The pharmacology of recombinant hirudin, a new anticoagulant

B. H. MEYER, H. G. LUUS, F. O. MÜLLER, P. N. BADENHORST, H.-J. RÖTHIG

Summary

A new anticoagulant, recombinant hirudin, was given to healthy volunteers (5 per test dose) in single intravenous doses of 0,01, 0,02, 0,04, 0,07 and 0,1 mg/kg to study its anticoagulant effects, how it was tolerated and its pharmacokinetics. Hirudin proved to be a potent anticoagulant with important effects on thrombin (increase in thrombin time and partial thromboplastin time). The maximum pharmacodynamic effect was achieved with the 0,07 mg/kg dose, and upwards. All doses of the compound were tolerated without side-effects. The mean elimination half-life is about 1 hour. Mean total clearance and volume of distribution are approximately 190 ml/min and 14 l, respectively. Hirudin obeys first-order pharmacokinetics.

S Afr Med J 1990; **78:** 268-270.

In 1884, J. B. Haycraft, working in Strasbourg, recognised that medicinal leeches (*Hirudo medicinalis*) contained a substance with anticoagulant properties. The pure anticoagulant substance from medicinal leeches was isolated in the late 1950s. Called hirudin, it was found to be a selective thrombin inhibitor with polypeptide structure. It is produced by the peripharyngeal glands of medicinal leeches. Hirudin is a polypeptide containing 65 amino acids with a molecular weight of about 8000.[1] Early studies were done with natural hirudin;[2] however, medicinal leeches are an endangered species, hence natural hirudin is not available in adequate quantities for therapeutic use.[1]

Progress in genetic engineering resulted in the availability of larger amounts of this anticoagulant. Recombinant hirudin has pharmacological properties similar to those of natural hirudin and is also a highly potent antithrombotic agent, which has been well tolerated *in vivo* in animal studies.[3]

A study was undertaken to investigate the tolerance, pharmacodynamics and pharmacokinetics of increasing doses of hirudin in healthy men.

Subjects and methods

Twenty-five consenting healthy male volunteers were recruited to participate in this study, which was performed at the Hoechst Research Clinic, Department of Pharmacology, University of the Orange Free State, with approval from the Medicines Control Council of South Africa and the University of the Orange Free State Ethics Committee.

Departments of Pharmacology and Haematology, University of the Orange Free State, Bloemfontein, OFS
B. H. MEYER, M.MED. (ANAESTH.) F.F.A. (S.A.), PH.D.
H. G. LUUS, PH.D.
F. O. MÜLLER, M.B. CH.B.
P. N. BADENHORST, M.MED. (ANAT. PATH.), M.D.
Hoechst Aktiengesellschaft, Klinische Forschung, Frankfurt, West Germany
H.-J. RÖTHIG, M.D.

Before being entered into the study, volunteers were screened by means of a physical examination and clinical chemistry measurements, haematological tests and urinalysis.

Five of the 25 volunteers received 0,01 mg/kg of the recombinant hirudin intravenously. Tolerance, effects on coagulation and pharmacokinetic values were measured. Doses of 0,02 mg/kg, 0,04 mg/kg, 0,07 mg/kg and 0,1 mg/kg were then administered sequentially to 4 sets of 5 different volunteers after it had been ascertained that the previous lower dose was well tolerated.

Volunteers had to have fasted before reporting at 07h00 to the clinic where they spent 12 hours. Medication was administered intravenously over 2 minutes into an arm and blood for the various tests was taken from the opposite arm.

The following variables were investigated:

Hirudin plasma and urine concentrations

Blood specimens were taken before medication and 10, 20, 30, 60, 90, 120, 180, 240, 300, 360 minutes and 24 hours after medication.

Fractionated urine specimens were collected 0-2, 2-4, 4-6, 6-8, 8-12, 12-18, 18-24 and 24-48 hours after medication.

Hirudin concentrations in serum and urine were measured by means of bioassay according to Griessbach *et al.*[4]

Coagulation

Thrombin time (TT) was measured by standard methods and partial thromboplastin time (PTT) by using Platelin-plus Activator (General Diagnostics, Morris Plains, New Jersey, USA).

Pharmacological safety

Measurement of plasma glucose, creatinine, sodium, potassium, uric acid, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, γ-glutamyl transferase and total protein values was performed before and 24 hours after medication by routine methods.

Haematological status (haemoglobin, haematocrit, red cell count, mean corpuscular haemoglobin, white blood cells and differential count, platelet count, and erythrocyte sedimentation rate) was measured at the same time as clinical chemistry tests were conducted.

Physical examination was performed before and 24 hours after medication. Recumbent systolic and diastolic blood pressure and heart rate were measured before and 15, 30, 60, 90, 120, 180, 240, 300, 360 minutes and 24 hours after medication. ECGs were performed immediately before and 1, 6 and 24 hours after medication.

Maximum hirudin plasma concentration (C_{max}) and time to maximum concentrations (T_{max}) were read directly from the hirudin plasma concentration-time curves.

Areas under the plasma concentration-time data pairs ($AUC_{0-1,5\,h}$ and $AUC_{0-5\,h}$) were calculated according to the linear trapezoidal rule.

Initial and second elimination half-lives ($t_{½;a}$ and $t_{½;b}$) were calculated by the adjustment of a double exponential function to the appropriate phases of the log-linear plasma concentration-time profile. The method of least squares was used for the adjustment. The value of z in the function Ce^{-zt} was thus found and $t_{½}$ calculated from:

$$t_{½} = 0{,}693/z.$$

Total clearance (Cl-tot) was calculated from the equation:

$$\text{Cl-tot} = \text{dose}/\text{AUC}_{0\text{-}5\,h}.$$

Total mean time (MT-vss) was calculated from:

$$\text{MT-vss} = \text{PAUC}_{1\ 0\text{-}5\,h}/\text{PAUC}_{0\ 0\text{-}5\,h}$$

where $PAUC_1$ and $PAUC_0$ are the first and zero order prospective AUCs for the period 0 - 5 hours.[5]

Total volume of distribution (V-ss) was calculated from:

$$\text{V-ss} = \text{Cl-tot}.\text{MT-vss}.$$

Results

The mean serum concentrations of hirudin for each of the 5 doses are shown in Fig. 1.

The mean ± SD values and ranges of the pharmacokinetic parameters of hirudin for the various doses are listed in Table I.

The mean ± SD total urinary excretion (from 0 - 48 hours after medication) of hirudin for the various doses is given in Table II.

Fig. 2 reflects the relationship between dose and $AUC_{0\text{-}1,5\,h}$.

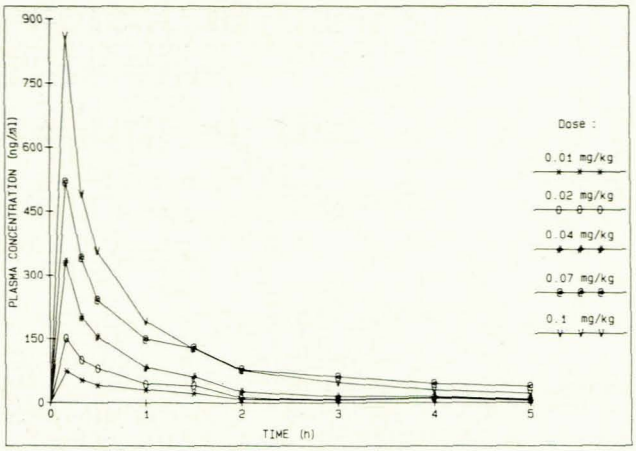

Fig. 1. Hirudin plasma concentrations for doses of 0,01 - 0,1 mg/kg.

The effects of hirudin on PTT and TT are shown in Table III. Fig. 3 reflects the relationship between hirudin plasma concentrations and TT and PTT.

Safety measurements (physical examination, blood pressure, clinical chemistry tests and haematological values) were not affected by the administration of hirudin. No trends or patterns of change associated with medication with hirudin could be discerned with regard to these variables.

TABLE I. SUMMARY OF MEAN VALUES ± SD AND RANGES OF THE PHARMACOKINETIC MEASUREMENTS OF HIRUDIN FOR THE VARIOUS DOSES

	0,01 mg/kg	0,02 mg/kg	0,04 mg/kg	0,07 mg/kg	0,1 mg/kg
C_{max} (ng/ml)	76,4±11,2	151±14,6*	331±42,5*	518±36,5*	859±106*
Range	63,0–89,7	135–168	281–395	461–559	752–996
$AUC_{0\text{-}1,5\,h}$ (ng.h/ml)	56,3±8,97	101±15,2	197±28,6	331±41,4	471±69,3
Range	44,7–65,4	86,0–120	175–243	293–392	395–550
$AUC_{0\text{-}1,5\,h}$ (ng.h/ml)	†	†	268±55,4	545±205	652±118
Range			214–347	389–890	551–834
$t_{½;a}$	0,08±0,05	0,13±0,08	0,09±0,04	0,14±0,04	0,10±0,04
Range	0,20–0,12	0,04–0,24	0,05–0,15	0,09–0,18	0,04–0,14
$t_{½;b}$	1,18±0,22	1,63±0,84	0,76±0,18	1,70±1,45	0,82±0,19
Range	0,99–1,43	0,71–2,74	0,53–1,02	0,83–4,28	0,62–1,09
Cl-tot (ml/min)	†	†	197±37,0	174±37,6	205±441
Range			150–246	115–216	158–256
Cl-renal (ml/min)	–	—	86,8±12,6	65,1±19,3	94,2±17,8
Range			73,9–103	33,5–81,4	72,5–115
MT-vss (h)	†	†	1,15±0,16	1,41±0,42	1,17±0,13
Range			0,91–1,33	0,99–2,02	1,06–1,38
V-ss (l)	†	†	13,3±1,21	14,1±2,01	14,3±3,08
Range			11,3–14,3	11,0–16,5	10,5–18,0

• First observed concentration.
† Plasma concentrations too low for calculation.

TABLE II. SUMMARY OF MEAN VALUES ±SD AND RANGES OF TOTAL URINARY EXCRETION OF HIRUDIN FOR THE VARIOUS DOSES

	0,01 mg/kg	0,02 mg/kg	0,04 mg/kg	0,07 mg/kg	0,1 mg/kg
Hirudin (μg)	484±122	560±120	1 455±250	2 252±901	3 855±715
Range	311–640	438–758	1 126–1 694	1 383–3 725	2 654–4 567
Hirudin (% of dose)	65,5±21,3	39,1±10,0	47,4±7,92	41,7±13,0	49,4±8,10
Range	42,5–92,7	28,1–54,5	38,0–54,9	24,7–60,5	38,5–57,8

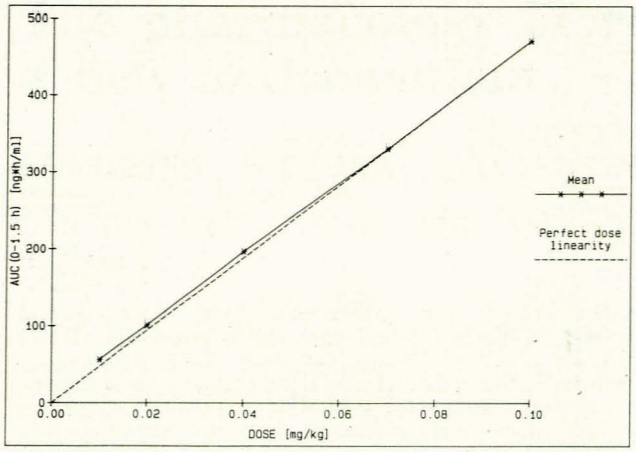

Fig. 2. Relationship between AUC $_{0-1,5}$ and dose of hirudin.

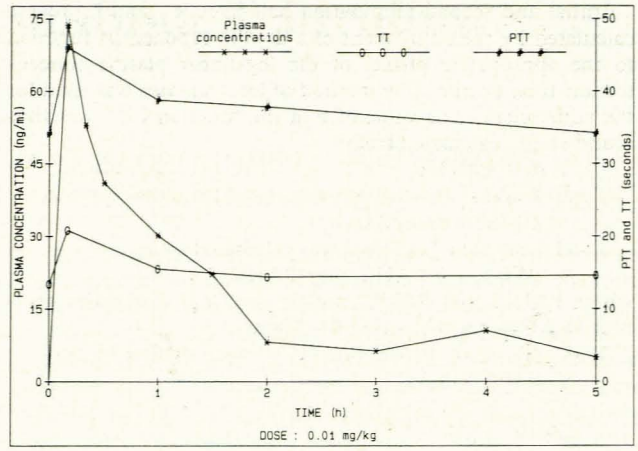

Fig. 3. Hirudin plasma concentrations v. PT and PTT.

TABLE III. MEAN ± SD VALUES OF PTT AND TT(s) FOR EACH OF THE 5 DOSES

Sampling time (h)	0,01 mg/kg	0,02 mg/kg	0,04 mg/kg	0,07 mg/kg	0,1 mg/kg
PTT					
0	34,2±3,27	31,5±1,73	32,4 ±0,89	36,0 ±4,95	32,8 ±2,17
0,17	46,0±4,80	47,8±4,44	60,4 ±4,22	82,2 ±15,3	76,2 ±9,96
1	38,8±2,75	39,2±2,28	45,2 ±2,39	57,6 ±11,5	52,2 ±6,72
2	37,8±2,59	36,8±2,86	38,8 ±2,22	51,0 ±12,0	44,6 ±5,81
5	34,2±2,39	33,6±2,51	33,8 ±1,30	39,8 ±6,54	35,6 ±3,29
TT					
0	13,4±1,34	13,0±0,82	14,2 ±0,45	13,6 ±0,89	12,8 ±0,84
0,17	20,8±3,19	57,4±5,81	60,0*	60,0*	60,0*
1	15,5±1,92	15,8±2,17	26,2 ±7,76	42,4 ±14,4	57,2 ±6,26
2	14,4±1,82	14,8±1,10	16,5 ±0,58	18,8 ±1,92	21,8 ±6,98
5	14,6±2,07	14,0±1,00	15,4 ±1,14	14,2 ±0,45	14,2 ±1,30

*All values above 60s.

Discussion

According to the data it is clear that hirudin is eliminated by a two-compartment body model, but the existence of a third compartment cannot be excluded. The mean $t_{½;b}$ was approximately 1 hour. Mean total clearance and total volume of distribution were about 180 ml/min and 12 l, respectively.

The pharmacokinetic parameters C_{max}, $AUC_{0-1,5h}$ and AUC_{0-5h} were dose-related — the mean values increasing with dose in a linear fashion. In addition, since the mean $t_{½;a}$ and $t_{½;b}$, total clearance and volume of distribution remained reasonably constant as dose increased, it may be assumed that hirudin obeys linear pharmacokinetics. This was confirmed by the fact that the total urinary excretion of hirudin also increased in an approximately linear fashion with an increasing dose.

The two most important pharmacodynamic parameters, PTT and TT, were markedly elevated after injection of hirudin and maximum values of both variables were achieved at 10 minutes, which was the first sampling time. The maximum effect on PTT and TT was achieved as from 0,07 mg/kg. Five hours after injection of hirudin, both parameters had returned to baseline levels.

Bleeding time tended to increase 30 minutes after injection, but statistical comparison with only five volunteers per group is not feasible.

In conclusion it may therefore be stated that all the doses of hirudin were tolerated very well. No signs of bleeding tendencies were observed and safety measurements, such as clinical chemistry tests and haematological values were unaffected. Hirudin seems to obey first-order kinetics and has a profound effect on the ability of blood to coagulate after transvenous administration, as can be seen from its effects on TT and PTT.

REFERENCES

1. Markwardt F. Pharmacology of hirudin: one hundred years after the first report of the anticoagulant agent in medicinal leeches. *Biomed Biochim Acta* 1985; **44:** 1007-1013.
2. Markwardt F, Nowak G, Stürzebecher J, Vogel G. Clinico-pharmacological studies with recombinant hirudin. *Thromb Res* 1988; **52:** 393-400.
3. Markwardt F, Fink G, Kaiser B *et al.* Pharmacological survey of recombinant hirudin. *Pharmazie* 1988; **43:** 202-208.
4. Griessbach U, Stürzebecher J, Markwardt F. Assay of hirudin in plasma using a chromogenic thrombin substrate. *Thromb Res* 1985; **37:** 347-350.
5. Brockmeier D. *In vitro/in vivo* correlation of dissolution using moments of dissolution and transit times. *Acta Pharm Technol* 1986; **32:** 164-174.