Oral Delivery of Anticoagulant Doses of Heparin
A Randomized, Double-Blind, Controlled Study in Humans

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Background—Parenteral heparin is the anticoagulant of choice in hospitalized patients. Continued anticoagulation is achieved by subcutaneous administration of low-molecular-weight heparin or with an orally active anticoagulant such as warfarin. An oral heparin formulation would avoid the inconvenience of subcutaneous injection and the unfavorable drug interactions and adverse events associated with warfarin. A candidate delivery agent, sodium N-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC), was evaluated with escalating oral heparin doses in a randomized, double-blind, controlled clinical study for safety, tolerability, and effects on indexes of anticoagulation.

Methods and Results—Increases in activated partial thromboplastin time (aPTT), anti-factors IIa and Xa, and tissue factor pathway inhibitor (TFPI) concentrations were detected when normal volunteers were dosed with 10.5 g SNAC/20 000 IU heparin by gavage in some subjects. For the entire group, 30 000 IU SNAC and heparin elevated TFPI from 74.9±7.6 to 254.2±12.3 mg/mL (P<0.001) 1 hour after dosing (P<0.001). Similar changes occurred in anti-factor IIa and anti-factor Xa. aPTT rose from 28±0.5 to 42.2±6.3 seconds 2 hours after dosing (P<0.01). No significant changes in vital signs, physical examination, ECGs, or clinical laboratory values were observed. Neither 30 000 IU heparin alone nor 10.5 g SNAC alone altered the hemostatic parameters. Emesis was associated with 10.5 g SNAC. A taste-masked preparation of SNAC 2.25 g was administered orally with heparin 30 000 to 150 000 IU. Both aPTT and anti-factor Xa increased with escalating doses of heparin. This preparation was well tolerated.

Conclusions—Heparin, administered orally in combination with the delivery agent SNAC, produces significant elevations in 4 indexes of anticoagulant effect in healthy human volunteers. These results establish the feasibility of oral delivery of anticoagulant doses of heparin in humans and may have broader implications for the absorption of macromolecules. (Circulation. 1998;98:1610-1615.)

Key Words: heparin • anticoagulants • oral administration

Heparin, a naturally occurring glycosaminoglycan, is a potent inhibitor of blood coagulation, primarily through formation of a protease inhibitor complex with antithrombin (AT) III. Parenteral administration of heparin has been shown to prevent venous thrombosis and pulmonary embolism in patients undergoing surgery. It has also been shown to prevent peripheral arterial embolism and reduce the incidence of myocardial infarction and death in patients with unstable angina. Heparin has been suggested to improve outcome in acute ischemic stroke. Heparin is composed of glucosamine and either l-iduronic acid or N-glucuronic acid in chains of variable length, having a molecular weight range of 5000 to 30 000. It is sulfated and highly acidic. Fractionated, low-molecular-weight heparins have also established clinical efficacy. Clinical prevention of thrombosis with heparin is attained at doses that modify tests of anticoagulant function. These include the activated partial thromboplastin time (aPTT) and tissue factor pathway inhibitor (TFPI). Clinical benefit has been associated with heparin-induced prolongation of aPTT by 1.5- to 2.5-fold. Less information is available that relates alterations in TFPI to clinical outcome.

Heparin is not absorbed in the gastrointestinal tract, presumably because of its size and ionic repulsion from negatively charged epithelial tissue. As such, it has poor oral bioavailability and is administered parenterally, either by continuous or intermittent infusion or by deep subcutaneous injection. Thus, parenteral heparin is usually replaced by the orally active anticoagulant warfarin for long-term outpatient therapy. However, the specific adverse effects of warfarin and pharmacokinetic interactions involving this highly protein-bound drug may limit its practical utility. Low-molecular-weight heparins may be self-administered by patients after hospital discharge. This approach does not require intensive monitoring and has established clinical efficacy, at least over short periods. Given this observation, continuation...
of heparin therapy with an oral dosage form might prove to be a clinically attractive alternative. Although thrombin generation may persist during parenteral heparin administration, abrupt cessation of therapy may result in a rebound increase in thrombin generation and reactivation of clinical syndromes of vascular occlusion.12

Several recent attempts to develop effective nonparenteral heparin formulations have been reported but have met with limited success.13–19 We have synthesized delivery agents based on N-acylated α-amino acids20 and n-acylated non–α-amino acids21 and demonstrated in animal models that they promote oral absorption of macromolecules. N-[(2-hydroxybenzoyl)amino]caprylate (SNAC)-mediated gastrointestinal absorption of heparin occurs in a passive transcellular process,22 without causing apparent damage to intestinal epithelium.23 Transport is thought to be facilitated by the formation of a noncovalent complex between SNAC and heparin.24 Investigations, both in vitro and in vivo, revealed that the n-acylated non–α-amino acid SNAC has no pharmacological activity.25 However, it increased aPTT in primates dosed orally with heparin. Hypoglycemia, nausea, and emesis were observed at high doses (1800 mg/kg) of SNAC with an unformulated solution in cynomolgus macaques.26

We now report that SNAC facilitated the gastrointestinal absorption of heparin in healthy volunteers, at tolerated doses, with no change in glucose or insulin levels. Furthermore, 4 indexes of anticoagulant effect were prolonged into the range associated with clinical utility by the SNAC-heparin solution. These observations suggest the potential for development of an orally available heparin preparation for clinical investigation.

Methods

Study Design

Three clinical studies were performed at the Clinical Research Unit of Harris Laboratories. All studies were approved by the Institutional Review Board. In the first study, healthy male (n = 27) and surgically sterile female (n = 15) volunteers were evaluated for inclusion in the study after providing written informed consent. All had unremarkable histories and physical examinations. No abnormalities were detected on 12-lead ECG, clinical chemistry, hematology, and urinalysis (including drug screen). Subjects were selected and randomly assigned to receive ascending doses (1.4 to 10.5 g) of SNAC by gavage (group 1), 10 000 IU heparin with ascending doses (1.4 to 10.5 g) of SNAC (group 2), or 20 000 or 30 000 IU heparin with a fixed dose (10.5 g) of SNAC (group 3). Subjects randomized to a heparin treatment (group 2 or 3) were screened with 4000 IU heparin (heparin sodium injection, USP, 1000 IU/mL SC, Schein Pharmaceuticals) for an atypical heparin response (aPTT increase >2 times baseline) or untoward reaction. In the second study, 4 volunteers (3 men, 1 surgically sterile woman) were administered 70 mL dosing solution via the stomach tube. In the third study, a formulated, taste-masked, oral SNAC-heparin solution was administered. Both the study physician and the subject were blinded as to which preparation was being administered. Similarly, the analyses were performed without knowledge of patient name, sex, or treatment.

Prothrombin time (PT), aPTT, AT III, and triglycerides were determined before dosing and at 2 and 4 hours after dosing in groups 2 and 3 of study 1. Arachidonic acid-induced platelet aggregation was measured before and 2 hours after dosing as previously described.26 Blood samples were drawn 30, 15, and 5 minutes before dosing and 0.33, 0.67, 1, 1.5, 2, 4, and 8 hours after dosing. Samples taken 30 minutes before and 1, 2, and 4 hours after dosing were analyzed for TFPI. Supplementary blood and urine samples for safety determination, including hematology (complete blood count, platelets), PT, aPTT, and urinalysis (dipstick), were collected 24 hours after dosing. In the third study, aPTT was measured before and 0.5, 1, 2, 4, and 24 hours after dosing. Anti-factor Xa was determined before and 0.08, 0.17, 0.33, 0.5, 0.67, 1.0, 1.33, 1.67, 2.0, 4.0, 6.0, and 8.0 hours after dosing.

Analytical Procedures

Anti-factors IIa and Xa and TFPI were assayed in the Center for Experimental Therapeutics, University of Pennsylvania School of Medicine, except for studies performed at Kabi Diagnostics. Anti-factors IIa and Xa activity was determined with a chromogenic substrate by use of a kit from Diagnostica. The assay has a coefficient of variation of <4% at a limit of detection (LOD) of 0.1 IU/mL. Values reported <0.1 IU/mL were generated from a standard curve by use of lower concentration standards. Anti-factor IIa activity was determined with a kit from Kabi Diagnostics. The coefficient of variation is <5% at an LOD of 0.05 IU/mL. TFPI concentrations were determined by use of an ELISA (American Diagnoctic, Inc). The LOD for this assay is 0.5 ng/mL. The intra-assay coefficient of variation is <5%. A subset of samples subjected to a previous freeze/thaw cycle was also assayed for TFPI. These data, however, were considered of qualitative value only.

Data Management

All clinical data were entered into an Oracle database. All computations and statistical analyses were carried out with the statistical software package SAS version 6.09 or greater. aPTT, anti-factors IIa and Xa, and TFPI values were analyzed by ANOVA, with subse-
TABLE 1. Subject Distribution and Gastrointestinal Side Effects by Treatment Group in Study 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Period</th>
<th>Dose Level (No. of Subjects)</th>
<th>Nausea</th>
<th>Emesis</th>
</tr>
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</table>

Results

Subjects in the first study were randomized to treatment groups as listed in Table 1; dropouts and no-shows were not replaced. There were no changes between preenrollment and discharge physical examinations in all subjects. All ECG results and vital sign measurements were considered unremarkable throughout the study.

Neither heparin nor SNAC induced significant changes in serum chemistry, glucose, insulin, hematology, or urinalysis. There were no trends observed for any dosing group. The PT, AT III levels, and triglycerides remained normal throughout the study.

Measures of anticoagulant effect were unaltered by SNAC alone (group 1) or by escalating doses of SNAC with 10 000 IU heparin. Specifically, TFPI did not change significantly from levels before (69.7±12.8 ng/mL) to 2 hours (79.9±8.8 ng/mL) and 4 hours (78.7±14.2 ng/mL) after 10.5 g SNAC alone. Similarly, anti-factor IIa and Xa levels were undetectable before or after 10.5 g SNAC. Administration of 20 000 and 30 000 IU heparin with the 10.5 g SNAC altered the indexes of anticoagulant effect. aPTT increased significantly (P<0.01) from a control level of 28.0±0.5 to 42.2±6.3 seconds 2 hours after dosing (Figure 1A) in subjects receiving 30 000 IU heparin. Anti-factor IIa activity was detected in 4 of 6 volunteers after the lower dose and in all but 1 of the 6 subjects receiving 30 000 IU heparin. An anti-factor Xa response was measured in 2 of 6 subjects at 20 000 IU heparin and 4 of the 5 volunteers at 30 000 IU heparin, reaching a peak concentration of 0.20±0.05 IU/mL 1 hour after dosing. Anti-factor IIa was significantly (P<0.05) elevated at 0.67, 1, and 1.5 hours after dosing, reaching a peak concentration of 0.22±0.08 IU/mL 1 hour after 30 000 IU heparin (Figure 1B). TFPI rose from 90.0±6.3 to 224.2±66.7 ng/mL 1 hour after dosing with 20 000 IU heparin (P<0.003) and from 74.9±7.6 to 254.2±12.3 ng/mL 1 hour after the 30 000 IU heparin dose (P<0.001). Following this observation, the −0.5-, 0.33-, 0.67-, and 1.5-hour samples remaining from the anti-factor IIa analysis were submitted for TFPI assay. This involved a second freeze/thaw cycle, so the data were viewed qualitatively. These data suggest 1.5- to 2-fold increases in TFPI as early as 0.33 hours after dosing in those subjects in whom a significant increase in anti-factor IIa was detected 1 and 1.5 hours after dosing with 30 000 IU heparin.

In the second study, TFPI levels were unaltered in any of the 4 subjects when 30 000 IU heparin was administered orally via nasogastric tube but without SNAC (Table 2). Consistent with these observations, neither anti-factor IIa nor anti-factor Xa was detectable at any time point in any individual.

In the third study, 4 of 12 subjects were excluded from further dosing for safety reasons, because their aPTT response to heparin 10 000 IU SC exceeded baseline values by a factor of ≥2.5-fold. The remaining 7 subjects (6 men) received ascending doses of oral SNAC-heparin. One patient was excluded because of an aPTT elevation >2.5-fold greater
after the third oral dose. The group as a whole exhibited dose-dependent prolongations of mean aPTT and anti-factor Xa (Figure 2). Thus, despite exclusion of the volunteers likely to exhibit a more sensitive response to heparin, mean aPTT was prolonged 172±22% above baseline values 30 minutes after dosing with 2.25 g SNAC and 150 000 IU heparin. One subject (included in the mean data) failed to exhibit a prolongation in aPTT at any oral dose of SNAC-heparin. After administration of heparin 10 000 IU SC, the aPTT in this individual was 27 seconds at baseline and 33, 34, 34, 37, and 27 seconds at 0.5, 1.0, 2.0, 4.0, and 24.0 hours after dosing. After oral SNAC-heparin, a detectable prolongation of anti-factor Xa was measured in this subject only at 0.5 (0.07 IU/mL) and 1 (0.06 IU/mL) hour after dosing with 2.25 g SNAC and 150 000 IU heparin.

There were no clinically important adverse events in 111 dosings in the 34 subjects. Nausea was reported after 29 of the 111 dosing events. Excluding the vehicle and heparin control groups (16 doses), nausea appeared to be unrelated to SNAC or heparin dosages. Emesis occurred after 14 of the 111 doses and was associated with higher SNAC dose levels only in study 1 (Table 1). Emesis was self described as mild (easily tolerated) in 13 of these individuals. One experienced emesis that interfered with usual activity. Less frequent side effects in study 1 were headache, diarrhea, abdominal pain, dyspepsia, and pharyngitis. Gastrointestinal side effects were not noted in the third study. Neither alterations in blood glucose or serum insulin nor bleeding complications were observed in any of the studies.

![Figure 2. Mean dose-dependent prolongation of anti-factor Xa activity and aPTT as percent at baseline (inset) in response to taste-masked syrup containing SNAC 2.25 g and rising doses of heparin ingested per os. ● indicates 30 000 IU heparin; ▲, 60 000 IU heparin; ■, 90 000 IU heparin; and □, 150 000 IU heparin.](image-url)
Discussion

This report describes the first controlled evidence for the oral absorption of an anticoagulant dose of heparin in humans. Our initial observations were obtained after drug delivery by gavage under double-blind conditions and involved 4 indexes of anticoagulant effect. The results indicate that SNAC does promote oral absorption of heparin in humans. The evidence that links the anticoagulant effect of heparin to prevention of thrombosis holds most strongly for aPTT. The changes in aPTT observed after the single oral dose of heparin administered by gavage were modest. However, TFPI may also reflect the effects of heparin in vivo.27 The maximal effects on TFPI that we observed after oral heparin were an increase of 2.5- to 3-fold. Such increases are within the range of those reported after intravenous dosing of 7500 U heparin (1.5- to 6.5-fold)27 and similar to that observed after 5000 anti-factor Xa units of a low-molecular-weight heparin.27 However, interindividual differences in the anticoagulant response to conventional heparins are well recognized.27,28 The peak TFPI concentration we observed was at 1 hour after dosing. This is similar to the response reported by Alban et al28 in healthy male and female human volunteers. Peak values for anti-factor IIa and Xa values were also observed 1 hour after dosing. Thus, the TFPI and anti-factor IIa and Xa concentrations suggest that the timing of the aPTT samples in the first study may have led to an underestimation of the maximum response to oral heparin on this index of anticoagulant effect. Similarly, plasma levels of heparin were not estimated by protamine titration.29 Such an approach may minimize variability in aPTT responses attributable to differences in test reagents.30

Preclinical data from cynomolgus macaques suggested that SNAC at high doses may lower fasting blood sugar.22 There was no evidence, as assessed by glucose and insulin, of a hypoglycemic response to SNAC in any individual at any dosing level. However, administration of a large volume (70 mL) of this unformulated solution to fasting volunteers in the initial study did produce digestive symptoms, most commonly nausea. These accounted for more than half the reported side effects. Twelve of the 30 subjects who received SNAC in combination with an anticoagulant dose of heparin in the first study reported gastrointestinal discomfort. Of the 30 subjects, 13 experienced mild emesis and 1 reported moderate emesis at this SNAC dose level. This last event was considered dose limiting. Although tolerated, SNAC has a bitter taste as an unformulated solution.

To address the issue of gastrointestinal intolerance, we developed a taste-masked preparation. Furthermore, we reduced the dose of SNAC to 2.25 g while evaluating the pharmacodynamic efficacy of increasing doses of heparin when the preparation was administered per os rather than by gavage. Given the interindividual variability in response to heparin, we wished to bias our experience against including those individuals most sensitive to heparin, for safety reasons. Thus, we excluded 5 of the 12 volunteers whose aPTT was prolonged by ≥2.5 times in response to 10 000 IU heparin SC from exposure to SNAC-heparin. Despite this, heparin dose dependently prolonged both indexes of anticoagulation. For example, 0.5 hours after dosing with 150 000 IU SNAC and 2.25 g heparin, aPTT had been prolonged from 30.3±2.4 to 51.7±8.6 seconds. Furthermore, with the individual who failed to prolong the aPTT with any dose of SNAC-heparin excluded, aPTT was elevated from 30.7±2.3 to 55.3±7.4 seconds. It is unclear why this 1 subject failed to respond to SNAC-heparin.

This study establishes the feasibility of oral heparin delivery in humans. Both unfractionated and low-molecular-weight heparins have been demonstrated to be efficacious in the prevention of thrombotic venous and arterial disease.1,2,7-31 Furthermore, nonanticoagulant properties of heparin may also reduce cardiovascular risk.32 Long-term therapy with subcutaneous administration of low-molecular-weight heparins is being evaluated currently in comparison with long-term oral warfarin therapy in the prevention of postoperative venous thrombosis.4 Indeed, long-term subcutaneous administration of specific ATs may prove even more efficacious than low-molecular-weight heparins.33 Although further modifications of drug delivery are desirable for clinical application, the present observations raise the possibility of extended oral dosing with heparins or specific ATs.34,35 They may also have a broader relevance to the absorption of macromolecules in general.

Acknowledgments

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References


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