Recombinant hirudin, a new anticoagulant, has no effect on faecal blood loss

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Hirudin is a selective thrombin inhibitor with strong anticoagulant properties which could elicit gastro-intestinal bleeding. A double-blind cross-over study of the effects of hirudin on gastro-intestinal bleeding was therefore conducted on 12 healthy, consenting males. After labelling erythrocytes with ⁵¹Cr and returning them intravenously, stools were collected for 2 days to measure radioactivity and hence baseline faecal blood loss. After injection of hirudin or placebo stools were collected for 3 days. Partial thromboplastin time was measured sequentially after medication with hirudin or placebo. This procedure was repeated after injection of the alternate medication 1 week later. Hirudin was tolerated well. Mean faecal blood loss associated with hirudin was slightly higher than with placebo (1.63 ml vs 1.15 ml over 3 days; 95% confidence interval for the difference between hirudin and placebo was –0.68 to 1.63) but these differences are clinically irrelevant. After hirudin injection PTT was elevated to about twice the baseline values but returned to baseline within 12 h after the last hirudin injection.

Keywords hirudin thrombin inhibitor faecal blood loss anticoagulant

Introduction

Hirudin, which is produced by the peripharyngeal glands of the medicinal leech (*Hirudo medicinalis*) is a selective thrombin inhibitor, a polypeptide containing 65 amino acids with a molecular weight of about 7000 (Markwardt, 1985). Its strong anticoagulant properties were recognised more than a century ago. Early studies were done with natural hirudin (Markwardt *et al.*, 1988b), but medicinal leeches are now an endangered species and natural hirudin is not available in adequate quantities for therapeutic use (Markwardt, 1985). However, progress in genetic engineering has resulted in the availability of large quantities of recombinant hirudin for research purposes (Meyer *et al.*, 1990). Recombinant hirudin has pharmacological properties similar to those of natural hirudin and is a highly potent thrombin inhibitor which is well tolerated *in vivo* by animals (Markwardt *et al.*, 1988a). It may prove to be very useful in therapeutics.

In view of its anticoagulant properties hirudin could elicit gastro-intestinal bleeding. This study was therefore conducted to measure the amount of blood loss in the gastro-intestinal tract after three subcutaneous injections

of hirudin, and to compare this blood loss with that occurring with a placebo.

Methods

Twelve healthy male volunteers, who gave written informed consent, participated in this double-blind cross-over study which was performed in the Hoechst Research Clinic, Department of Pharmacology, University of the Orange Free State. Approval was obtained from the Ethics Committee of the University as well as the South African Medicines Control Council. The mean age of the subjects was 20 years (range 18 to 23 years) and their mean weight was 72 kg (range 61 to 77 kg). Before being entered into the study the volunteers were screened by means of a physical examination, clinical chemistry measurements, haematological tests and urinalysis. These tests were repeated at the end of the study.

The subjects reported to the clinic at 06.30 h on day 1 of the study. Blood (10 ml) was drawn for erythrocyte labelling with ^{51}Cr and was returned intravenously at 07.30 h. For 48 h thereafter (starting at 07.30 h on day 1) all stools were collected in order to measure radioactivity and thus to determine baseline faecal blood loss. On day 3 hirudin (0.35 mg kg^{-1}) or placebo, depending on the randomisation schedule, was injected subcutaneously into the abdominal wall at 07.30 h, 13.30 h, and 19.30 h. Stools were collected for the 72 h period following the first hirudin or placebo injection at 07.30 h. Subjects returned to the clinic at 07.30 h on the morning of day 10 for the alternate injections (placebo or hirudin) and stools were again collected for 72 h after the first injection.

The International Committee for Standardisation in Haematology (1971) technique was used for labelling erythrocytes. In order to evaluate faecal blood loss, radio activity in stools was measured by means of standard methods (Hine, 1967). During the 13 days regular blood specimens were taken for measurement of blood radioactivity in stools was measured by means of standard methods of faecal blood loss.

In addition, partial thromboplastin time (PTT) was measured using Platelin-plus Activator (General Diagnostics, Morris Plains, New Jersey, USA). This was done before and 1, 3 and 6 h after the first two injections as well as 12 h after the last injection on days 3 and 10.

The total faecal blood loss over days 1 and 2 was multiplied by 1.5 to make the time period covered comparable with that of the blood loss measured for the 72 h following hirudin and placebo injections on days 3 and 10. Two analyses of variance were performed: one (with treatment and subject effects) to compare baseline, placebo and hirudin, and one (with treatment, subject, period and sequence effects) to compare only placebo and hirudin. Results are expressed as means or mean differences and associated 95% confidence intervals.

Results

Safety measurements (physical examination, blood pressure, clinical chemistry tests and haematological values) were not affected by hirudin or placebo administration.

In all subjects but one, both hirudin and placebo were associated with higher blood loss than was observed at

baseline. The highest amount of blood loss after hirudin injection was 8 ml over 3 days. The means, mean differences and associated 95% confidence intervals for total faecal blood loss over 3 days are given in Table 1. PTT values are reflected in Figure 1.

Both the period and sequence effects were statistically not significant (P values were 0.11 and 0.3 respectively). The corresponding 95% confidence interval for the difference 'hirudin-placebo' was -0.9 ml to 1.85 ml .

Discussion

The mean total amount of faecal blood loss over 3 days after three 6 hourly subcutaneous injections of 0.35 mg kg^{-1} hirudin was 0.48 ml more than after placebo injections (1.63 vs 1.15 ml) but this difference is clinically irrelevant. Both these values compare well with 0.6 ml/day found in untreated patients by Leonards *et al.* (1973). The mean baseline value in our study was even lower at 0.25 ml/3 days . The difference of 0.9 ml over 3 days between mean faecal blood loss for baseline and placebo should probably be attributed to random fluctuation. Eight subjects on placebo had blood losses of less than 1.1 ml and the difference of 0.9 ml was due mainly to two subjects with values of 2.85 and 3.53 ml . Partial thromboplastin time after hirudin injection was elevated to about twice the baseline values. However, by 12 h after the third injection the mean PTT had decreased to 36.8 s , which is close to the baseline value of 33.3 s .

No similar studies in healthy volunteers receiving heparin have been reported, consequently no comparison between hirudin and heparin is possible. However, based on our findings we conclude that hirudin was tolerated well and did not cause any clinically relevant increase in faecal blood loss. More prolonged studies will be required to exclude a chronic effect on gastro-intestinal bleeding.

Table 1 Means, mean differences and associated 95% confidence intervals of faecal blood loss

	Mean (ml)	95% confidence interval (ml)
<i>Treatment</i>		
Baseline	0.25	0.05–0.46
Placebo	1.15	0.48–1.82
Hirudin	1.63	0.19–3.06
<i>Difference</i>		
Placebo-baseline	0.90	-0.25 – 2.05
Hirudin-baseline	1.37	0.22 – 2.52
Hirudin-placebo	0.47	-0.68 – 1.63

Figure 1 Mean partial thromboplastin time associated with three 6-hourly doses of placebo (○), and hirudin 0.35 mg kg^{-1} (×).

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