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CLINICAL PHARMACOLOGY OF HIRUDIN (HBW 023)

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INTRODUCTION

Leeches are small fresh water animals sucking blood from other animals or man. The capacity to suck blood is enormous. After an opulent meal, leeches can remain without food for many months. This would not be possible without a mechanism that prevents blood from clotting during and after the sucking process. In 1884, John Haycraft discovered that leeches secrete a substance which inhibits blood clotting (1). This substance was later on called hirudin. It is a single chain polypeptide with a molecular weight of 7000 daltons, and it consists of 65 amino acids (Figure 1) (2). Hirudin binds specifically to thrombin with a very high affinity (3). This is surprising because thrombin is only a slightly modified trypsin like proteinase. The carboxy terminal end of the hirudin molecule binds near the active centre of thrombin and other parts of the hirudin molecule to other sites of the thrombin molecule . So far, no other enzymes were found to be inhibited by hirudin. There are a few reports of additional actions of hirudin: acceleration of the displacement of Factor Xa from the vascular endothelium and association with Factor Xa (4). Hirudin is a rather stabile peptide, quite resistant against proteinase and heat degradation and well soluble in water.

MATERIALS AND METHODS

Hirudin (HBW 023) was produced by r-DNA technology in yeast cells. After purification, the product had a purity of more than 97 %. Special investigations were undertaken to assure the quality of the product (Table 1). The product was supplied in vials containing 10 mg of lyophilyzed hirudin to be dissolved in 1 ml of physiological saline.

In rats, dogs and monkeys, hirudin showed dose-dependent increases of thrombin time and activated partial thromboplastin time after i.v. and s.c. application in doses of 0.01 - 10 mg/kg. In single dose toxicological studies the LD_{50} could not be determined due to good tolerance. In repeated dose administration over 4 weeks and 3 months in rats and monkeys

Table 1

Quality Control of Hirudin (HBW 023)

Peptide Characterisation	
Biologic Activity	Thrombin Inhibition
Peptide Sequence	Amino Acid Analysis
Molecular Weight	SDS-Gel Electrophoresis
Molecular Charge	Isoelectric Focussing
Molecular Size	Gel Permeation Chromatography

Table 2

Phase I Program of Hirudin (HBW 023)

Single Dose Studies	0.01	0.025	0.05	0.07 mg/kg	i.v.
	0.1	0.2	0.3	0.5 mg/kg	i.v.
Single Dose Studies	0.05	0.1	0.15	mg/kg	s.c.
	0.2	0.35	0.5	mg/kg	s.c.
Multiple Dose Studies	5 x 0.1 mg/kg			every 24 hours	i.v.
	5 x 0.1 mg/kg			every 12 hours	i.v.
	5 x 0.5 mg/kg			every 24 hours	s.c.
	5 x 0.5 mg/kg			every 12 hours	s.c.

Table 3

Pharmacokinetics of Hirudin (HBW 023)

Means and standard deviations of 5 volunteers per dose group

Dose mg/kg	0.1	0.2	0.3	0.5	0.1	0.2	0.35	0.5
Route of administration	i.v.	i.v.	i.v.	i.v.	s.c.	s.c.	s.c.	s.c.
C_{max} (ng/ml)	859*	1177*	1818*	3443*	125	231	346	382
	106	133	219	327	12.5	47.2	108	61.3
AUC (ng.h/ml)	652	1098	1562	2959	498	1098	1695	2064
	118	111	351	473	65.2	188	234	249
t_2 (h)	0.89	1.16	1.10	1.15	1.80	1.92	2.31	2.78
	0.15	0.07	0.29	0.18	0.77	0.14	0.78	0.92
Vss (l)	14.3	22.4	19.0	20.5				
	3.08	3.57	2.01	3.37				
Cl-tot (ml/min)	205	241	254	227	257	241	236	269
	44.1	30.2	56.3	28.3	28.3	36.7	38.6	31.8
Cl-ren (ml/min)	94.2	90.4	110	115	97.6	73.5	-	-
	17.8	15.2	30.9	14.7	15.4	10.2	-	-
Ae (% of dose)	49.4	38.6	44.0	51.8	43.7	35.4	-	-
	8.10	5.64	5.60	4.80	5.77	1.86	-	-

* First observed concentration

Figure 1

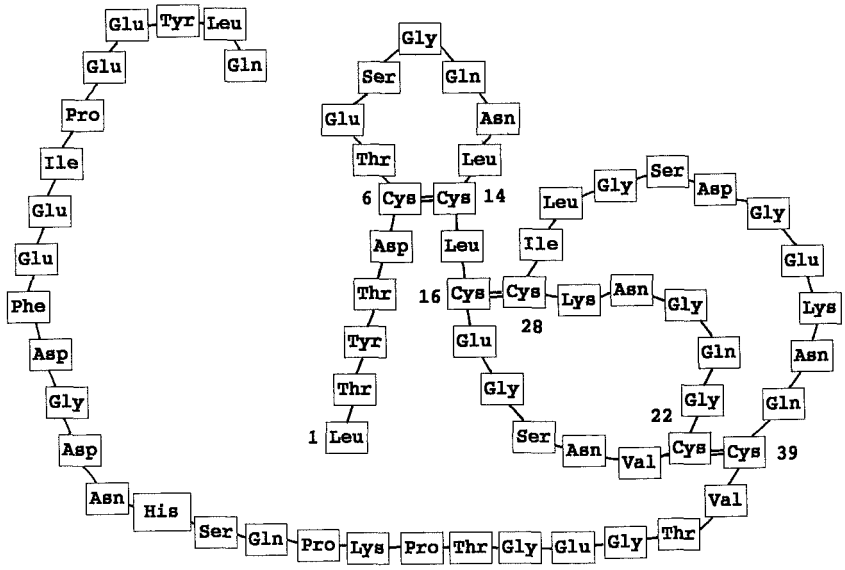
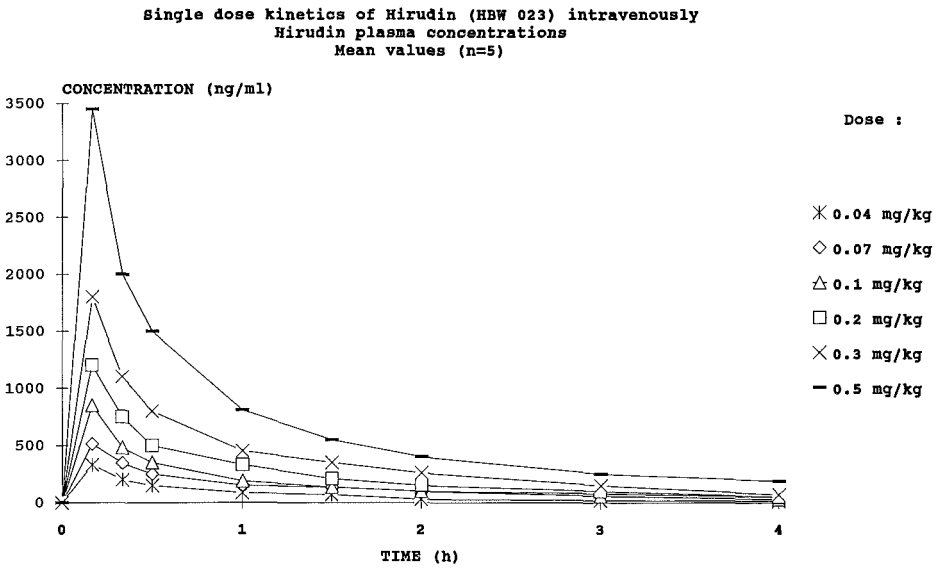


Figure 2



intravenously and subcutaneously, no toxic effects could be observed, except the anticoagulant effect of hirudin with higher doses. The local tolerance of hirudin was also good. There were also no hints for mutagenic effects.

According to Blackwell (5), the first dose in man was calculated to be 0.01 mg/kg.

For the determination of hirudin in plasma and urine, we used a thrombin inhibition assay (6). In order to have adequate precision and sensitivity, individual calibration curves had to be established for each volunteer from his plasma blanks. The detection limit of this assay is 9 ng/ml for plasma and 60 ng/ml for urine.

RESULTS

The Phase I program included single dose studies i.v. and s.c. and multiple dose studies i.v. and s.c. (Table 2). The results of single dose kinetic studies are given in Figure 2 for intravenously applied hirudin and Figure 3 for subcutaneously applied hirudin. The pharmacokinetic parameters are summarized in Table 3. Hirudin has dose-linear kinetics with a half-life of approximately 1 hour. Major route of excretion is renal elimination, probably mainly by glomerular filtration. It becomes evident that renal clearance accounts only for 50 % of the total body clearance. Around 30 to 50 % of the administered hirudin was found unchanged in urine. This is in contrast to former reports of natural hirudin and kinetics in animals where renal elimination was found to contribute more than 90 % to elimination (7).

After subcutanous administration, hirudin is well absorbed from the tissue with C_{max} values occurring around 2 hours. Comparison of AUC and urinary excretion with i.v. dosing indicates 80-90 % bioavailability.

In multiple dose studies intravenous doses of 0.1 mg/kg every 12 or every 24 hours for 5 times were well tolerated. The concentration time curves were rather identical for all days. So, no accumulation of hirudin occurred. The same holds true for subcutanous administration.

In total, more than 50 volunteers participated in the Phase I trials. Hirudin was very well tolerated, no side effects were noted. No specific antibody induction could be detected against hirudin or yeast proteins.

Figure 3

Single dose kinetics of Hirudin (HBW 023) subcutanously
Hirudin plasma concentrations
Mean values (n=5)

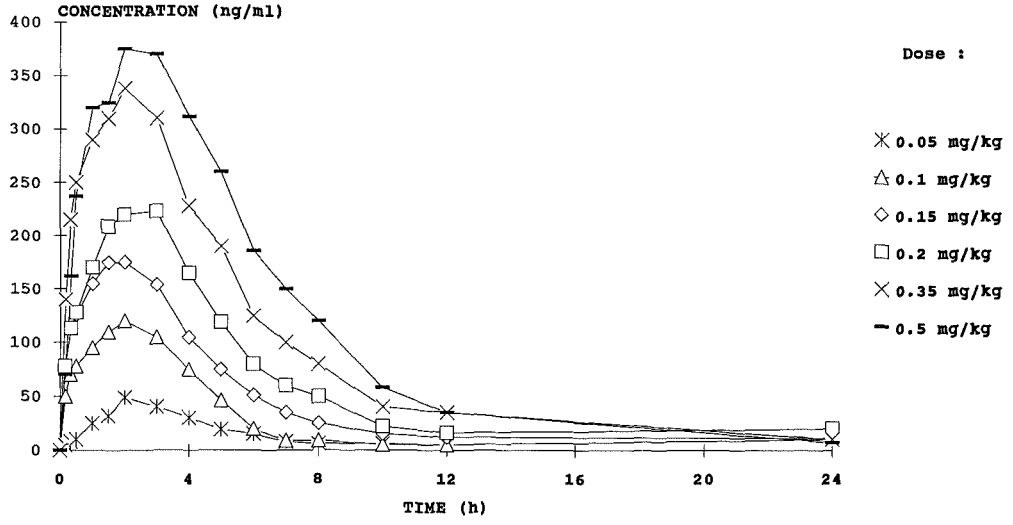


Figure 4

Single doses of Hirudin (HBW 023) intravenously
Activated partial thromboplastin time (aPTT)
Mean values (n=5)

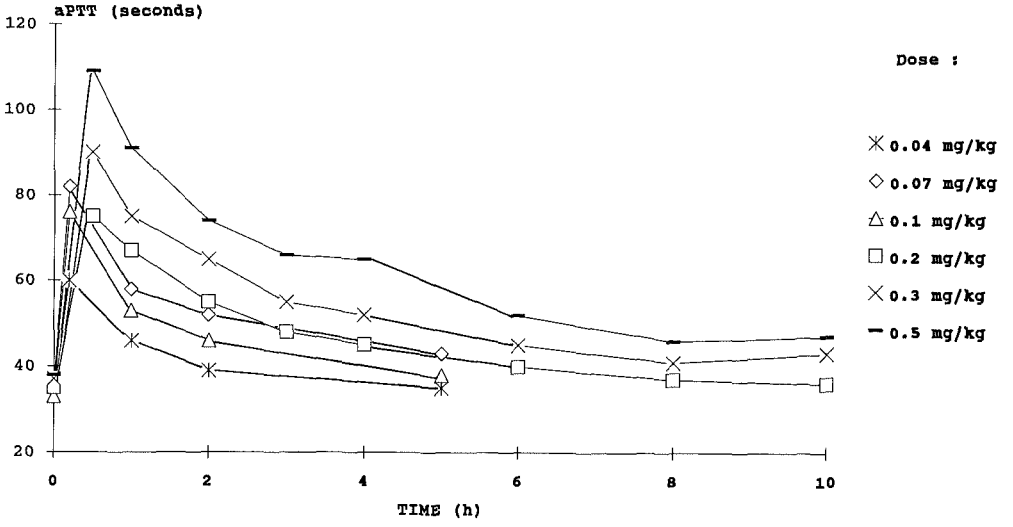


Figure 5

Single doses of Hirudin (HBW 023) subcutanously
Activated patial thromboplastin time (aPTT)
Mean values (n=5)

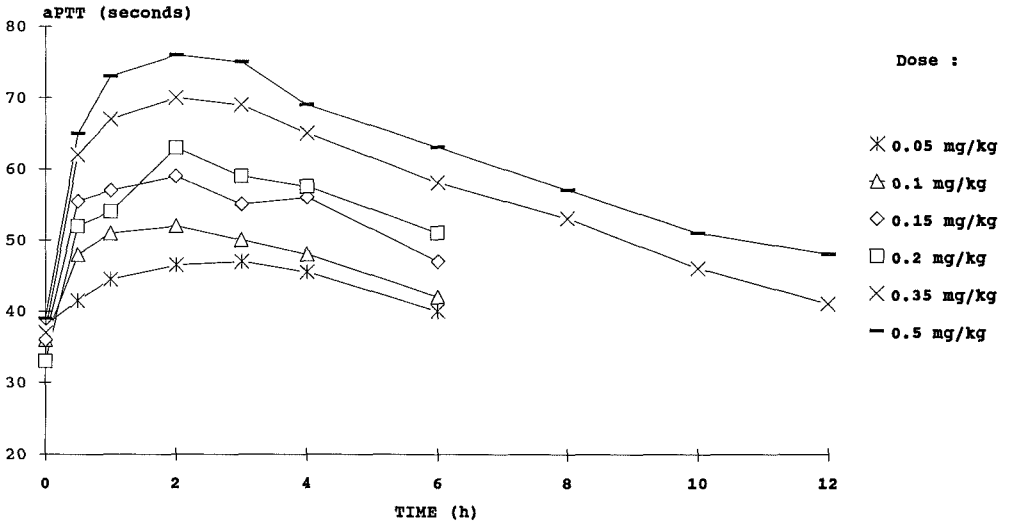


Figure 6

Multiple doses of Hirudin (HBW 023)
Activated partial thromboplastin time (aPTT)
Mean values (n=5)

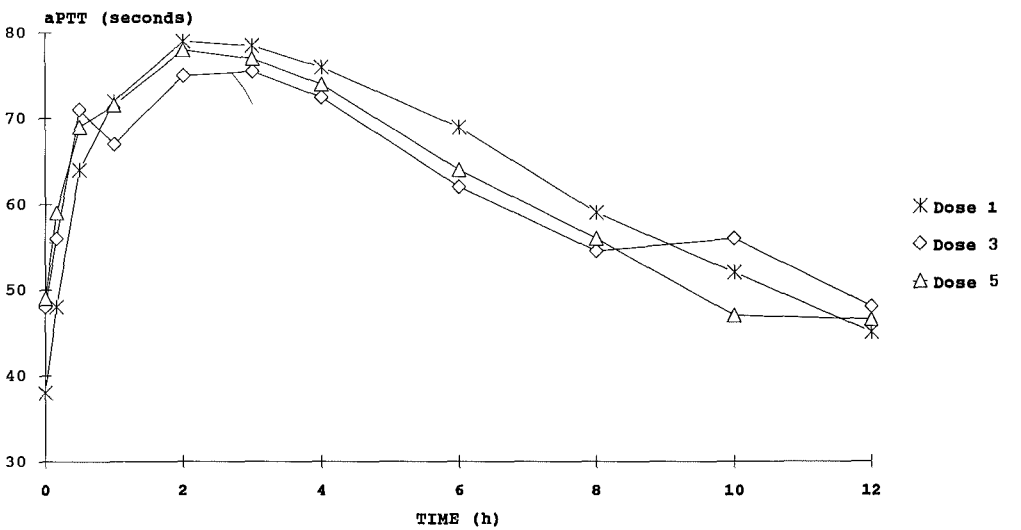
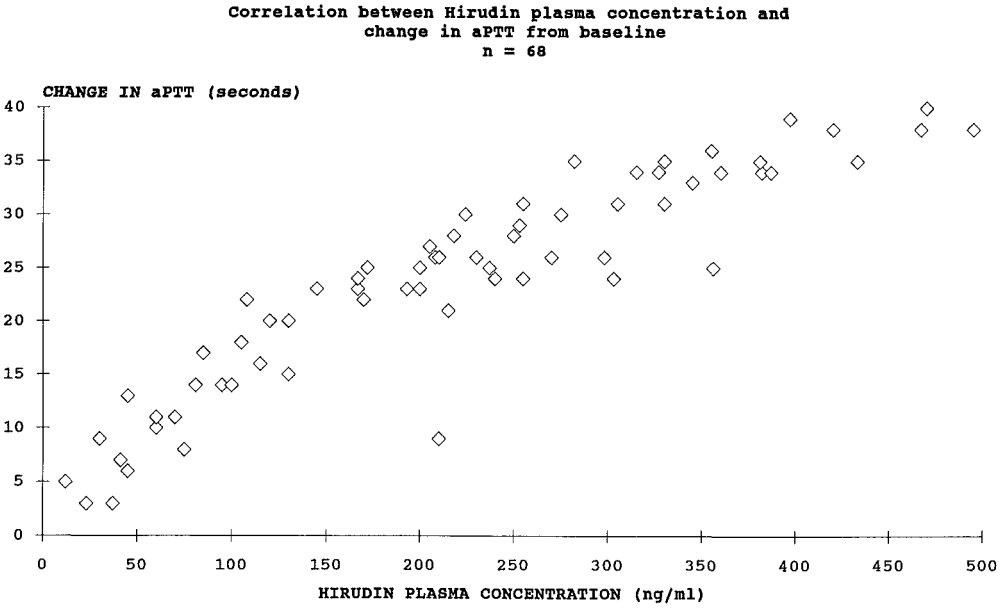


Figure 7



Pharmacodynamics were measured by several assays. As pharmacodynamic variables for in vivo effects we used bleeding time, thrombin time and activated partial thromboplastin time (aPTT). There were no effects, even not in the highest dose on bleeding time. The thrombin time showed a very steep dose response, so that for most of the time points the values were above 100 seconds. Thus, the thrombin time does not seem to be an appropriate method for the pharmacodynamic measurement of hirudin. The effect of single doses of hirudin on aPTT can be seen in Figures 4 and 5. After multiple dosing i.v., the response on aPTT was identical for doses 1, 3 and 5 (Figure 6). Similar results were seen after multiple doses s.c.

The effect of hirudin was clearly related to plasma levels. After a linear increase with low levels, the curve flattens with higher levels (Figure 7). This is in contrast to the effects seen with other anticoagulants (8). The effect of hirudin on aPTT can be described by the formula: aPTT = A-m/log concentration of hirudin.

Hirudin application had no influence on the spontaneous thrombin generation. This can be concluded from constant F_{1+2} levels as marker of thrombin generation. But hirudin competes with AT III for thrombin as can be seen by slightly decreasing levels of AT III/thrombin complexes during hirudin therapy.

DISCUSSION

Haemostasis in blood is normally well regulated in man through a number of systems including activators, inhibitors and feed-back magnification. Nevertheless, under certain circumstances situations occur when the clotting of blood leads to disease rather than being beneficial. In these cases, anticoagulants are needed. But they all bear the risk of bleeding depending on the therapeutic range of the drug. Heparin is a widely used drug with well-proven clinical efficacy. Unfortunately, heparin is a mixture of molecules with different affinities for binding; heparin has multiple sites for binding, some are specific, others are unspecific. Heparin also binds to many proteins in blood and can also induce thrombocyte aggregation. The kinetics of heparin are non-linear and influenced by liver and kidney function. The individual anticoagulative response is very variable. The dynamic action can heavily be disturbed by histidin-rich protein. Patients with thrombosis need more heparin than healthy volunteers. The non-linear kinetics are accompanied by a poor correlation of plasma levels and pharmacodynamic effect. Thus it can be concluded that heparin is an anticoagulant with a very narrow therapeutic range (9).

In contrast, for a very specific thrombin inhibitor like hirudin a much wider therapeutic range is anticipated. From the data presented here we can see that the kinetics are linear with low variability and are very predictable. In contrast to heparin, hirudin effects correlate well with plasma levels. The effect is clearly dose-related. The flattening of the dose-response curve with higher hirudin levels might be a major point guaranteeing safety because overdosing would not necessarily mean bleeding. This is in contrast to heparin where the dose-response curve is much steeper with higher doses.

In conclusion, the presented Phase I data support the hypothesis that hirudin by its high specificity is a valuable anticoagulant which seems to have a much wider therapeutic range as compared to heparin and other anticoagulants. The safety profile of hirudin therapy is expected to be better than that of heparin.

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Discussion - CLINICAL PHARMACOLOGY OF HIRUDIN (HBW 023)

J.A. Galloway

Does hirudin have any antimicrobial effect? Because one rarely sees infections in people who have leeches attached to them.

H.J. Röthig

So far, I don't think so. Hirudin is a highly specific thrombin inhibitor. It has been assumed that it may also act on factor Xa, but this is unclear.

M.M. Reidenberg

You mentioned that half life of subcutaneously administered hirudin is more prolonged than that of IV hirudin. I tend to think of half life as being related to elimination and I am curious if you think the subcutaneous route actually modified the elimination of the drug or if the prolonged half life was simply an artifact caused by continuing absorption.

H.J. Röthig

Sure, it is an effect of retarded absorption. It has nothing to do with elimination, but it is clinically relevant. The interesting thing is that the absorption seems to be slower with increasing doses. At present, we haven't found out if this is a result of a reduced perfusion of the tissue, but that could be an explanation.

P. Tanswell

In your healthy volunteer studies you used the activated partial thromboplastin time as an end point. I would be interested to know what the end point was in your phase II studies, because I would imagine you would be looking for an antithrombotic effect rather than an effect on the coagulation system, and there you may not see such a clear cut relationship between plasma levels and pharmacological effect.

H.J. Röthig

We look for the prevention of thrombosis, that is the end point. There are certain clinical models, such as hip replacement surgery. There is a high percentage of thrombosis and that can be used in the quantification of therapeutic effects.

R.G. Werner

You mentioned that there are natural variants of hirudin and that there are also second generation hirudins. Is there any improvement in specificity or activity compared to the natural compound?

H.J. Röthig

Actually not. This molecule has such a high affinity to thrombin that by changing the molecule one can no longer improve that. Any reported difference on binding constants lies in the methodology used.

D.C. Brater

I want to make a comment concerning pharmacokinetics. The data that you already have could be used to answer the question as to whether or not the prolonged half life following subcutaneous administration is due to absorption. You can look at the mean residence time after the subcutaneous administration and subtract the mean residence time after intravenous administration and that gives you a parameter called absorption time. If that absorption time is greater than the mean residence time intravenously, then you have what is called "flip-flop kinetics" and the half life that you are actually looking at is the absorption half life, which is longer than the elimination half life. I also have a question: With heparin, if people get into trouble we have an antidote, that is protamine, but if someone who is anticoagulated with hirudin starts to bleed, what could you do to stop that?

H.J. Röthig

THARA is an antidote available. It is possible to administer activated factors like "autoplex" which is available in most countries. One can normalize the APTT and probably the bleeding tendency. In general, we think that the risk of bleeding with hirudin is not higher than with "aspirin".

M. Levy

Let me ask you a question concerning the antigenicity of hirudin. Did anyone look at people who were treated with leeches to see whether they formed antibodies?

H.J. Röthig

Yes, this was done and there one can find antibodies. When we first started we had this information and we spent a lot of time measuring possible formation of

antibodies, particularly in our multiple dose subcutaneous studies. None of the volunteers developed specific antibodies against hirudin. We saw some unspecific effects but in our assay we can clearly discriminate between specific and non-specific antibodies.