Orally Administered Unfractionated Heparin With Carrier Agent Is Therapeutic for Deep Venous Thrombosis

Mark D. Gonze, MD; Khashayar Salartash, MD; W. Charles Sternbergh III, MD; Robert A. Baughman, PhD; Andrea Leone-Bay, PhD; Samuel R. Money, MD

Background—Orally administered heparin (OHEP) is unreliable because of poor absorption. Sodium N-(8[2-hydroxybenzoyl]amino) caprylate (SNAC) is an amido acid that facilitates the gastrointestinal absorption of heparin. We evaluated the effectiveness of OHEP combined with SNAC (OHEP/SNAC) in the treatment of deep-vein thrombosis (DVT).

Methods and Results—An internal jugular DVT was produced in 54 male Sprague-Dawley rats. Animals were assigned to 6 different groups for 7 days of treatment: untreated control, subcutaneous heparin (SC HEP) (300 U/kg SC TID), SNAC only (300 mg/kg PO TID), OHEP only (30 mg/kg PO TID), low-molecular-weight heparin (LMWH) (enoxaparin 5 mg/kg SC QD), and OHEP/SNAC (30 mg/kg:300 mg/kg PO TID). The activated partial thromboplastin time (aPTT) and anti–factor X (anti-Xa) levels were measured. The incidence of residual DVT after 1 week of treatment was 100% (9 of 9) in the control group versus 10% (1 of 10) in the OHEP/SNAC and 10% (1 of 10) in the LMWH groups (P<0.001). There was also a significant reduction in clot weights between these groups. Compared with controls, there were no significant differences in the residual DVT in the SNAC-only (6 of 6), OHEP-only (9 of 9), or SC HEP (8 of 10) groups. Combination OHEP/SNAC was as effective in the resolution of the clot and reducing clot weight as LMWH. The aPTT levels in the OHEP/SNAC group peaked at 30 minutes and were significantly higher than in all other groups (P<0.01). Anti-Xa levels were elevated at 15 minutes after dosing in the OHEP/SNAC group and remained significantly elevated at 4 hours (P<0.001).

Conclusions—OHEP combined with a novel carrier agent (SNAC) successfully treated DVT in this rat model.

Key Words: thrombosis ▪ veins ▪ heparin

Deep-vein thrombosis (DVT) is a major cause of death and morbidity in hospitalized patients. The traditional treatment of a DVT involves initial therapy with continuous, intravenous (unfractionated) heparin. Because long-term use of continuous intravenous heparin is not practical, this is done with oral agents (warfarin). Unfortunately, individual patient response to warfarin therapy varies, and the dosage must be monitored closely for the duration of treatment.

An orally administered heparin (OHEP) would have several advantages over warfarin therapy. Heparin does not have teratogenic effects, and its half-life of <4 hours allows reversal of anticoagulation if necessary. Also, the pain associated with intravenous or subcutaneous routes of administration would be avoided.1 For all of these reasons, OHEP would facilitate DVT treatment in an outpatient setting.

Because of its large size and anionic structure, heparin is not reliably absorbed when taken orally. Sodium N-(8[2-hydroxybenzoyl]amino) caprylate, or SNAC (Emisphere Technologies), is an amido acid compound that facilitates the gastrointestinal absorption of heparin. Previous work has shown that the OHEP/SNAC combination is effective at reducing the incidence of DVT in a rat model of venous thrombosis.2 This present study was undertaken to evaluate the effects of orally administered OHEP/SNAC in treating an established DVT in a standard rat model. In addition, the effect of combination OHEP/SNAC on activated partial prothrombin times (aPTTs) was evaluated.

Methods

Male Sprague-Dawley rats (250 to 300 g) were provided standard rat chow and water ad libitum. The animals were maintained in accordance with the recommendations of the Guidelines for the Care and Use of Laboratory Animals. This project was approved by the Institutional Animal Care and Use Committee.

All animals were anesthetized with an intraperitoneal injection of ketamine (72 mg/kg) and acepromazine (3 mg/kg). A standard rat model of venous thrombosis3 was used in 54 rats. The skin over the
right neck was prepped with 70% alcohol, and a transverse cervical incision was made. The internal jugular vein was identified, and a 2-cm segment was isolated with 4-0 silk sutures. All tributaries draining into the vein were alsoatraumatically controlled. After traction was placed on the sutures to produce venous stasis, the internal jugular vein was bathed in a sclerosant mixture of 10% formalin and absolute ethanol for 2 minutes. After this time, the sclerosant mixture was removed, flow restored within the jugular vein, and the incision closed. After 120 minutes, the neck incision was reexplored, and the presence or absence of thrombus was noted. Thrombus was consistently located within the 2-cm segment of the internal jugular vein. A species-specific dose-response curve was performed by Emisphere Technologies to determine the appropriate combination of OHEP and SNAC (unpublished data) to reach a therapeutic elevation in the anti-Xa levels.

If a thrombus was present, the animal was randomly assigned into 1 of 6 different treatment groups: group 1, untreated control; group 2, subcutaneous heparin (SC HEP) (300 U/kg SC TID); group 3, SNAC only (300 mg/kg PO TID); group 4, OHEP only (30 mg/kg PO TID); group 5, low-molecular-weight heparin (LMWH) (enoxaparin 5 mg/kg SC QD); and group 6, combination OHEP/SNAC (30 mg/kg:300 mg/kg PO TID). Oral gavage was performed with a Rusch catheter passed down the esophagus 10 cm from the incisors. The solution was then slowly expressed into the stomach. After 7 days of treatment, the animals were reanesthetized. In a blinded fashion, the previous incision was reopened and the jugular vein inspected. Under an operating microscope, the presence of intraluminal thrombus was determined. Next, the 2-cm segment of jugular vein was excised, and the thrombus was extracted and weighed.

The second phase of this experiment evaluated the effects of combination heparin/SNAC (30 mg/kg:300 mg/kg), SNAC only (300 mg/kg PO), and OHEP only (30 mg/kg PO) on the activated partial thromboplastin time (aPTT) and anti-factor Xa. This was performed on 30 animals equally divided among the 3 groups. Venous blood samples were obtained at serial time points after a single dosing for aPTT and anti-Xa levels. The aPTT was determined in the citrated samples with a BBL Fibrometer (VWR Scientific) (aPTT reagents purchased from Sigma Diagnostics). Anti–factor Xa levels were determined in plasma with a colorimetric quantitative assay (Chromogenix). Statistical analysis for the incidence of thrombus was performed with χ² analysis with Yates’ correction. The weight of the thrombus (mg), aPTT (seconds), and anti-Xa (IU/mL) are expressed as mean ± SEM. Statistical analysis was performed with ANOVA and Student’s unpaired t test. A value of P<0.05 was considered significant.

**Results**

After 7 days of treatment, there was a 100% incidence of residual DVT in the saline gavage control group (9 of 9), the SNAC-only group (6 of 6), and the OHEP-only group (9 of 9). The SC HEP group had an 80% (8 of 10) incidence of residual thrombus, which was not significantly different from the control group. A significant resolution of the DVT was noted in the combination OHEP/SNAC group (1 of 10) and LMWH group (1 of 10) compared with controls (Figure 1).

The mean thrombus weight was 9.35±0.46 mg in the control group (Figure 1). No significant differences in mean thrombus weight were noted in the OHEP-only group (7.70±0.65 mg), the SNAC-only group (10.16±2.01 mg), or the SC HEP–only group (7.72±1.47 mg) compared with controls. Reductions in the mean clot weight were noted in the combination OHEP/SNAC (0.46±0.01 mg) and the LMWH heparin (0.53±0.01 mg) groups that were significantly less than in controls (P<0.001) but not statistically different from each other (P=NS).

After a single oral dose of combination OHEP/SNAC, the anti-Xa levels were significantly elevated at 15 minutes (1.1±0.05 IU/mL) after dosing and remained significantly elevated for as long as 4 hours (0.8±0.02 IU/mL) (P<0.0005). There was no significant elevation in anti-Xa levels detected in the OHEP-only or SNAC-only groups (Figure 2). Likewise, the aPTTs were significantly elevated as soon as 15 minutes (36.3±2.28 seconds) after dosing in the combination OHEP/SNAC group compared with predose aPTT (19.8±1.65 seconds). However, unlike the anti-Xa levels, the aPTTs values returned to baseline 1 hour after dosing (Figure 3). The aPTT did not change significantly after single dosing with SNAC only or OHEP only. Coagulation studies were not performed on all study groups because of the excessive costs of these tests.

Five animals died of the anesthesia early in the study. After consultation with the Institutional Animal Care and Utilization Committee, additional animals were not approved, because statistical significance was already achieved on the basis of the completed sample sizes. No animal in the study died as a result of bleeding complications.

**Discussion**

In this study, we demonstrated that a combination of heparin and SNAC given orally is as effective as subcutaneous LMWH in the treatment of DVT in a standard rat model. Orally administered combination heparin/SNAC not only significantly elevated the aPTT level but also elevated the anti-Xa level for a prolonged period after a single dose, in contrast with OHEP or SNAC alone.

Venous thromboembolism is recognized in ≈250 000 hospitalized patients annually.4 Unfortunately, this disease is often silent until symptoms develop, and ≈100 000 patients die each year of pulmonary embolism.5 Without therapy, there is a 50% chance of recurrent thromboembolism.6 The use of unfractionated heparin followed by 3 months of warfarin therapy successfully prevents pulmonary embolism in 95% of patients with proximal DVT.7 There is great variability in this anticoagulation response between patients; therefore, the dosage of heparin needs to be monitored by the aPTT.8 The in-hospital time required for conversion from intravenous heparin to therapeutic warfarin has been reduced.
to 5 days if oral anticoagulation is started on admission.9 Challenging this “gold-standard” treatment of DVT are reports of various low-molecular-weight heparins administered parenterally at home for the DVT treatment. These studies by Levine et al10 and Koopman et al11 show that the treatment for DVT can be moved to the outpatient setting.

Hiebert et al12 demonstrated that less than half of an orally administered dose of heparin reaches the systemic circulation. Even suprapharmacological doses of OHEP, significantly higher doses than those used in this study, did not elevate aPTT. The splanchnic bed may be responsible for sequestering the OHEP and preventing its anticoagulation effect.13 Because of its large size (12 000 to 16 000 Da) and negative charge, orally administered unfractionated heparin cannot reach therapeutic levels.14

SNAC is a small (301-Da), synthetically derived amido acid. Research has shown that SNAC binds noncovalently to heparin. The bound heparin becomes more lipophilic and can pass across the intestinal lumen into the systemic circulation. Once in the circulation, SNAC dissociates from the heparin, allowing heparin to exert its anticoagulation effect. Approximately 15% of the oral SNAC dose can be detected in the circulation until the SNAC is cleared by the kidneys. The precise molecular interaction between heparin, the gastrointestinal mucosal cells, and SNAC is not well understood.

Heparin binding to antithrombin III induces a conformational change in the antithrombin III. This change allows the complex to increase its reactivity with coagulation enzymes, particularly factors II (thrombin) and X. The anticoagulant activity of heparin is typically measured by an increase in the aPTT with the therapeutic target range of 1.5 to 2.5 times above baseline clinically.15 In our study, the oral combination of heparin and SNAC elevated aPTT significantly; however, this effect was only transient, because the aPTT levels returned to baseline after 1 hour. The anti-Xa activity was also significantly elevated above baseline with the initial dose, and this elevation persisted even 4 hours after the initial dose. An anti–factor Xa activity of 0.35 to 0.7 IU/mL correlates to an aPTT of 1.5 to 2.5 times baseline.16 Baughman et al17 evaluated several other parameters of anticoagulation in which SNAC was combined with OHEP in humans. In this study, significant elevations were found in anti-IIa, anti-Xa, tissue factor pathway inhibitor, and aPTT after a single-dose combination of oral unfractionated heparin and SNAC. Although the LMWHs are more specific for anti-Xa activity, this study and our own used unfractionated heparin. Despite being composed of a broad spectrum of molecular weights (even those in the range of the LMWH), unfractionated heparin was able to cause an elevation of anti-Xa levels. Because the combination of OHEP/SNAC does not need to be regulated on the basis of aPTT levels, serial coagulation tests would not need to be followed during therapy.

In conclusion, the combination OHEP/SNAC and the subcutaneous LMWH groups demonstrated a significant increase in both the percentage of animals with resolution of their DVT and a reduction in the mean thrombus weight compared with the untreated controls. In addition, we have...
demonstrated that combination OHEP/SNAC significantly elevated anti-Xa levels up to 4 hours after dosing. Because LMWH requires parenteral administration, the potential clinical role of OHEP/SNAC could be a more attractive and convenient alternative.

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References
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