Kinetics of Heparin Removal from Circulation of the Minipig

P. A. HARRIS[x] and K. L. HARRIS

Abstract □ Techniques were developed for kinetic studies in unanesthetized, unrestrained minipigs weighing less than 45.4 kg (100 lb). By using ^{35}S-heparin and ^{3}H-heparin, the disappearance from the plasma of different amounts of heparin injected intravenously into the minipig was determined. Serial plasma samples were assayed for radioactivity, and the resulting plasma radioactivity-time curves were analyzed for calculation of half-lives of disappearance ($t_{1/2}$) and volume of distribution (V_d). The plasma radioactivity followed a monoexponential decay. Over the 1–50-mg heparin range, an increase in $t_{1/2}$ from 3.4 to 39 min was observed. Essentially no differences in the V_d were observed among the various doses. This volume was approximately 1.5 times the estimated plasma volume. The observed kinetics are consistent with previous suggestions that the reticuloendothelial system may be involved in the disposition of heparin.

Keyphrases □ Heparin—kinetics of removal from circulation of minipig □ Minipig—kinetics of heparin removal from circulation, suitability as test animal □ Anticoagulants—kinetics of heparin removal from circulation of minipig □ Pharmacokinetics—heparin removal from circulation of minipig

Heparin, a strongly acidic mucopolysaccharide, is well known and is exploited for its pharmacological properties as an anticoagulant and a lipemia-clearing factor. Although these effects of administered heparin are well characterized, the physiological role of endogenous heparin remains unclear. Little also is known regarding the relationship between heparin blood concentrations and its pharmacological effect. The kinetics of heparin removal from the blood were reported in a retrospective study and literature analysis (1). It appears clear that there are species differences in both quantitative and qualitative parameters of heparin removal. In man, the $t_{1/2}$ of removal varies with the dose. With a dose of 100 units/kg iv, the $t_{1/2}$ is approximately 1 hr; with 400 units/kg iv, it is approximately 2.5 hr. The plasma concentration-time curves decline exponentially in any case; therefore, a saturation mechanism does not seem likely.

Another report (2) showed that, although the $t_{1/2}$ of heparin removal is inversely related to dose, the $t_{1/2}$ of heparin's anticoagulant effect is not affected by dose. The former was measured by bioassay with a whole blood partial thromboplastin time assay, while the latter was estimated by heparin's effect on the whole blood partial thromboplastin of patients. *Recently*, a study (3) showed that heparin is removed biexponentially in man. The method utilized for heparin analysis was a metachromatic assay combined with aminoacridine precipitation of heparin from the plasma. This study did not examine the dose dependency of heparin kinetics. Nearly all dose dependency studies to date have used coagulation assays such as blood clotting time or partial thromboplastin time for heparin, which only indirectly estimates heparin concentration (1).

The present study was undertaken as part of an effort to define the suitability of the minipig as an experimental animal in investigations of the physiological role of heparin.

EXPERIMENTAL

Animal Procedures—Techniques were developed for kinetic studies in unanesthetized, unrestrained minipigs[1] weighing less than 45.4 kg (100 lb). The animal was placed on its back in a V-shaped trough and held in position while a 13-gauge needle was introduced into the jugular vein by percutaneous puncture. A Teflon catheter was passed through the needle for a distance of 20 cm toward the heart. The needle was removed and a stopcock was attached to the catheter. The catheter extending from the skin was placed dorsally around the neck to the back and protected by securing it to the neck with adhesive tape. After the catheter and stopcock were secured, the animal was returned to its feet and released. It was then possible to approach the animal at any time to administer the dose or collect blood samples without unduly disturbing it. The dose of heparin was injected into the catheter, and the catheter was flushed well with 0.15 *M* saline. Twelve to 15 blood samples (3 ml) were collected serially up

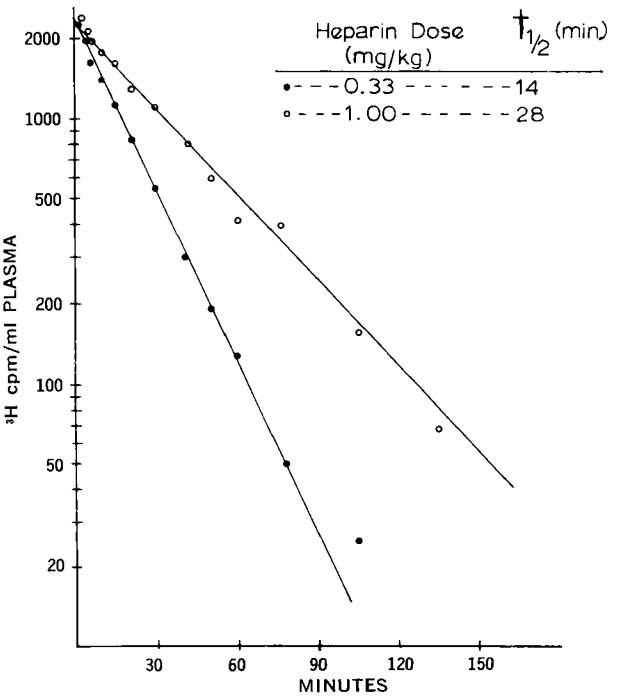

Figure 1—*Radioactivity in the plasma with time resulting from the intravenous injection of 2.76 × 10⁶ cpm of ^{3}H-heparin at two dose levels of heparin.*

[1] The minipigs used were of the genetic line produced by the Hormel Institute of the University of Minnesota.

to 180 min. At the end of the experiments, the catheter was removed. No evidence of infection developed at the site of catheterization. In subsequent experiments, catheters were positioned in a similar manner. Five animals were studied, three of them with both ^{3}H-heparin and ^{35}S-heparin.

Dose Preparation—^{35}S-Heparin[2] was purified by a procedure involving precipitation with 9-aminoacridine. A literature method (4) was modified slightly to redissolve the 9-aminoacridine–heparin complex with 4 *M* NaCl instead of a strongly alkaline solution. The high salt concentration was removed by dialysis. Recovery was approximately 90%. ^{3}H-Heparin[3] was purified in a similar manner with 75% yield. The authenticity of the purified labeled heparins was verified by agarose gel electrophoresis[4] against a reference standard of nonradioactive heparin[2]. Solutions of heparin containing 1 μg/μl in barbital buffer (0.1 *M*, pH 8.6) were prepared and subjected to electrophoresis according to the method of Jaques *et al.* (5). The electrophoretograms were stained with toluidine blue and scanned for radioactivity with a silicon avalanche detector[5]. Doses were prepared in 0.15 *M* saline. Appropriate amounts of unlabeled heparin were added to the ^{3}H-heparin and ^{35}S-heparin for total dosage adjustment.

Sample Analysis—Blood was collected into tubes containing edetate (EDTA) as an anticoagulant. The plasma was separated by centrifugation. One milliliter plasma was added to 12 ml scintillation fluid[6] for analysis by liquid scintillation spectrometry[7]. Counts were corrected for quenching.

RESULTS AND DISCUSSION

The purified ^{3}H-heparin and ^{35}S-heparin on electrophoresis yielded a single band each when stained with toluidine blue. The radioactive heparin mobility and the hue of their stains corresponded to unlabeled heparin. All radioactivity was associated with the band corresponding with the stains.

Curves of plasma radioactivity *versus* time are shown in Fig. 1. The plasma radioactivity followed a monoexponential decay. Data utilizing the ^{3}H-heparin are shown, but similar curves were obtained with ^{35}S-heparin.

The resulting plasma radioactivity–time curves were analyzed for calculating $t_{1/2}$ and V_d (Table I). It is apparent that $t_{1/2}$ increases with increasing dose, but at higher doses there appears to be much less change in $t_{1/2}$ as indicated by the similarity in the $t_{1/2}$ of the 0.95–2.80-mg/kg doses. There was no significant change in the V_d after different doses. The V_d was approximately 1.5 times the plasma volume. Results obtained with ^{3}H-heparin are similar to those obtained with ^{35}S-heparin.

The results of the electrophoresis of the labeled heparins suggest a high degree of purity and authenticity of the heparin used in these experiments. The magnitude of the V_d suggests also that the measured radioactivity was associated with intact heparin because the volumes are similar to those reported by others using unlabeled heparin (2, 3, 6).

The lack of differences in the kinetics of removal of ^{3}H-heparin and ^{35}S-heparin suggests that heparin is removed from the circulation intact rather than being broken down by sulfatases or mucopolysaccharidases in plasma. The kinetics in swine are similar to those reported in man in that the $t_{1/2}$ increases with increasing doses while the V_d remains unchanged. The removal rate in swine appears to be more rapid than those reported in man (2). However, the dose range used in this study was much smaller than those studied in man. The upper range in this study corresponds with the lower range studied in man. The $t_{1/2}$ of these higher doses in swine approach those encountered in man.

Table I—Disposition Kinetics of ^{3}H-Heparin and ^{35}S-Heparin in the Minipig

Animal	Isotope	Dose, mg/kg	$t_{1/2}$, min	V_d, ml/kg
1	^{35}S	0.06	3.4	65
	^{35}S	2.80	38	65
2	^{35}S	0.30	9.2	68
	^{35}S	1.47	32	71
3	^{35}S	0.20	8.5	69
	^{35}S	0.95	28	77
4	^{35}S	0.32	13	61
	^{35}S	1.27	25	59
5	^{3}H	0.33	14	53
	^{3}H	1.00	28	60
1	^{3}H	0.25	11.6	65
	^{3}H	1.25	31	67
2	^{3}H	0.25	12.6	72
	^{3}H	1.25	22	66
3	^{3}H	0.05	4.3	58
	^{3}H	2.50	39	65

Estes *et al.* (1) believed that their compiled data were consistent with the hypothesis that heparin may leave the plasma by uptake into the reticuloendothelial system. Piper (7) and Monkhouse (8) also suggested involvement of the reticuloendothelial system in the elimination of heparin. Another study (9) implicated mast cells in the uptake of heparin. It has been speculated that the combination of the large "stiff" heparin molecule with plasma protein molecules may be capable of uptake as a foreign particle (1). If this is true, then models for the uptake of particles (10) may be expected to apply to heparin. Our kinetic data are consistent with previous studies in that they indicate that heparin may be removed from the plasma by the reticuloendothelial system in the minipig.

REFERENCES

(1) J. W. Estes, E. W. Pelikan, and E. Krüger-Thiemer, *Clin. Pharmacol. Ther.*, **10**, 329(1969).

(2) J. W. Estes, *Ann. N.Y. Acad. Sci.*, **179**, 187(1971).

(3) N. Zöllner, C. Burger, and R. Braun, *A. Physiol. Chem.*, **329**, 76(1962).

(4) N. Zöllner and W. Kaiser, *Res. Exp. Med.*, **158**, 89(1972).

(5) L. B. Jaques, R. E. Ballieux, C. P. Dietrich, and L. W. Kavanagh, *Can. J. Physiol. Pharmacol.*, **46**, 351(1968).

(6) P. Olsson, H. Lagergren, and S. Ek, *Acta Med. Scand.*, **173**, 619(1963).

(7) J. Piper, *Acta Pharmacol.*, **3**, 373(1947).

(8) F. C. Monkhouse, *Amer. J. Physiol.*, **178**, 223(1954).

(9) T. A. Loomis, *Proc. Soc. Exp. Biol. Med.*, **106**, 490(1961).

(10) B. Benacerraf and P. Miescher, *Ann. N.Y. Acad. Sci.*, **88**, 184(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 25, 1973, from the *Sections of Clinical Pharmacology and Pharmacokinetics, Schools of Pharmacy and Medicine, University of Southern California, Los Angeles, CA 90033*

Accepted for publication September 5, 1973.

Supported by U.S. Public Health Service Grant HE-13620.

The provision of the ^{3}H-heparin by Dr. G. H. Barlow of Abbott Laboratories is greatly appreciated.

[x] To whom inquiries should be directed.

[2] Cal-Biochem, Los Angeles, Calif.
[3] Abbott, Chicago, Ill.
[4] Turner, Palo Alto, Calif.
[5] Nucleye, General Electric Co., King of Prussia, Pa.
[6] Aquasol, New England Nuclear, Boston, Mass.
[7] Mark II spectrometer, Nuclear-Chicago, Chicago, Ill.