Parenteral low molecular weight heparin (LMWH) is the standard of care for the prevention of deep vein thrombosis in patients undergoing joint replacement or abdominal surgery. LMWH is not absorbed after oral dosing. This article describes the delivery agent–mediated transport of LMWH across Caco-2 cells. These agents are shown to facilitate oral LMWH absorption in rats, dogs, and monkeys.

Parenteral low molecular weight heparin (LMWH) has replaced warfarin as the standard of care for the prevention of deep vein thrombosis (DVT) and pulmonary embolism in high-risk, hospitalized patients undergoing joint replacement or abdominal surgery (1). LMWH is favored over antivitamin K oral anticoagulants such as warfarin because it produces a rapid onset of anticoagulant activity and has a short physiological half-life (2). Compared with warfarin, LMWH has a significantly lower incidence of drug–drug interaction. Anticoagulation with LMWH typically is evidenced by its effect on plasma LMWH concentrations measured by the anti-Factor Xa assay. The target therapeutic range for DVT prophylaxis is 0.1–0.2 IU/mL (2). With the use of fixed doses, continuous monitoring generally is unnecessary, and untoward hemorrhage rarely occurs. The major disadvantage of LMWH therapy is that it must be parenterally administered because it is ineffective when dosed orally (3,4). Thus, LMWH usually is replaced by oral warfarin for outpatient therapy. Unfortunately, this switch from parenteral LMWH to oral warfarin often requires prolonged hospitalization for the patient because the delayed onset of action, the prolonged half-life, and the variable response to warfarin necessitate a gradual increase in dose as the LMWH dose is slowly decreased. An oral LMWH formulation would allow for continuous LMWH treatment of outpatients, thereby eliminating the need to change to warfarin.

Several recent attempts to develop effective oral LMWH formulations have been reported. For example, LMWH complexes with tertiary diamines have shown limited oral bioavailability...
upon intraduodenal administration to rabbits (5). Administration of LMWH in a lipid matrix composed of phosphatidylcholine from soy protein and medium-chain monoacyl-glycerols has improved absorption in the small intestine of rabbits (6). Other approaches have included the use of glycerol esters of fatty acids (7) and non-ionic surfactants (8) to increase the absorption of orally dosed LMWH. Recently, studies have been conducted in which high doses of LMWH alone have been administered orally to rabbits (9,10). In general, these experiments have met marginal success.

Oral unfractionated heparin (UFH) absorption in rats (11), monkeys (12), and healthy human subjects (13) following the administration of an aqueous solution containing UFH and a delivery agent have been reported. These delivery agents are low molecular weight compounds that can be dissolved in water with UFH (11). Oral administration of this solution mixture results in the gastrointestinal absorption of UFH. One of these delivery agents, sodium 8-[N-(2-hydroxybenzoyl)amino] caprylate (SNAC), is the subject of advanced clinical studies (13). Thus, therapeutic levels of anticoagulation, as measured by increased activated partial thromboplastin time (APTT) and anti-Factor Xa activity, were obtained following single oral doses of the SNAC–UFH combination as a flavored syrup. We also have demonstrated the efficacy of this oral SNAC–UFH combination for the prevention (14) and treatment (15) of DVT in a rat model of venous thrombosis. These studies show that orally delivered UFH is as effective as injectable UFH in this model.

As part of our continued research in this therapeutic, we have investigated the delivery agent–facilitated gastrointestinal absorption of LMWH. In this article, we report on the effects of oral LMWH delivery facilitated by SNAC and a more efficient delivery agent, sodium 10-[N-(2-hydroxybenzoyl)amino]decanoate (SNAD). SNAC and SNAD are compared for their abilities to transport LMWH across Caco-2 cells in vitro as well as to facilitate oral LMWH absorption in rats, dogs, and monkeys from aqueous solutions and tablets.

Materials and methods

Materials. SNAC and SNAD were synthesized at Regis Laboratories (Chicago, IL). LMWH (Parnaparin) (91 IU/mg) was purchased from Opocrin Laboratories (Modena, Italy). Propylene glycol was purchased from Aldrich Chemical Company (Milwaukee, WI). Plasma samples and cell culture samples were analyzed using the anti-Factor Xa activity assay. The integrity of the cell monolayers was monitored by measuring transepithelial electrical resistance (TEER) immediately before the addition of donor solutions (apical side) and again after 2 h. In addition, trypan blue staining of the monolayers at the end of each experiment was used to verify monolayer integrity.

Preparation of dosing solutions. Solutions of SNAC/LMWH and SNAD/LMWH were prepared in Hank’s buffered saline solution supplemented with 11 mM glucose and 25 mM N-[2-hydroxyethyl]piperazine-N’-[2-ethanesulfonic] acid. Final concentrations of the delivery agent (SNAC or SNAD) and LMWH applied to the apical side of the monolayers were 5.0 mg/mL and 60 mg/mL (5460 IU/mL), respectively. Basolateral samples were taken every 30 min for 2 h and replaced with fresh buffer. These were analyzed using the anti-Factor Xa activity assay. The integrity of the cell monolayers was monitored by measuring transepithelial electrical resistance (TEER) immediately before the addition of donor solutions (apical side) and again after 2 h. In addition, trypan blue staining of the monolayers at the end of each experiment was used to verify monolayer integrity.

Determination of the permeability coefficients and enhancement ratios. The apparent permeability coefficient ($P_{app}$, cm/s) was calculated according to Kotz et al. (18) using the following equation:

$$\frac{dC}{dt} = \frac{V_k}{A_{C_0}} = P_{app}$$

in which $dC/dt$ is the steady-state rate of change in the drug concentration in the receiver chamber ($\mu$g mL$^{-1}$ s$^{-1}$), $V_k$ is the volume in the receiver chamber (mL), $A$ is the surface area of the cell monolayer (1.13 cm$^2$), and $C_0$ is the initial concentration in the donor chamber ($\mu$g/mL). All experiments were carried out under “sink” conditions. The enhancement ratio was calculated by dividing the $P_{app}$ of LMWH obtained in the presence of the delivery agent by the control $P_{app}$ of LMWH alone.

Animal studies. Animal protocols (rats and dogs) were reviewed and approved, where appropriate, in advance by the Institutional Animal Care and Use Committee. Animal protocols (monkeys) were approved in advance by ITR Labora-
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Male Sprague-Dawley rats (Taconic Farms, Germantown, NY), housed in the animal care facility at New York Medical College (Valhalla, NY), were fasted for 12 h before dosing. Groups of five or six rats weighing 300 to 350 g each were anesthetized with 44 mg/kg ketamine hydrochloride (Fort Dodge Laboratories, Inc., Fort Dodge, IA) intramuscularly. One dose of either SNAC/LMWH or SNAD/LMWH in 25% v/v aqueous propylene glycol was administered orally via an 8-fr. Nelaton catheter (Rusch, Kernen, Germany) attached to a 1-ml syringe. Control animals were dosed with 25% v/v aqueous propylene glycol solution containing either LMWH alone, SNAC, or SNAD alone. Citrated blood samples (0.5 mL) were collected serially by cardiac puncture for 1.5 h, the plasma was harvested, and the anti-Factor Xa activity was measured.

Rat studies. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY), housed in the animal care facility at New York Medical College (Valhalla, NY), were fasted for 12 h before dosing. Groups of five or six rats weighing 300 to 350 g each were anesthetized with 44 mg/kg ketamine hydrochloride (Fort Dodge Laboratories, Inc., Fort Dodge, IA) intramuscularly. One dose of either SNAC/LMWH or SNAD/LMWH in 25% v/v aqueous propylene glycol was administered orally via an 8-fr. Nelaton catheter (Rusch, Kernen, Germany) attached to a 1-ml syringe. Control animals were dosed with 25% v/v aqueous propylene glycol solution containing either LMWH alone, SNAC, or SNAD alone. Citrated blood samples (0.5 mL) were collected serially by cardiac puncture for 1.5 h, the plasma was harvested, and the anti-Factor Xa activity was measured.

Monkey studies. Groups of four cynomolgus monkeys, two males and two females, weighing 2–3 kg each and housed in the animal care facility at ITR Laboratories Canada, Inc., were fasted for 4 h before dosing and as long as 2 h after dosing. The animals were sedated with an intramuscular injection of 10 mg/kg ketamine hydrochloride immediately before dosing. Three mL/kg or 1 mL/kg of the dosing solution was administered to each animal via oral gavage. Citrated blood samples (1 mL each) were collected by venipuncture at 1 h before dosing and at 10, 20, 30, 40, and 50 min and 1, 1.5, 2, 3, 4, and 6 h after dosing. The harvested plasma was frozen at −80 °C and shipped to Emisphere Technologies, Inc., for anti-Factor Xa analysis.

Dog studies. Conscious, male beagle dogs (Taconic Farms), housed in the animal care facility at New York Medical College, were fasted for 12 h before dosing. One oral dose of SNAD/LMWH either as a solution in water (5 mL) or as one tablet was administered to groups of six dogs weighing ~15–18 kg each. Blood samples (0.9 mL) were removed via a saphenous vein catheter before dosing and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, and 6 h after dosing. Anti-Factor Xa assays were conducted.

Results and discussion

Both SNAC and SNAD previously were identified as effective oral and colonic delivery agents for UFH (11). To test their abilities to facilitate oral LMWH absorption across intestinal tissue, the SNAC/LMWH and SNAD/LMWH combinations were evaluated in vitro (cell culture studies) and in vivo (rats, dogs, and monkeys).

Cell culture studies. Both SNAC and SNAD were effective at increasing the permeation of LMWH across Caco-2 cell monolayers compared with controls run with LMWH alone. Data in Table I show that the apparent permeability coefficient of LMWH alone was 5.3–8.4 × 10⁻⁹ ± 0.7. In the presence of SNAC, P_app of LMWH (27.2 × 10⁻⁹ ± 0.7) was increased greater than three-fold in comparison with the value of the control study. In the presence of SNAD, P_app of LMWH (22.9 × 10⁻⁹ ± 0.8) was increased more than four-fold in comparison with the value of the control study. In both cases, the monolayers were not adversely affected. Thus, no significant decreases in Caco-2 TEER were observed by the end of the experiments (see Table I).

Caco-2 monolayer integrity was verified by trypan blue staining after each experiment. No increase in dye uptake was observed at the conclusion of the control, SNAD/LMWH, or SNAC/LMWH studies. These studies indicate that SNAC and SNAD do not behave as classical penetration enhancers (e.g., sodium dodecylsulfate). Absorption that occurs as a result of traditional penetration enhancement is accompanied by significant reductions in TEER and significant increases in trypan blue uptake (19–22). The data show that SNAC and SNAD do not cause these changes. The enhancement ratio for LMWH in the presence of SNAD (4.32) was higher than that obtained in the presence of SNAC (3.26). These studies suggest that SNAD is a more efficient oral delivery agent for LMWH. The following animal experiments were conducted to test this observation in vivo.

In vivo administration of SNAC/LMWH and SNAD/LMWH solutions. Having verified that SNAC and SNAD were effective at promoting the membrane transport of LMWH, in vivo studies were initiated. Thus, either SNAC/LMWH or SNAD/LMWH as an aqueous propylene glycol solution was orally administered to rats. Following a single oral dose, both the SNAC/LMWH and SNAD/LMWH combinations produced increased plasma LMWH concentrations as measured by anti-Factor Xa assay (see Figure 1). Mean peak anti-Factor Xa levels of 2.0 ± 0.3 IU/mL and 2.2 ± 0.2 IU/mL were observed following oral administration of solutions containing SNAC/LMWH and SNAD/LMWH, respectively. The SNAC-facilitated bioavailability (relative to subcutaneous injection [23]) of LMWH was 59%, and the SNAD-facilitated bioavailability of LMWH was 9%. Control animals dosed with either LMWH, SNAC, or SNAD alone did not show any increase in plasma LMWH concentrations.

To confirm the abilities of SNAC and SNAD to facilitate oral LMWH delivery in a second species, single oral doses of either the SNAC/LMWH or SNAD/LMWH heparin combinations as aqueous solutions were administered to monkeys. Both combinations caused significant elevations in plasma LMWH concentrations. In the presence of a constant dose of LMWH, a dose-dependent response was evident for SNAC delivery agent

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<th>Table I: Summary of Caco-2 studies.*</th>
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<td><strong>Delivery Agent</strong></td>
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*% TEER = percentage transepithelial electrical resistance remaining ± SEM. Enhancement ratio was calculated by dividing P_app of LMWH in the presence of delivery agent by the P_app of LMWH alone. For each study, n = 3.
doses ranging from 50 to 300 mg/kg (see Figure 2). Mean peak anti-Factor Xa levels for all monkey studies were achieved between 0.5 and 1.5 h after dosing. Mean peak plasma LMWH concentrations were 0.17–0.96 IU/mL. A similar dose–response relationship was observed with SNAD at lower delivery agent doses. Figure 3 shows a comparison of the responses following single oral doses of the SNAC/LMWH and SNAD/LMWH combinations at a delivery agent dose of 50 mg/kg and a LMWH dose of 1000 IU/kg. The relative bioavailability of LMWH was 3.1% with SNAC and 38.2% with SNAD. Thus, SNAD is ~10 times more effective than SNAC for oral LMWH delivery in the monkey model.

**Solid dose formulation in vivo studies.** Having confirmed in both in vitro cell culture and in vivo models that SNAD is more effective than SNAC for facilitating oral LMWH absorption from unformulated aqueous solutions, studies were initiated to evaluate a SNAD/LMWH solid dose form. Dogs were selected as the large-animal model for this work. Single oral doses of either aqueous solutions or tablets containing SNAD (275 mg) in combination with LMWH (45,000 IU) were administered to groups of six dogs. Figure 4 shows the response following a single oral dose of the SNAD/LMWH solutions or the SNAD/LMWH tablets. Both dosage forms produced increases in anti-Factor Xa activity. Similar pharmacokinetic profiles were obtained from the aqueous dosing solution and the tablets, both producing a peak response at ~0.5 to 1.5 h. The area under the curve of plasma LMWH concentration versus time indicates that the solution elicited a 1.5-fold greater response than did the tablets. Oral administration of LMWH or SNAD alone did not produce elevations in anti-Factor Xa activity. The bioavailability of LMWH, administered orally as a solid dose form, relative to subcutaneous injection was 3%.

**Conclusion**
The studies presented in this article have shown that both SNAC
and SNAD facilitate the transport of LMWH across Caco-2 epithelial cells without opening the tight junctions or adversely affecting the structural integrity of the cell monolayer. Administration of aqueous solutions of the SNAC/LMWH and SNAD/LMWH combinations to rats and monkeys indicated that the Caco-2 cell model correctly predicted the relative transport abilities of both SNAC and SNAD. Thus, these delivery agents were effective at promoting oral LMWH absorption in these animal models, and SNAD was more effective than SNAC. The SNAD/LMWH combination was evaluated further as a tablet formulation. Increased plasma LMWH concentrations were also measured following the oral administration of these tablets to dogs. Overall, these studies demonstrate that SNAC and SNAD facilitate oral LMWH absorption in two species, and that the SNAC/LMWH and SNAD/LMWH combinations are not cytotoxic in a Caco-2 cell culture model.

References