Randomized, double-blind, placebo-controlled, interventional phase IV investigation to assess the efficacy and safety of r-hirudin gel (1120I.U) in patients with hematomas

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Abstract
Background: Hirudin is the most potent direct thrombin inhibitor, and recombinant forms are routinely used in anticoagulation therapy. Recombinant hirudin gels are commercially available for the treatment of hematomas and associated symptoms.

Objectives: To assess the efficacy and safety of a topically administered recombinant hirudin gel in patients with hematomas.

Patients/Methods: This double-blind, placebo-controlled, phase IV investigation recruited patients presenting with at least one hematoma. Subjects were randomly assigned (1:1) recombinant hirudin gel (1120 IU/100 g) or a placebo, administered 2-3 times daily for 16 days. Changes in hematoma size, flare, and the proportion of patients achieving complete resolution of hematomas and associated edemas were investigated.

Results: By study end, a greater proportion of subjects in the treatment group achieved a complete resolution of hematomas versus placebo (98.0% vs 71.9%; \( P < .001 \)) and edemas (99% vs 50%; \( P < .001 \)). Patients in the recombinant hirudin group exhibited a marginally larger, yet significant, reduction in mean hematoma size versus placebo (99.9% vs 96.6%; \( P < .001 \)) and flare (93.6% vs 78.6%; \( P < .001 \)). Median time to hematoma resolution for the recombinant hirudin and placebo administered cohorts was 8 and 16 days, respectively (\( P < .001 \)). No adverse events were reported for the recombinant hirudin cohort.

Conclusions: Topical recombinant hirudin is an effective, safe, and well tolerated intervention for the symptomatic treatment of hematomas. This trial was registered at www.clinicaltrials.gov as NCT01960569.

KEYWORDS
administration, antithrombin, hematoma, hirudins, topical, trauma

Essentials
- Efficacy and safety data on recombinant hirudin gels for the treatment of hematomas is limited.
- We assessed the clinical efficacy of a topical r-hirudin gel in 199 patients with hematomas.
- Treated patients exhibited significant reductions in hematoma size and flare within 16 days.
- r-hirudin gel treatment induces a complete resolution of hematomas and associated edema in 98%, and 99% of patients, respectively.
1 | INTRODUCTION

Hirudin is the most potent naturally-occurring direct thrombin inhibitor (DTI), and the first parenteral anticoagulant used on humans. Originally derived from the medicinal leech (Hirudo medicinalis), it consists of a 65 amino acids polypeptide chain, forming non-covalent, equimolar, non-reversible 1:1 complexes with \( \alpha \) thrombin. When hirudin-bound, thrombin-catalyzed reactions and fibrinogen clotting are blocked, and coagulation is subsequently inhibited. Hirudin was previously produced in limited amounts, however, recombinant DNA technology allowed its mass production. These recombinant forms bind bivalent to thrombin with pharmacokinetic and anticoagulant profile similar to that of the native form.

Hematoma, a localized mass of extravagated blood that is relatively or completely confined within an organ or tissue, is a common result of physical trauma. Topical applications are frequently used to treat subdermal hematomas and accompanying symptoms, and include anti-inflammatory and antioedematous treatments like heparin and heparinoid gels and creams.

Heparins have proven to be an effective topical treatment for patients with hematomas due to blunt injuries. However, \( \alpha \)-hirudin proved to be more effective than heparin in percutaneous penetration following topical application due to its relatively small size. Percutaneous penetration of hirudin was demonstrated in pigs and guinea pigs. Furthermore, the thrombolytic effect of topically administered \( \alpha \)-hirudin was shown in a rabbit model for thrombosis. There are several other benefits to the alternative use of hirudin; it does not require a cofactor to produce a coagulant effect, and it does not induce platelet activation. Unlike heparin, hirudin cannot be bound and inactivated by platelet factors and other substances, and its small size also allows it to block thrombus-bound thrombin which is inaccessible to heparin-antithrombin complexes.

Studies assessing the clinical effectiveness of topical \( \alpha \)-hirudin in treatment of hematomas are scarce. The extent to which \( \alpha \)-hirudin facilitates the resolution of hematomas and alleviates hematoma-associated clinical symptoms requires further investigation. This randomized, double-blind, placebo-controlled investigation was carried out to assess the efficacy and safety of topical yeast-derived \( \alpha \)-hirudin in the treatment of all types of superficial hematomas and associated symptoms.

2 | MATERIALS AND METHODS

2.1 | Study design

This single-center, randomized, double-blind, placebo-controlled phase IV investigation (www.clinicaltrials.gov identifier: NCT01960569) was scheduled to recruit patients presenting to the Mansoura University Orthopedic Surgery Department with any type of hematoma. In order to detect a reduction in hematoma size with a two-sided 5% significance level and a power of 80%, an estimated sample size of 100 patients per group (200 total) was necessary, considering an anticipated dropout rate of 10%. Enrollment was open for 1 month, and the first and last patients were recruited on December 2014 and January 2015. The experimental protocol was approved by the local ethics committee/institutional review board–based on all applicable local laws and regulations and the principles established by the 18th World Medical Assembly (Helsinki, 1964)—prior to study commencement. All patients gave written informed consent.

The study consisted of a total of four visits. The primary and co-investigators determined subject eligibility during the screening visit (defined as day 1). Eligible patients were randomized, using a simple randomization scheme (1:1), to receive either hirudin gel (Hirudo medicinalis extract 1120 IU/100 g, Thrombexx, Minapharm Pharmaceutical, Heliopolis, CAI, Egypt) or placebo. All randomization procedures were conducted by the study monitors. Our investigation adopted a double-blind design to limit bias; only the study monitors and statistician were aware of treatment allocation. During visits 2-4 (days 4, 8, and 16) patients were provided with their respective intervention in the dose prescribed, and target parameters were assessed.

2.2 | Inclusion and exclusion criteria

The study population comprised male and female adult patients (20–60 years of age) presenting with any type of superficial hematoma. Patients were excluded if they presented with an infection or wound requiring hospitalization or a surgical procedure, had a history of allergy towards \( \alpha \)-hirudin or any component of the \( \alpha \)-hirudin gel, had coagulation disorders (e.g., hemophilia), or were receiving additional anticoagulants (warfarin and/or acetylsalicylic acid) or digestive enzymes such as \( \alpha \) chemotrypsin, during the study period.

2.3 | Treatments

Study medications and the placebo were packaged in tubes of identical appearance. These tubes were subsequently numbered for each patient according to the randomization schedule. Patients were instructed to apply 2-3 cm of \( \alpha \)-hirudin gel 2-3 times daily, for a total of 32-48 administrations throughout the 16-day study period. Due to ethical considerations, treatment with concomitant analgesics/NSAIDs was permitted, when required. Diclofenac sodium and paracetamol were prescribed for patients needing additional treatment.

2.4 | Study outcomes

The primary endpoints were assessed during each study visit, and included: change in hematoma size (cm²), calculated utilizing the longest two intersecting lines, measured using a ruler; The color of hematomas (the following color grades were utilized: A = Bluish red, B = Blue, and C = Faint); the number of patients achieving a complete resolution of hematomas (hematoma status); time taken for patients to achieve a complete resolution of hematomas (days); the change in flare (pain intensity), assessed by means of a 10 cm visual assessment scale (VAS), where 0 represented a complete absence of pain, and 10 signified the worst possible pain; and the number of
patients achieving a complete resolution of hematoma associated edemas (edema status).

The secondary endpoint was to assess the safety of topical r-hirudin gel. Safety analysis included assessing the incidence and frequency of adverse and serious adverse events (AE and SAE, respectively). Any abnormal reaction, side effect, intercurrent disease or unexpected event or abnormal laboratory finding that occurred during the course of the clinical trial, whether or not considered therapy medication related, was defined as an AE. These fell into one of several categories, including mild, moderate, or severe, and their relationship to the trial medications was determined by the investigator. Any untoward medical occurrence that, following the administration of any dose of the drug, resulted in death, was life threatening, required inpatient hospitalization or caused a prolongation of an existing hospitalization was defined as a SAE.

2.5 | Statistical analysis

The safety population included all randomized patients who had signed an informed consent form. The intent to treat (ITT) population consisted of all patients who had met the study’s eligibility criteria, and received one or more dose/s of the study medication. The per-protocol (PP) or modified intent to treat (mit) population comprised all treated patients who had completed all study visits without major protocol violations and was used only in the analysis of the end of the study visit results as five patients in the placebo group were lost to follow up at this visit (visit 4) (Figure 1). Descriptive analysis was carried out for the ITT population.

Descriptive summary statistics were provided for quantitative data, and were summarized using count, mean with 95% confidence interval (CI), standard deviation (SD), median, minimum, and maximum. The frequency, percentage and 95% CI were applied for qualitative categorical variables. The Mann-Whitney test was used to compare changes in quantitative variables between the two arms. Wilcoxin signed-rank and Friedman tests were employed to estimate the paired changes in quantitative variables throughout the study. Chi-square and Fisher’s exact tests were utilized to compare the independent change in qualitative variables between the two arms. McNemar-Bowker and Cochran’s Q tests were used to estimate the paired changes in qualitative variables throughout the study. Any relative difference in the time to complete resolution was assessed using the Kaplan-Meier estimator. All statistical tests were performed at a two tailed 5% level of significance, using SPSS version 18 (IBM, Chicago, IL, USA).

3 | RESULTS

3.1 | Patient status and characteristics

A flow diagram of the current study is shown in Figure 1. The study population comprised 200 patients, diagnosed with at least one subdermal hematoma. One patient committed a major protocol violation (patient was below the specified inclusion age range; 20-60 years). The remaining 199 patients, presenting with 200 hematomas (one patient had two hematomas) were subsequently randomized: 49.5% of cases (n = 99) were allocated to the r-hirudin treatment group (arm 1), and 50.5% of cases (n = 101) in the placebo group (arm 2). Both

![Figure 1](image-url)
sets of patients encompass the ITT population, and were included in the demographics and efficacy analysis. Five patients from the placebo arm were lost to follow-up at visit 4 (day 16). The remaining 195 patients comprised the PP study population (Figure 1).

Patient characteristics of the ITT population are summarized in Table 1. For the treatment arm of the study, 48.5% of subjects were female (n = 48) and the mean age of patients was 35.7 ± 12.3 years. As for the placebo arm, 32.7% of patients were female (n = 33), and the mean age of constituents was 34.1 ± 11.9 years. While there were no significant age differences between the two arms of the study, a significant gender difference was observed (p < 0.05). No significant differences in the position of musculoskeletal injuries were observed, with the majority of participants presenting with lower limb trauma (r-hirudin arm: n = 78, 78.8% vs. placebo arm: n = 87, 86.1%; Table 1). Moreover, no significant inter-arm differences, in regards to the color of hematomas and the concomitantly prescribed analgesic, were noted. However, significant differences in mean hematoma size and mean pain intensity were observed at baseline; the r-hirudin treated

| Comparative Factors | r-Hirudin | Placebo |
|---------------------|----------|---------|
|                     | Mean/Count ±SD/% | Mean/Count ± SD/% | P-value |
| **Patient demographics** | | | |
| Age (years) | 35.7 ±12.3 | 34.1 ±11.9 | .376 |
| Gender | | | |
| Male | 51 | 51.5% | 68 | 67.3% | .023 |
| Female | 48 | 48.5% | 33 | 32.7% | |
| **Position of trauma** | | | |
| Longitudinal plane | | | |
| Upper limb | 21 | 21.2% | 14 | 13.9% | .171 |
| Lower limb | 78 | 78.8% | 87 | 86.1% | |
| Horizontal plane | | | |
| Right side | 50 | 50.5% | 49 | 48.5% | .888 |
| Left side | 49 | 49.5% | 52 | 51.5% | |
| **Anatomical location** | | | |
| Ankle | 43 | 43.4% | 52 | 51.5% | .750 |
| Foot | 24 | 24.2% | 24 | 23.8% | |
| Hand | 11 | 11.1% | 8 | 7.9% | |
| Knee | 9 | 9.1% | 7 | 6.9% | |
| Forearm | 4 | 4% | 1 | 1% | |
| Shoulder | 3 | 3% | 1 | 1% | |
| Elbow | 2 | 2% | 3 | 3% | |
| Leg | 2 | 2% | 4 | 4% | |
| Arm | 1 | 1% | 1 | 1% | |
| **Concomitant analgesic prescribed** | | | |
| Paracetamol | 5 | 5.1% | 3 | 3% | .551 |
| Diclofenac sodium | 80 | 80.8% | 79 | 78.2% | |
| None prescribed | 14 | 14.1% | 19 | 18.8% | |
| **Hematoma characteristics (Baseline)** | | | |
| Size (cm²) | 31.82 ±38.55 | 20.32 ±12.39 | .019 |
| Edema status | | | |
| Present | 99 | 100% | 101 | 100% | .000 |
| Absent | 0 | 0% | 0 | 0% | |
| Pain intensity (VAS) | 6.88 ±1.59 | 7.43 ±1.47 | .008 |
| Color grade | | | |
| Bluish red | 23 | 23.2% | 16 | 15.8% | .292 |
| Blue | 5 | 5.1% | 3 | 3% | |
| Faint | 71 | 71.7% | 82 | 81.2% | |

P, probability; r, recombinant; SD, standard deviation; VAS, visual assessment scale.

**TABLE 1** Baseline characteristics of study participants
cohort presented with significantly larger hematomas (r-hirudin: 31.82 ± 38.55 cm² vs placebo: 20.32 ± 12.39 cm²; P < .05), whilst the placebo group reported a higher mean pain intensity (r-hirudin: 6.88 ± 0.16 cm vs placebo: 7.43 ± 0.15 cm; P < .01; Table 1). Although there was a statistical significant difference in pain intensity between the two groups, it was of no clinical significance. For the aforementioned variables, the change from baseline values was used to determine efficacy.

3.2 | Change in hematoma size

A significant baseline-to-end of study reduction in mean hematoma size was observed in the r-hirudin cohort (P < .001). While a significant decrease was also observed in the placebo group (P < .001), it was less pronounced; significant inter-group differences were noted. Reductions in hematoma size were initially observed on day 4, with r-hirudin administered patients exhibiting a significantly larger reduction in mean hematoma size (r-hirudin: 87.0 ± 20.9% vs placebo: 52.8 ± 52.0%; P < .001; Figure 2A). r-hirudin treated patients continued to exhibit significantly larger declines in hematoma size at day 8 (r-hirudin: 99.0 ± 4.5% vs placebo: 82.9 ± 30.8%; P < .001; Figure 2A), and at the end of study (r-hirudin: 99.9 ± 0.6% vs placebo: 96.6 ± 7.3%, P < .001; Figure 2A).

3.3 | Change in hematoma color

Inter-arm differences in hematoma color were initially observed at day 4, and a statistically higher number of patients receiving r-hirudin presented with faint hematomas (r-hirudin: n = 76, 76.8% vs placebo: n = 61, 60.4%; P < .05; Figure 2B). These inter-group differences remained significant at day 8, with 96% (n = 95) and 62.4% (n = 63) of r-hirudin and placebo administered patients exhibiting faint hematomas, respectively (P < .001; Figure 2B). By study end, a significantly higher number of r-hirudin administered patients presented with faint hematomas, when compared to control (r-hirudin: n = 98, 99% vs placebo: n = 69, 71.9%; P < .001; Figure 2B). Overall, significant baseline-to-end of study changes in hematoma color were observed for r-hirudin (P < .001) as well as placebo administered subjects (P < .001).

3.4 | Resolution of hematomas

Significant inter-group differences in the number of subjects who had achieved complete hematoma resolution were observed throughout the treatment period, with a statistically higher number of patients in the r-hirudin arm reaching this therapeutic target. This difference favoring the treatment arm was initially observed at day 4, with a higher proportion of r-hirudin administered with resolved hematomas (r-hirudin: n = 42, 42.4% vs placebo: n = 13, 12.9%; P < .001; Figure 2C). By day 8, the majority of patients in the r-hirudin arm achieved resolution (r-hirudin: n = 93, 93.9%, placebo: n = 39, 38.6%; P < .001; Figure 2C), and inter-group differences remained significant until the final visit (r-hirudin: n = 97, 98% vs placebo: n = 69, 71.9%; P < .001; Figure 2C). While the number of patients achieving this therapeutic target was distinctly higher for the treatment arm, significant baseline-to-end of study changes in hematoma status were observed in both groups (r-hirudin: P < .001 vs placebo: P < .001).

3.5 | Time to complete resolution of hematomas

Median time to resolution for r-hirudin administered patients was significantly lower in the treatment arm (r-hirudin: 8 days vs placebo: 16 days; P < .001). At day 4, 42.4% (n = 42) of r-hirudin administered patients achieved their target, whilst only 12.9% (n = 13) did so in the placebo arm. By day 8, 93.9% (n = 93) of r-hirudin administered patients achieved complete resolution, compared to 38.6% (n = 39) for the placebo arm. At the end of study (day 16), 98% (n = 97) and 71.9% (n = 69) of r-hirudin and placebo administered patients, respectively, achieved complete hematoma resolution.

**FIGURE 2** The effect of topical r-hirudin on hematomas: (A) mean hematoma size. (B) hematoma color. (C) proportion of patients without complete resolution of hematoma. Arrows show the percent decrease relative to baseline. *P < .05; **P < .001; NS, P > .05.
3.6 | Change in pain intensity (improvement of flare)

$r$-hirudin administered patients exhibited a significant baseline-to-end of study reduction in mean VAS scores ($P < .001$). While placebo administered patients also displayed significant reductions ($P < .001$), the decline observed in the treatment group was significantly larger throughout the study. Reductions in VAS scores were initially observed at day 4 with $r$-hirudin administered patients exhibiting significantly higher reductions ($r$-hirudin: 43.5 ± 16.9% vs. placebo: 30.4 ± 17.3%; $P < .001$; Figure 3A). Further decline in mean VAS scores was observed during day 8 ($r$-hirudin: 70.9 ± 16.3% vs. placebo: 54.7 ± 21.5%; Figure 3A) and at the end of study ($r$-hirudin: 93.6 ± 12.4% vs. placebo: 78.6 ± 21.6%; Figure 3A). The difference favoring the $r$-hirudin cohort of patients was statistically significant during both visits (day 8: $P < .001$; day 16: $P < .001$).

3.7 | Resolution of hematoma-associated edemas

A statistically significant number of $r$-hirudin administered patients achieved complete resolution of edemas by study end ($P < .001$). While a significant change in edema status was also observed in the control group, the proportion of patients with resolved hematomas was significantly larger in the $r$-hirudin cohort. Resolution of edemas was initially observed at day 4, with a significantly larger number of patients in the $r$-hirudin arm achieving this therapeutic goal ($r$-hirudin: $n = 30$, 30.3% vs placebo: $n = 11$, 10.9%; $P < .01$; Figure 3B). This trend was consistent throughout the study, with a statistically higher number of treatment administered subjects achieving edema resolution at day 8 ($r$-hirudin: $n = 92$, 92.9% vs placebo: $n = 34$, 33.7%; $P < .001$; Figure 3B) and 16 ($r$-hirudin: $n = 98$, 99% vs. placebo: $n = 48$, 50%; $P < .001$; Figure 3B).

3.8 | Safety and tolerability

Data from safety population ($n = 200$) was included in this analysis. The overall treatment exposure was 16 days. None of the patients in either arm exhibited any AEs, including allergic cutaneous reactions or local skin events. Furthermore, no SAEs occurred during the study and/or follow-up period. Hirudin gel (1120 IU/100 g), applied 2-3 times daily, was well tolerated.

4 | DISCUSSION

Treatments of hematomas include the use of analgesics, anti-inflammatory, and in some cases, anticoagulants. The use of topical anticoagulants such as heparin gels has been comprehensively investigated, and the safety, tolerability, as well as the symptom reducing effects for superficial vein thrombosis. However, some studies have demonstrated a limited systemic anticoagulant effect of topically administrated heparin, which is due to the large molecular weight of heparin, its negative charge, and hydrophilic nature.

In contrast, hirudin binds directly to thrombin without a co-factor to exert its anticoagulant effect. Moreover, hirudin is highly specific, and acts on both soluble and fibrin-bound thrombin to produce a sustained thrombolytic effect that lasts beyond its plasma clearance. $r$-hirudin is relatively small when compared to heparin, and thus has the ability to percutaneous penetration following topical application. The antithrombotic effect of topically administrated $r$-hirudin has been clinically assessed in patients with extravasations caused by internal fistula during maintaining-blood purification treatment, a curative effect was observed following a 7-day hirudin cream treatment, with or without ultrashort wave therapy and low-frequency magnetic therapy. Moreover, in an investigation by Stamenova et al. (2001), local application of a $Hirudo medicinalis$ extract-containing cream produced a significant and rapid alleviation of pain in patients affected with bruises, with or without hematomas.

The aim of our study was to assess the efficacy and safety of $r$-hirudin gel in patients with hematomas. $r$-hirudin administered-patients demonstrated significantly larger reductions in mean hematoma size when compared to their placebo administered counterparts at the study end. Moreover, topical treatment with $r$-hirudin resulted in a more pronounced improvement in flare, a finding that is consistent with previous reports. A significantly higher number of subjects in the $r$-hirudin cohort exhibited faint bruises following allocation of treatment, and a larger proportion of these patients achieved complete resolution of hematomas and edemas by end of study. These improvements were observed as early as day 4 with $r$-hirudin. $r$-hirudin gel showed a faster resolution of hematomas, with inter-arm comparisons revealing a statistically shorter median time in the treatment arm.

The study duration (16 days) was sufficient to evaluate the safety of topically applied $r$-hirudin. $r$-hirudin gel was well tolerated, and no
local skin reactions or mild irritations were reported. Our findings are consistent with safety reports from previous investigations, including one study where a hirudin-containing ointment was administered for 4.5 months. One disadvantage of hirudin is that it is highly immunogenic when taken intravenously, thus topical administration limits systemic exposure of r-hirudin. However, some studies showed that r-hirudin has weak immunogenicity.

There are several limitations faced our current investigation. Although the study was a double-blind, there was a baseline disparity between the two arms. In addition to the significant differences in gender composition for the two arms, r-hirudin administered patients also had, on average, larger hematomas when compared to placebo. These initial dissimilarities may play a role in the observed inter-arm differences for these variables. Furthermore, due to ethical concerns, the use of a concomitant analgesic was permitted, if needed by patients. The effect of topical hirudin administration on pain intensity is likely to have been confounded by the use of these analgesics.

In our study the majority of patients presented with hematomas caused by musculoskeletal injuries. The effect of topically applied r-hirudin in the treatment of other types of hematomas requires further investigation. For instance, patients undergoing plastic surgery may develop secondary complications requiring surgical intervention. Furthermore, topical anticoagulants can be used to treat burn injuries. While some studies have demonstrated the efficacy of topically applied heparin for the treatment of such injuries, the effect of topical r-hirudin for the treatment of burn injuries is less understood. Moreover, prosthetic devices or non-biological surfaces coming into contact with blood are subject to thrombosis. For instance, patients with vein catheters can develop superficial venous thrombosis (SVT). Studies have shown that the prophylactic administration of topical low-molecular-weight heparin may reduce the prevalence of SVT. Furthermore, Minar et al. have demonstrated that the prophylactic administration of r-hirudin ointment was effective in preventing a shunt thrombosis in patients undergoing a shunt thrombectomy while on maintenance heparin.

While the use of parenteral r-hirudin has been extensively investigated, the number of studies assessing the efficacy and safety of topical r-hirudin are limited. Findings from the current study suggest that topical 1120 IU/100 g r-hirudin gel is an effective approach for the symptomatic treatment of hematomas.

**AUTHOR CONTRIBUTIONS**

All listed authors contributed to study design and manuscript review. H. El-Mowafi: Principal investigator, responsible for all the study procedures. A. El Araby: Sub investigator, patient screening and CRF filling. Y. Kandil: Co investigator, data collection and patient follow-up. A. Zaghloul: Co investigator, patient education and drug dispensing.

**RELATIONSHIP DISCLOSURE**

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**REFERENCES**

1. Greinacher A, Warkentin TE. The direct thrombin inhibitor hirudin. Thromb Haemost. 2008;99:819–29.
2. Markwardt F. Hirudin as alternative anticoagulant–a historical review. Semin Thromb Hemost. 2002;28:405–14.
3. Markwardt F. Hirudin as an inhibitor of thrombin. Methods Enzymol. 1970;19:924–32.
4. García DA, Baglin TP, Weitz JI, Samama MM. Parenteral coagulants: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141:e245–43S.
5. Capper C. The language of forensic medicine. The meaning of some terms employed. Med Sci Law. 2001;41:256–9.
6. Vecchio C, Frisinghelli A. Topically applied heparins for the treatment of vascular disorders. Clin Drug Invest. 2008;28:603–14.
7. Weitz JI, Hudoba M, Massel D, Maraganore J, Hirsh J. Clot-bound thrombin is protected from inhibition by heparin-antithrombin III but is susceptible to inactivation by antithrombin III-independent inhibitors. J Clin Invest. 1990;86:385–91.
8. Serruys PW, Herman JP, Simon R, et al. Comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. N Engl J Med. 1995;333:757–63.
9. Stamenova PK, Marchetti T, Simeonov I. Efficacy and safety of topical hirudin (Hirudex®): a double-blind, placebo-controlled study. Eur Rev Med Pharmacol Sci. 2001;5:37–42.
10. Pirkle H. Hemostasis and animal venoms (hematology). New York: CRC Press; 1988.
11. Fu K, Izquierdo R, Walenga JM, Fareed J. Comparative study on the use of anticoagulants heparin and recombinant hirudin in a rabbit traumatic anastomosis model. Thromb Res. 1995;78:421–8.
12. Belcaro G, Cesarone MR, Rugoli A, et al. Topical formulation of heparin is effective in reducing the symptoms of superficial venous thrombosis: a monocenter, observer-blind, placebo-controlled randomized study. Panminerva Med. 2011;53:3–11.
13. Katzenschlager R, Hirschl M, Minar E, Ugurluoglu A, Liposomal heparin-spray gel in comparison with subcutaneous low molecular weight heparin in patients with superficial venous thrombosis. A randomized, controlled, open multicentre study. Austrian. J Cardiology. 2003;10:375–8.
14. Górski G, Szopiński P, Michalak J, et al. Liposomal heparin spray: a new formula in adjunctive treatment of superficial venous thrombosis. Angiology. 2005;56:9–17.
15. Bichler J, Fritz H, Hirudin, a new therapeutic tool? Ann Hematol. 1991;63:67–76.
16. Bates SM, Weitz JI. The mechanism of action of thrombin inhibitors. J Invasive Cardiol. 2000;12:27F–32F.
17. Agnelli G, Renga A, Weitz JI, Nenci GG, Hirsh J. Sustained antithrombotic activity of hirudin after its plasma clearance: comparison with heparin. Blood. 1992;80:960–5.
18. Stringer KA. Hirudins: antithrombin anticoagulants. Ann Pharmacother. 1992;26:1535–40.
19. Zhang L, Huang B, Ding T. Curative effects of physical methods combined with hirudin cream on arteriovenous fistula. Journal of Kunming Medical University. 2014;35:156–7.
20. Minar E, Zagornik J. Local hirudin application–an aid in preventing occlusion of an arteriovenous fistula in dialysis patients? Klin Wochenschr. 1985;63:190–1.
21. Eichler P, Friesen HJ, Lubenow N, et al. Antihirudin antibodies in patients with heparin-induced thrombocytopenia treated with lepirudin: incidence, effects on aPTT, and clinical relevance. Blood. 2000;96:2373–8.

22. Spinner S, Stöffler G, Fink E. Quantitative enzyme-linked immunosorbent assay (ELISA) for hirudin. J Immunol Methods. 1986;87:79–83.

23. Verstraete M, Hoet B, Tornai I, Stockmans P, Arnout J. Hirudin, a specific thrombin inhibitor: pharmacokinetics and hemostatic effects in man. Circulation. 1989;80:421.

24. Byrd HS, Barton FE, Orenstein HH, et al. Safety and efficacy in an accredited outpatient plastic surgery facility: a review of 5316 consecutive cases. Plast Reconstr Surg. 2003;112:636–41.

25. Venakatachalapathy TS, Mohan Kumar S, Saliba MJ. A comparative study of burns treated with topical heparin and without heparin. Ann Burns Fire Disasters. 2007;20:189.

26. Agbenorku P, Fugar S, Akipaloo J, Hoyte-Williams PE, Alhassan Z, Agyei F. Management of severe burn injuries with topical heparin: the first evidence-based study in Ghana. Int J Burns Trauma. 2013;3:30.

27. Mason RG, Shermer RW, Rodman NF. Reactions of blood with nonbiologic surfaces. Ultrastructural and clotting studies with normal and coagulation factor deficient bloods. Am J Pathol. 1972;69:271–88.

28. Litzendorf ME, Satiani B. Superficial venous thrombosis: disease progression and evolving treatment approaches. Vasc Health Risk Manag. 2011;7:569.

29. Cesarone MR, Belcaro G, Agus G, et al. Management of superficial vein thrombosis and thrombophlebitis: status and expert opinion document. Angiology. 2007;58:75–145.

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