Oral Delivery of Heparin in Combination with Sodium *N*-[8-(2-Hydroxybenzoyl)amino]caprylate: Pharmacological Considerations

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INTRODUCTION

Heparin sodium, USP (heparin), is a heterogeneous mixture of oligosaccharides with an average molecular weight of about 20,000 Daltons (1). It is administered parenterally to hospitalized patients to prevent deep venous thrombosis (DVT) and pulmonary embolism, two common post-surgical complications (2). Oral heparin administration is ineffective because its high charge density and large molecular size preclude its absorption from the gastrointestinal tract.

Numerous efforts to develop an oral heparin formulation have been reported. Formulations using enteric-coated heparin-amine combinations, heparin complexes or salts prepared with organic acids, heparin derivatives produced by partial desulfation and methylation, mixed micelles, oil/water emulsions, and absorption enhancers such as EDTA have been described (3). Dosage forms based on hydrophobic organic bases (4,5), spermine and lysine salts (6), liposomes (7,9), hydrogel nanospheres (9), or bile salts (10,11) have also been investigated. These approaches have been largely unsuccessful. Consequently, the need for an oral heparin dosage form remains.

We have synthesized a family of novel compounds that are effective at promoting the oral absorption of various drugs (12–15) without causing intestinal damage. As part of this research program, sodium *N*-[8-(2-hydroxybenzoyl)amino]caprylate (SNAC) was identified as a potent promoter of heparin absorption from the gastrointestinal tract. To demonstrate the effectiveness of this delivery agent, heparin absorption was assessed by APTT and/or anti-Factor Xa assay following oral administration of heparin, SNAC, or a combination of both in rats and monkeys. These data were compared with those obtained following subcutaneous heparin administration in each animal species.

MATERIALS AND METHODS

Preparation of SNAC

N-[8-(2-hydroxybenzoyl)amino]caprylic acid was synthesized at Cambridge Labs, Germantown WI and converted to SNAC (the sodium salt) at ProCyte Corporation, Kirkland WA.

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Pharmacology Screen

The general pharmacological activity of SNAC (without heparin) was assessed in 75 functional and receptor-mediated *in vitro* and *in vivo* assays (Pharmascreen®; Panlabs Inc., Bothell WA) along with vehicle and reference controls. These 75 assays included the following therapeutic areas: MTD/autonomic signs (1 *in vivo* assay), central nervous system (14 *in vivo* assays), cardiovascular (9 *in vivo* and 17 *in vitro* assays), metabolism (8 *in vivo* assays), allergy/inflammatory (3 *in vivo* and 5 *in vitro* assays), gastrointestinal (5 *in vivo* and 4 *in vitro* assays), and microbiological (9 *in vitro* assays). SNAC was screened at a single dose or concentration, and positive responses in the primary assays were further evaluated. The *in vivo* studies were conducted at 300 mg/kg in 2.0% Tween 80; the *in vitro* studies were conducted at 3 to 30 μg/mL in 0.1 to 0.5% DMSO. The concentrations of SNAC used in these *in vitro* assays were within the range observed in plasma within the first hour following oral administration of 300 mg/kg SNAC to cynomolgus monkeys.

Preparation of Dosing Solutions

Solutions of SNAC, heparin (164 to 165.7 IU/mg; Scientific Protein Laboratories, Waunakee, Wisconsin), or a combination of both for oral administration were prepared in 25% (v/v) aqueous propylene glycol as follows. A solution of 25% (v/v) aqueous propylene glycol was added to a dry mix of the compounds to be dosed. The mixture was vortexed and then placed in a 37°C ultrasonic waterbath for 10 to 15 minutes until the solution became clear. The solution was brought to its final volume with 25% v/v aqueous propylene glycol. Typically, the final solutions contained 100 mg/mL SNAC and 33.3 mg/mL heparin, pH 7.5–8.5. Heparin solutions (6 mg/mL) for subcutaneous administration were prepared in sterile physiological saline. All solutions were dosed within 2 hours of preparation.

Animal Studies

All animal protocols adhered to the "Principles of Laboratory Animal Care" and were approved by Emisphere Technologies, Inc. and the outside test sites' (New York Medical College, Valhalla NY; Sierra Biomedical Inc., Sparks NV; and, ITR Laboratories Canada Inc., Baie d'Urfé, Québec) respective Institutional Animal Care and Use Committees.

Rat Experiments

Male Sprague-Dawley rats (Taconic Farms, Germantown NY), housed in the animal care facility at New York Medical College, Valhalla, New York, were fasted for 12 hours before dosing. Groups of five or six rats weighing 300 to 350 g were anesthetized with 44 mg/kg ketamine hydrochloride (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) intramuscularly (IM) and administered a single oral dose of SNAC, heparin, or a combination of both, in a vehicle of 25% aqueous propylene glycol via a 8 fr. Nelaton catheter (Rusch, Kernen, Germany) attached to a 1 mL syringe. Citrated blood samples (0.5 mL) were collected serially by cardiac puncture for 1.5 hours, the plasma harvested, and the APTT values measured immediately.

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Table I. Mean APTT Values in Rats Following Oral or Subcutaneous Administration of SNAC, Heparin, or a Combination of Both

SNAC Dose (mg/kg)	Heparin Dose		Route of Administration	Mean Peak APTT Response ± SEM
	mg/kg	IU/kg		
300	0	0	oral	23.5 ± 0.5
0	100	16400	oral	25.0 ± 1.7
0	3	492	subcutaneous	39.7 ± 3.6
300	3	492	subcutaneous heparin followed by oral SNAC	44.5 ± 3.5
300	101	16564	oral	101.9 ± 12.0
200	101	16564	oral	57.1 ± 8.9
133.3	101	16564	oral	31.3 ± 2.8
88.8	101	16564	oral	41.1 ± 7.3

Histological Evaluation of the Gastrointestinal Tract

Rats were dosed by oral gavage as described above. At 0.5, 1, and 2 hours after dosing, the animals were anesthetized with 44 mg/kg ketamine and 1.5 mg/kg thorazine and then exsanguinated. Gastric, duodenal, jejunal, and ileal tissues were removed from the animals and fixed in neutral buffered formalin. The tissues were sent to a clinical laboratory (Cenvet Laboratory, Woodside, New York) for processing and histological evaluation by a DCAVP-certified pathologist. Representative slides were then sent to a second veterinary pathologist for corroboration. At least 4 rats were used for each time point.

Monkey Experiments

Groups of 2 male and 2 female cynomolgus monkeys, housed at Sierra Biomedical Inc., Sparks NV or ITR Laboratories Canada Inc., Baie d'Urfé, Québec were acclimated for at least 2 weeks prior to dosing. Following an overnight fast, fully-conscious monkeys received a single oral dose of SNAC, heparin, or a combination of both, in a vehicle of 25% aqueous propylene glycol via nasogastric intubation or oral gavage. Citrated blood samples (1.0 mL) were collected serially by femoral venipuncture at 1 and 0.5 hours before dosing and for 6 hours after dosing. The plasma was harvested and divided into two aliquots. One aliquot was used for automated APTT analysis at the test site and the other was frozen and shipped to Emisphere Technologies, Inc. for anti-Factor Xa analysis.

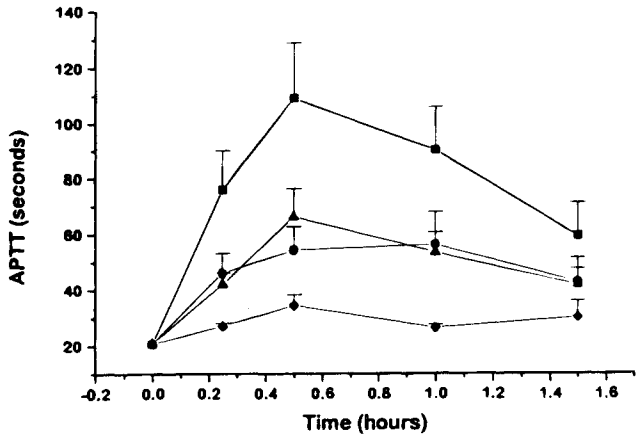

Fig. 1. Pharmacodynamic profile following the oral administration of 300 mg/kg SNAC in combination with varying heparin doses in 50 rats. Squares represent 101 mg/kg (16564 IU/kg) heparin. Circles represent 67.5 mg/kg (11070 IU/kg) heparin. Triangles represent 45 mg/kg (7380 IU/kg) heparin. Diamonds represent 30 mg/kg (4920 IU/kg) heparin. The data are plotted as mean ± SEM.

Heparin Assays

The APTT assay of citrated plasma (2.7% citrate) from rats was performed as described by Henry *et al.* (16) using a BBL fibrometer (VWR Scientific, South Plainfield NJ), and APTT reagents purchased from Sigma Diagnostics, St. Louis MO. Anti-Factor Xa activity in plasma was measured using an assay kit from Chromogenix, MoIndal, Sweden. The limit of detection was 0.1 IU/mL. There was no interference caused by SNAC in either of these assays.

Pharmacokinetic Analysis

C_{max} and T_{max} were determined from the pharmacodynamic or pharmacokinetic profiles generated by plotting plasma APTT values or anti-Factor Xa activity, respectively, versus time. The areas under the curves, mean values, and standard errors of the means ($n \geq 3$) were calculated using Microcal™ Origin™ Software, version 4.1. Relative bioavailability was calculated from the dose-corrected truncated areas under the plasma concentration-time curves for the oral versus subcutaneous routes of administration (17).

RESULTS AND DISCUSSION

SNAC was screened for potential unwanted pharmacological activities in a battery of 75 *in vitro* and *in vivo* assays. The vehicles used in these assays were different from the 25% aqueous propylene glycol used in the absorption studies in order to solubilize the reference compounds used in the pharmacological screens. SNAC elicited a significant response (>50 % inhibition) in the tracheal relaxation (allergy/inflammation) *in vitro* assay at 30 and 10 μg/mL. This reduction of spontaneous tone in an isolated, open ring, cut guinea pig trachea assay had no correlation with similar cardiovascular assays [antiarrhythmic (*in vivo*), (+) inotropic response, (+) chronotropic response, and adrenoceptor β_1 agonism], which were negative. This suggests that the effect is unrelated to β-adrenoceptor agonism. The effect was insignificant at 3 μg/mL SNAC. There was also a significant response reported in the arachidonate-induced platelet aggregation *in vitro* assay. At 10 μg/mL SNAC there was complete inhibition of platelet aggregation induced by arachidonic acid, while at 3 μg/mL there was no inhibition. Platelet aggregation induced by two other agents, thromboxane A_2 and platelet aggregation factor, was not inhibited by SNAC. These results suggest the effect on aggregation may be due to cyclooxygenase inhibition. The reference compound, indomethacin, had an ED_{100} at 0.1 μg/mL indicating SNAC is not a potent inhibitor in this arachidonate-induced platelet aggregation assay.

Oral administration of SNAC and heparin in combination resulted in increased heparin absorption in rats as shown by elevated APTT values (Table 1). Baseline APTT values averaged 21 seconds. When heparin was dosed alone at 100 mg/

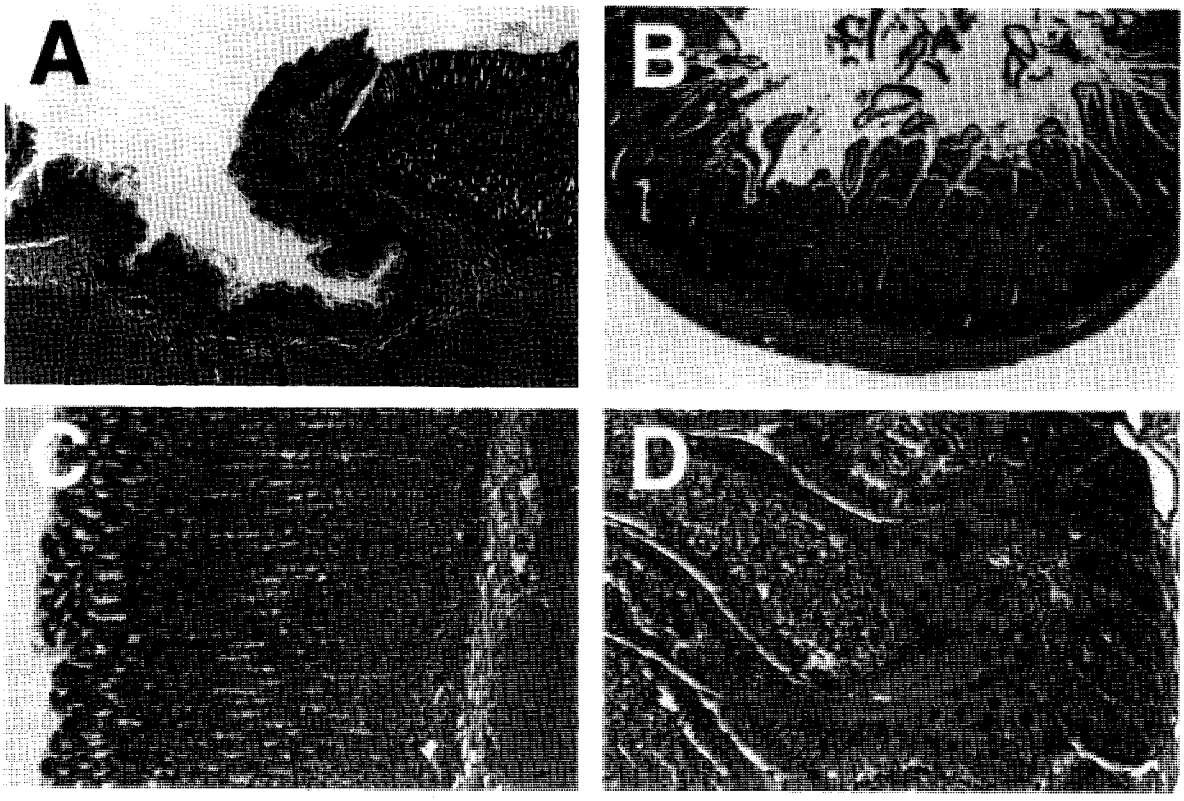

Fig. 2. Representative micrographs of hematoxylin and eosin stained gastrointestinal tissue isolated from a rat 1 hour after oral administration of 300 mg/kg SNAC. Panel A shows a cross section of the limiting ridge of the stomach with the keratinized forestomach and the glandular stomach. In panel B, the glandular stomach is shown in greater detail. A full-thickness cross section of the duodenum is shown in panel C, and detail of the crypts and Brunner's glands can be seen in panel D. The original magnification is 40× in panels A and C and 100× in panels B and D.

kg, the peak plasma APTT value was 22.9 ± 1.2 seconds. However, when SNAC (300 mg/kg) was administered orally in combination with heparin at 100 mg/kg, the peak APTT value was 101.9 ± 12 seconds with a T_{max} of 30 minutes. SNAC dosed orally at 300 mg/kg alone caused no change in clotting time, demonstrating that the increase in APTT was not due to a pharmacological effect of SNAC (Table I). Furthermore, rats administered a subcutaneous dose of heparin followed by SNAC orally had the same APTT response as rats administered only subcutaneous heparin. This would indicate that SNAC facilitates the gastrointestinal absorption of heparin rather than changing its pharmacodynamics.

The data show a direct relationship between the dose of heparin, at a fixed dose of SNAC, and the APTT response. As expected, this relationship was non-linear due to heparin's dose-dependent clearance (18,19). There was also a direct relationship between the dose of SNAC, at a fixed dose of heparin, and the APTT response (Table I). Heparin dosed at 30, 45, 67.5, and 100 mg/kg in combination with a fixed dose of 300 mg/kg SNAC gave mean peak APTT responses of 34.4 ± 3.9, 66.2 ± 10.1, 56.1 ± 11.7, and 109 ± 19.7 seconds, respectively (Figure 1). At the doses used in these studies, the anticoagulant response observed as a result of heparin absorption did not reach a saturated level. All of the heparin doses led to APTT values that were above the target range for a clinical effect in humans (1.5 times baseline APTT).

The increased oral absorption of various drugs that has been achieved with "traditional penetration enhancers" has been reported to correlate directly with the extent of damage caused to the gastrointestinal tract (11). Histological evaluation of the gastrointestinal tract of rats was performed following a single oral administration of SNAC to ensure that the increased absorption of heparin observed in these studies was not due to tissue damage. Representative micrographs of the stomach and small intestine are shown in Figure 2. At no time point was there detectable pathology caused by SNAC. These data confirm that the increased absorption of heparin in the presence of SNAC was not due to disruption of the intestinal epithelium (20). Furthermore, acute toxicity studies conducted in Crl:CD-1(ICR)BR mice demonstrated that multiple (28 days) oral doses up to 1200 mg/kg/day SNAC did not cause damage to any of the tissues examined.

The mechanism by which SNAC facilitates the absorption of heparin is not yet fully understood. Our working hypothesis is that SNAC interacts with heparin in some way that creates more favorable physicochemical properties for absorption. We have recently shown that a series of delivery agents chemically similar to SNAC affect the association of methylene blue with heparin. These compounds also bind to an affinity column of heparin linked to Sepharose CL-6B (21).

To confirm these data in a second species, oral absorption studies were performed in cynomolgus monkeys. Monkeys that

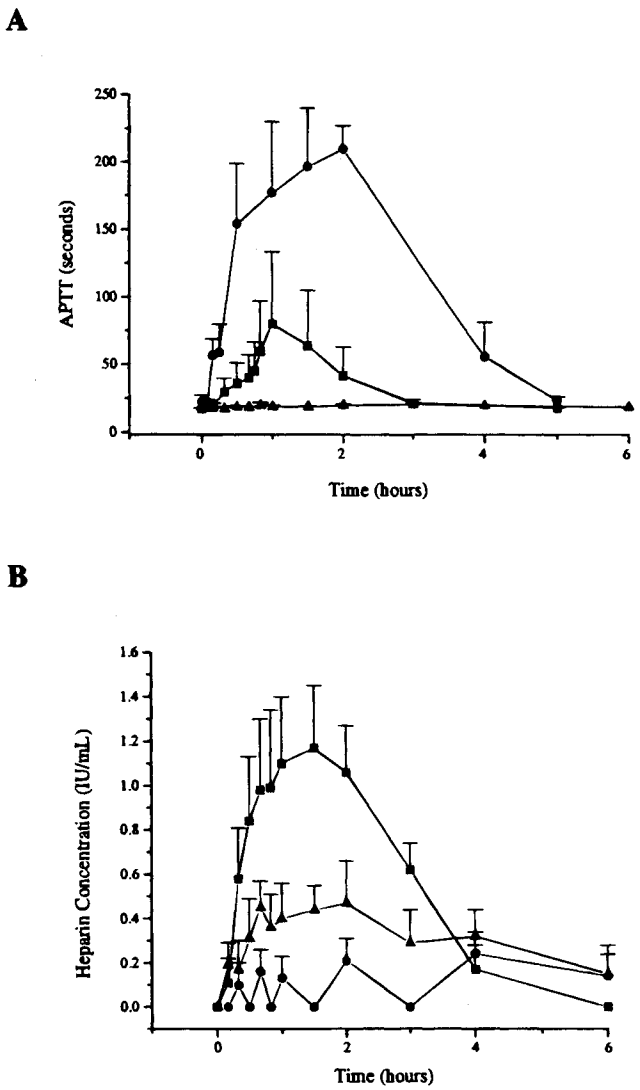

Fig. 3. *Panel A.* Pharmacodynamic profile following the oral administration of SNAC in combination with varying heparin doses in cynomolgus monkeys. *Panel B.* Pharmacokinetic profile following the oral administration of SNAC in combination with varying heparin doses in cynomolgus monkeys. Squares represent 150 mg/kg SNAC in combination with 30 mg/kg (4971 IU/kg) heparin. Circles represent 100 mg/kg (16570 IU/kg) heparin. Triangles represent 150 mg/kg SNAC in combination with 15 mg/kg (2486 IU/kg) heparin. The data are plotted as mean ± SEM.

received a single oral dose of 150 mg/kg SNAC in combination with 30 mg/kg heparin had plasma APTT above the detectable range of the assay (>240 seconds). This response was much greater than that observed when rats were administered the same dose (Table I), and underscores the fact that different animal models for oral drug absorption do not always give the same results. The pharmacodynamic response following oral administration of 150 mg/kg SNAC and 30 mg/kg heparin is shown in Figure 3A. A large increase in clotting time demonstrated that significant absorption of heparin was achieved in the presence of SNAC. Neither SNAC (300 mg/kg) nor heparin (100 mg/kg) dosed alone elicited any change in APTT.

When the dose of heparin was reduced by one-half (150 mg/kg SNAC and 15 mg/kg heparin), the pharmacodynamic response was greatly reduced (Figure 3A). However, the direct plasma heparin measurement by anti-Factor Xa activity showed that the drug levels were approximately half those measured following a heparin dose of 30 mg/kg (Figure 3B). As with the rat experiments, the dependence of the pharmacological response (clotting time) to increasing heparin concentrations is not a linear function due to heparin's dose-dependent clearance (18,19). At higher heparin doses, relatively small changes in plasma heparin concentrations will lead to proportionately larger changes in APTT.

Given the effective oral delivery of heparin in the presence of SNAC in two species, the safety of this combination dosing solution was further assessed in primates. Cynomolgus monkeys dosed orally with SNAC and heparin (30 mg/kg) in combination for 28 consecutive days showed no observable effects at a dose of SNAC up to 800 mg/kg. Microscopic examination of the gastrointestinal tract at the end of this period did not reveal pathological changes in the tissue. Also, there were no significant changes in serum chemistries.

CONCLUSIONS

A novel compound, SNAC, has been shown to effectively promote the oral absorption of heparin. The combination of SNAC and heparin was well-tolerated and did not exhibit unwanted pharmacological effects. The increased oral absorption of heparin was not caused by damage to the gastrointestinal tract.

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