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Efficacy and safety of topical Hirudin (Hirudex®): a double-blind, placebo-controlled study

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Abstract. – **Hirudin, a thrombin inhibitor isolated from the leech Hirudo medicinalis, has been long known for its anticoagulant effects. In 1990, the former German BGA has published a monograph on Hirudo medicinalis extract (containing Hirudin and Eglin), stating that its local use is indicated in bruises with or without hematoma.**

The aim of this double-blind, placebo-controlled clinical trial was to evaluate the efficacy and safety of a Hirudin-containing cream (Hirudex® cream; Hirudo medicinalis extract 280 UI/100 g) in patients affected with bruises with or without hematoma.

60 men and women between the ages of 18 and 65 years with a unilateral acute musculoskeletal injury (bruise) with or without hematoma were included. Dosage schedule and application route for both treatments were the following: 3-4 daily applications for 5 days for a total of 15-20 administrations during the whole study period.

In the Hirudin group, a highly statistically and clinically significant improvement were noted. Although a statistical improvement was also seen in patients treated with placebo, this was less pronounced, and a highly significant between-group difference was noted for all three major efficacy parameters at each follow-up visit in favour of Hirudin. Both the patients and the investigator considered the overall assessment of efficacy at the end of the study to be significantly better ($p < 0.001$) in the Hirudin group than in the placebo group.

Results of this study suggest that Hirudin is an effective local treatment in patients with mild to moderate bruises.

Key Words:

Hirudin, Bruises, Hematoma, Topical therapy.

Introduction

The potent blood coagulation inhibitor Hirudin is produced by, and was originally isolated from, the salivary glands of medicinal leeches (Hirudo medicinalis)[1].

Naturally occurring Hirudin consists of a number of different isoforms depending on the tissues from which it is extracted. Desirudin, a recombinant form of Hirudin, is a potent inhibitor of human α-thrombin with a very low dissociation constant ($Ki = 2 \times 10^{-13}$ M), although this is approximately 10-fold higher than that of the naturally occurring molecule. Desirudin inhibits thrombin by forming a 1:1 complex with the molecule. Naturally occurring Hirudin variants differ in primary amino acid sequence but they are identical in their biological activity against thrombin. All actions of thrombin are inhibited by Hirudin: fibrinogen cleavage, activation of factors V, VIII, and XIII, platelet activation, binding to thrombomodulin, and mitogenic and contractile effect on vascular smooth muscle[2].

Hirudin has no effect on other enzymes of the coagulation or fibrinolytic systems and the only effect observed is inhibition of thrombin. Hirudin reacts with thrombin in a 1:1 molar ratio to form an enzyme inhibitor complex in which the proteolytic activity of the enzyme is totally blocked[3].

In a rat model of thrombosis the ID_{50} of Hirudin was 0.01 mg/kg intravenously and 0.45 mg/kg subcutaneously; the figures for total inhibition were 0.03 and 1.0 mg/kg respectively[4]. The principal effect of Hirudin is regarded as anticoagulant but some procoagulant effect will arise because activation of protein C by thrombin, in conjunction with thrombomodulin, is inhibited.

The pharmacokinetics (half-life time of absorption and elimination, total clearance, distribution volume etc.), effects on hemostasis (clotting times, blood cell counts) and renal

excretion of hirudin have been extensively investigated in healthy volunteers after single subcutaneous (600, 800 or 1000 antithrombin units (AT-U)/kg; n = 3 each dose) or intravenous (1000 AT-U/kg; n = 3) injections[5].

Although recombinant Hirudin is therapeutically used as an alternative to heparin and requires parenteral administration[6], Hirudin, extracted from leeches, is available in some European countries (such as Germany and Italy) for topical application after acute musculoskeletal injuries.

The aim of this double-blind, placebo-controlled clinical trial was to evaluate the efficacy and safety of a Hirudin-containing cream (Hirudex® cream; Hirudo medicinalis extract 280 UI/100 g) in patients affected with bruises with or without hematoma. Patients were drawn from the outpatients clinic of the Queen Joanna Hospital in Sofia (Bulgaria) and scheduled to receive treatment for a period of 5 days.

Patients and Methods

This was a double-blind, placebo-controlled, unmasked, parallel-group, randomised study. According to the protocol, this trial was designed as single-centre, double-blind, randomised, parallel group, placebo-controlled, where the 95% confidence interval had to lie entirely to the right of the value -Δ. A full analysis, based on the intention-to-treat principle, was planned and performed, taking into account a regulatory equivalence margin of ± 15%.

This study design was chosen because of its reliability in medical research, taking into account the therapeutic non-inferiority hypothesis, according to the "Points to consider on biostatistical/methodological issues arising from recent CPMP discussions on licensing applications: superiority, non-inferiority and equivalence" (CPMP/EWP/482/99 draft corr, 23 September 1999).

A 3-arm design, comparing placebo, the new product and the product already licensed (which contains a combination of Hirudin and esculoside) was discarded because it should prove unlikely to be performed in these setting of out-patients. A 2-arm study was then preferred, according to the "Note for Guidance on the clinical requirements for locally applied, locally acting products containing know constituents" (CPMP/EWP/239/95 final).

Thirty patients per group (60 male and female patients in total) had been calculated to detect, if it exists, a difference of ± 15% between the two groups. Since the lesion is self-limiting, the use of placebo (vehicle) was judged ethical.

Men and women between the ages of 18 and 65 years with a unilateral acute musculoskeletal injury (bruise) with or without hematoma were included. The presence of at least one of the following mild to moderate symptoms was required: localised tenderness, pain on movement, and limitation of movement (defined as a reduction in range of joint motion of at least 20%). At the day of enrolment, at least one of the clinical evaluation parameters (calor, rubor and pain) had to be greater than 50 mm as assessed by means of a 10-cm visual analogue scale (VAS).

Patients were excluded from the trial if they presented with infected bruises, or required any drug therapy that would interfere with the evaluation of the acute disorder; had suspected bone fracture; had any injury requiring hospitalisation, surgical intervention, or a cast; had demonstrated a history of allergy or hypersensitivity to Hirudin. Other reasons for exclusion were: antagonistic personality, poor motivation, or other emotional or intellectual problems likely to either invalidate the informed consent or limit the ability to comply with protocol requirements; participation in other studies involving investigational or marketed products within 1 month before study entry, or concomitant with the study.

Dosage schedule and application route for both treatments were the following: 3-4 daily applications for 5 days for a total of 15-20 administrations during the whole study period. To ensure that patients were taking their medication according to the protocol, patients had to return the medication at each follow-up visits (T1-T2), and the investigator recorded the start and stop dates of the medication, as well as all changes in dose, missed doses, and days of therapy. No concomitant therapy with analgesics or NSAIDs, systemic or local steroids, or any agents altering pain perception were permitted during the study.

Drug therapy could be interrupted if symptoms were aggravated, or if severe adverse events occurred. Non-pharmacologic treatment was allowed at the discretion of the investigator, standardised as follows: ice application, and compression.

Patients underwent clinical assessments at baseline (T0) and at clinic visits on days 2 (T1) and 5 (T2). Three major efficacy variables were measured: Pain, flare, and caumesthesia were assessed by means of a 10-cm visual analogue scale (VAS) just before application of medication (T0), on day 2 (T1), and on day 5 (T2). In order to simplify statistical analysis, the following score ratings were adopted: 0-25 mm = 0; 26-50 mm = 1; 51-75 mm = 2; 76-100 mm = 3.

As a secondary efficacy variable, a score evaluation was carried out to quantify the patients' subjective feelings (local discomfort from 0 = absent, 1 = mild, 2 = moderate, 3 = severe).

Overall efficacy and tolerability of treatments were assessed by both patients and investigators at the end of the study on a 4-point scale, ranging from excellent to poor. Adverse events were recorded at each visit.

Efficacy was defined as the difference between the VAS assessment of bruise symptoms by the patient at baseline and the assessment made at T2.

The safety profile was based on the number of observed or volunteered adverse events, the number of patients with serious adverse events, the severity of the reported adverse events, and the relationship between the adverse events and the study medication. Laboratory tests (vital signs and standard clinical chemistry) were recorded on the case report form.

The description and comparison of baseline data and the safety assessment were based on intent-to-treat population.

The study was approved by the appropriate institutional review board and was conducted according to good clinical practice requirements. All patients provided written informed consent before entering the study.

Statistical analysis

Efficacy parameters (VAS and score) were analysed by comparing the two groups using the Kruskal-Wallis test at Visit T1 and T2 vs. T0. A Dunn's multiple comparison test was coupled comparing the difference in the sum of ranks between all study periods with the expected average difference (based on the number of groups and their size).

For the intra-group analysis, the Wilcoxon test was used. 95% confidence intervals were also calculated. All statistical analyses, blinding, and randomisation, were carried our according to the "Note for Guidance on Statistical Principles for Clinical Trials" (CPMP/ICH/363/96, 18 March 1998), taking into account that for confirmatory trials the parallel group design is the recommended trial design. Since a non-inferiority trial is conservative in nature, a special attention has been paid at avoiding (or at least minimising) the incidence of violations of the entry criteria, non-compliance, withdrawals, losses of follow-up, missing data and other deviations from the protocol, and also at minimising their impact on the subsequent analyses.

The significance level was set at 0.05. Vital signs and clinical laboratory tests results were compared by the analysis of variance (anova), coupled with post-test analyses or the unpaired Student t Test with Welch correction (parametric values) when needed. Abnormal values were judged by the investigator as clinically or non clinically relevant.

Results

Seventy-three patients were screened for entry into the study; 4 patients had insufficient data on the case report form, and 3 patients did not give informed consent at the baseline visit. Six other patients were on analgesic or NSAIDs at baseline. The remaining 60 patients (32 men and 28 women; mean age, 34.9 (10.4 years) comprised the intent-to-treat population and were randomly allocated to receive Hirudin (n = 30) or placebo cream (n = 30). Treatment lasted 5 days in both groups.

Patient characteristics of the intent-to-treat group are summarised in Table I.

Mean body weight and height were 71.7 (8.5 kg and 173.1 (8.3 cm) respectively. All vital signs (body temperature, heart rate, and blood pressure) were in the normal range. The two treatment groups were comparable in terms of disease characteristics at baseline.

Table I. Baseline patient characteristics (intent-to-treat population).

	Hirudin (n = 30)	*Placebo (n = 0)*	*All Patients*
Sex no. (%)			
Male	18	14	32
Female	16	12	28
Age	34.7 ± 8.0	35.0 ± 11.3	34.9 ± 10.4

Results obtained in patients treated with Hirudin are shown in Table II. In this group, a highly statistically and clinically significant improvement were noted. Although a statistical improvement was also seen in patients treated with placebo (Table III), this was less pronounced, and a highly significant between-group difference was noted for all three major efficacy parameters at each follow-up visits in favour of Hirudin (Figures 1-3).

Both the patients and the investigator considered the overall assessment of efficacy at the end of the study to be significantly better ($p < 0.001$) in the Hirudin group than in the placebo group.

Although difficult to assess, hematoma, when present (50% of patients in each group), seemed to be reabsorbed more quickly after Hirudin than after placebo.

Tolerability was based on recorded adverse events for all randomised patients. An overall assessment of tolerability was also made by patients and investigator at the end of the treatment period; no significant difference was noted between the treatment groups. During the whole study period no serious adverse events occurred. No patients in either treatment group discontinued the study because of an abnormal laboratory test, vital sign measurements or physical examination findings.

Three adverse events were reported throughout the entire study period. All of them were mild local intolerance (erythema) after topical application (2 cases in the Hirudin group and 1 case in the placebo group). These adverse events were self-limiting, did not cause withdrawal from the study or temporary discontinuation of treatment.

Discussion

Hirudin, a thrombin inhibitor isolated from the leech Hirudo medicinalis, has been long known for its anticoagulant effects , but a wide clinical use of Hirudin was prevented by difficulties in isolating sufficient amounts of this substance. Advanced methods of peptide isolation have provided the opportunity of obtaining Hirudin in purified form and in sufficient yield.

The percutaneous penetration of Hirudin was demonstrated by immunological methods in guinea-pigs and with radiolabelled Hirudin in pigs[7]. The skin permeability of these animals is similar to that of man.

Other experiences showed an accelerated permeation of this substance in man after application of iontophoresis and the presence of Hirudin in human subcutaneous tissue after cutaneous application of the substance. Clinically, the efficacy of local application of an Hirudin ointment in preventing shunt-thrombosis in dialysis patients with recurrent and impending occlusion of their arteriovenous fistula has been demonstrated[8].

Table II. Assessment of pain, flare, and caumesthesia in patients treated with Hirudin (mean ± SD).

Symptom	*T0*	*T1*	*T2*
Pain	2.700 ± 0.46*	1.200 ± 0.76*	0.3333 ± 0.47*
Flare	2.433 ± 0.56	1.400 ± 0.49*	0.2667 ± 0.44*
Caumesthesia	2.467 ± 0.62	1.067 ± 0.69*	0.2667 ± 0.44*

*P < 0.0001 vs. T0.

Table III. Assessment of pain, flare, and caumesthesia in patients treated with placebo (mean ± SD).

Symptom	*T0*	*T1*	*T2*
Pain	2.733 ± 0.44	2.500 ± 0.57°	1.968 ± 0.61*
Flare	2.567 ± 0.50	2.268 ± 0.44**	1.801 ± 0.66***
Caumesthesia	2.600 ± 0.49	2.301 ± 0.59**	1.867 ± 0.77*

*P < 0.0001 vs. T0; **P = 0.0039 vs. T0; ***P = 0.0001 vs. T0; °P = 0.0156 vs. T0.

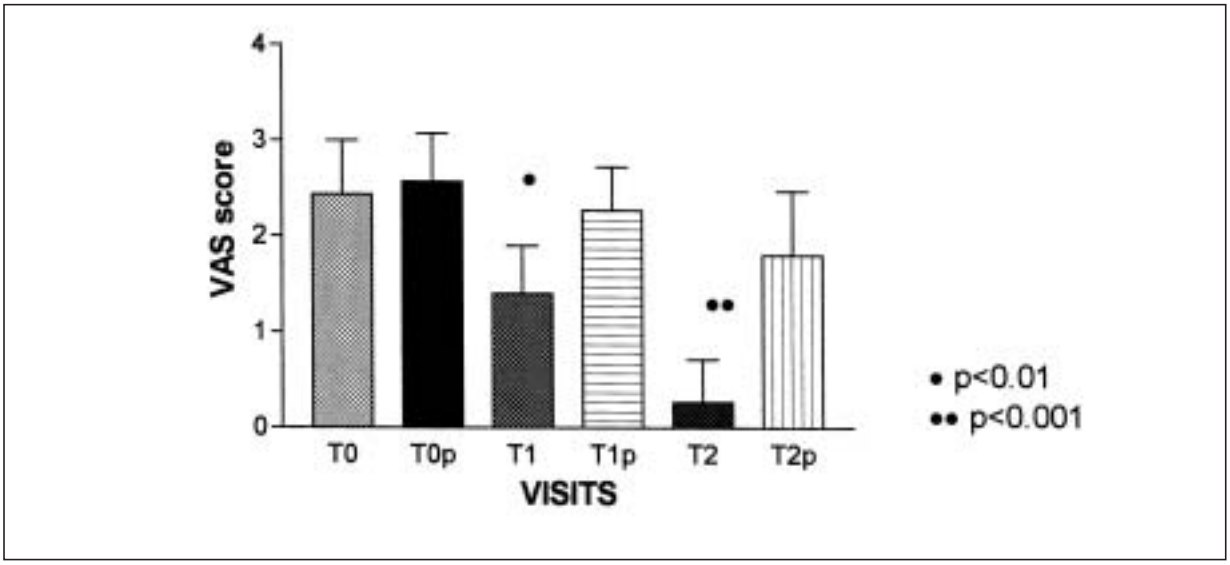

Figure 1. Change in VAS assessment of flare between treatment groups (p = placebo group).

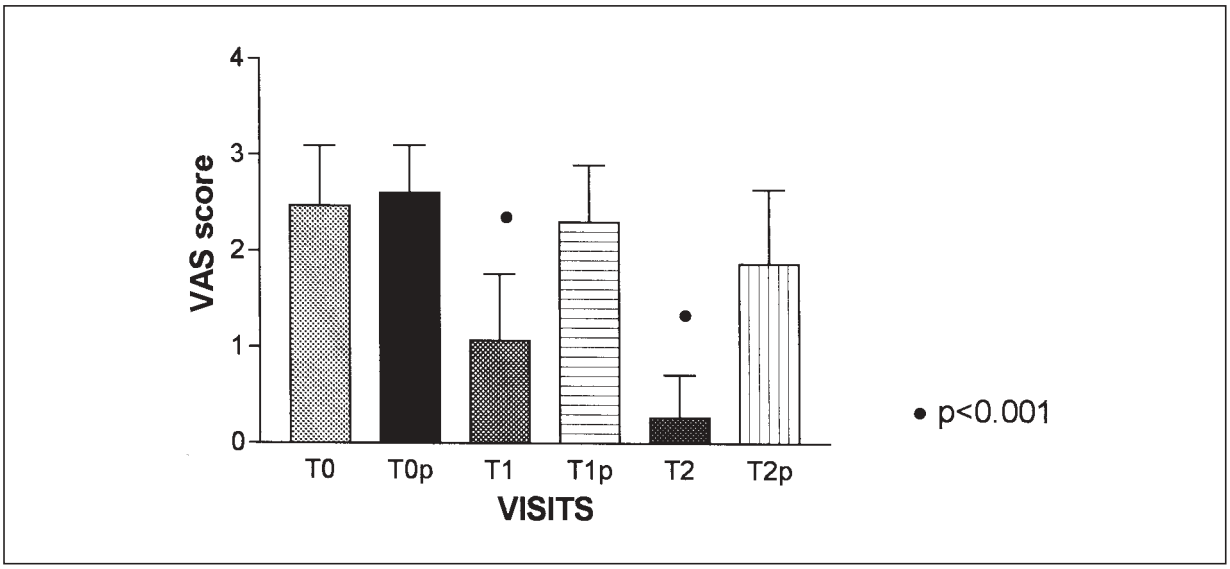

Figure 2. Change in VAS assessment of caumesthesia between treatment groups.

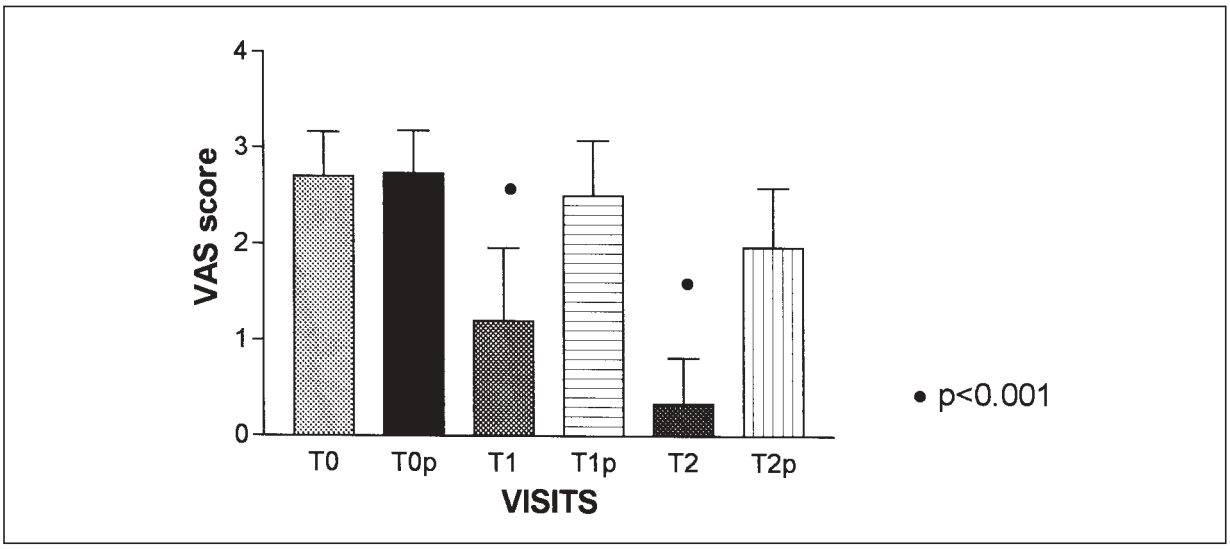

Figure 3. Change in VAS assessment of pain between treatment groups.

In 1990, the former German BGA has published a monograph on Hirudo medicinalis extract (containing Hirudin and Eglin, a substance with antiinflammatoris properties) Hirudin, stating that its local use is indicated in bruises with or without hematoma.

In the present study, symptoms of bruises (pain, flare, and caumesthesia) were effectively cured by local application of a Hirudo medicinalis extract-containing cream. Change in symptoms was significantly better at each visit in the active group than in the placebo (veihicle) group.

In conclusion rapid onset of action and good tolerability are important properties of systemic or locally applied drugs to treat acute musculoskeletal injuries. Results of this study suggest that Hirudo medicinalis extract is an effective local treatment in patients with mild to moderate bruises.

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