P.; Degryse, E.; Stefani, L.; Schamber, F.; Cazenave, J. P.; Courtney, M.; Tolsthev, P.; Lecocq, J. P. Cloning and expression of c DNA coding for the anticoagulant hirudin from the blood sucking leech, *Hirudo medicinalis*. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1084−1088.
3. Kelly, A. B.; Marzec, U. M.; Krupski, W.; Bass, A.; Cadroy, Y.; Hanson, S. R.; Harker, L. A. Hirudin interruption of heparin-resistant arterial thrombus formation in baboons. *Blood* **1991**, *77*, 1006−1012.
4. Kotzé, H. F.; Lamprecht, S.; Badenhorst, P. N. A 4-h infusion of recombinant hirudin results in long-term inhibition of arterial-type thrombosis in baboons. *Blood* **1995**, *85*, 3158−3163.
5. Kotzé, H. F.; Lamprecht, S.; Van Wyk, V.; Roodt, J. P.; Badenhorst, P. N. Differences in platelet-dependent thrombosis and thrombin production does not affect the pharmacokinetics of r-hirudin (HBW 023) in baboons. *Thromb. Haemost.* **1995**, *73*, 1308 (abstract).
6. Nowak, G. Pharmacokinetics of hirudin. *Semin. Thromb. Hemost.* **1991**, *17*, 145−149.
7. Harker, L. A.; Hanson, S. R. Experimental arterial thromboembolism in baboons: Mechanism, quantification, and pharmacologic prevention. *J. Clin. Invest.* **1979**, *64*, 559−569.
8. Hanson, S. R.; Kotzé, H. F.; Savage, B.; Harker, L. A. Platelet interactions with Dacron vascular grafts. *Arteriosclerosis* **1985**, *5*, 595−603.
9. Salacinski, P. R. P.; Mclean, C.; Sykes, J. E. C.; Clement-Jones, V. V.; Lowry, P. J. Iodination of proteins, glycoproteins and peptides using a solid-phase oxidising agent, 1,3,4,5-tetrachloro-3α, 6α-diphenyl glycoluril (IODO-GEN). *Anal. Biochem* **1981**, *117*, 136−146.

10. Nowak, G.; Bucha, E. Quantitative determination of hirudin in blood and body fluids. *Semin. Thromb. Hemost.* **1996**, *22*, 197−202.
11. Kotzé, H. F.; Lötter, M. G.; Badenhorst, P. N.; Heyns, A. du P. Kinetics of ^{111}In-platelets in the baboons. II. In vivo distribution and sites of sequestration. *Thromb. Haemost.* **1985**, *53*, 408−410.
12. Lyer, L.; Koza, M.; Iqbal, O.; Calabria, R.; Fareed, J. Studies on the pharmacokinetics and pharmacodynamics of recombinant hirudin (rHV-Lys47) after intravenous and subcutaneous administration in dogs. *Thromb. Res.* **1993**, *69*, 259−269.
13. Saha, G. B. Uses of radiochemicals in nuclear medicine. In *Fundamentals of Nuclear Pharmacy*; Saha, G. B., Ed.; Springer-Verlag: New York, 1992; pp 227−241.
14. Markwardt, F.; Nowak, G.; Stürzebecher, J.; Griessbach, U.; Walsmann, P.; Vogel, G. Parmacokinetics and anticoagulant effect of hirudin in man. *Thromb. Haemost.* **1984**, *52*, 160−163.
15. Meyer, B. H.; Luus, H. G.; Müller, F. O.; Badenhorst, P. N.; Röthig, H.−J. The pharmacology of recombinant hirudin, a new anticoagulant. *S. Afr. Med. J.* **1990**, *78*, 268−270.
16. Markwardt, F.; Fink, G.; Kaiser, B.; Klöking, H, P.; Nowak, G.; Richter, M.; Stürzebecher, J. Pharmacological survey of recombinant hirudin. *Pharmazie* **1988**, *43*, 202−207.
17. Nowak, G.; Markwardt, F.; Fink, E. Pharmacokinetic studies with recombinant hirudin in dogs. *Folia Haematol.* **1988**, *115*, 70−74.
18. Grötsch, H.; Hropot, M.; Berscheid, G.; Crause, P.; Malerczyk, G.; Apidopoulos, G.; Haun, G.; Husak, B. Pharmacokinetic investigations of the α-human thrombin-hirudin complex in rhesus monkeys. *Thromb. Res.* **1992**, *66*, 271−275.
19. Grötsch, H.; Hropot, M. Degradation of rDNA hirudin and α-human thrombin-lepirudin complex in liver and kidney homogenates from rat. *Thromb. Res.* **1991**, *64*, 763−767.

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