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Research Paper

Papain: An Effective Permeation Enhancer for Orally Administered Low Molecular Weight Heparin

Vjera Grabovac,[1] Thierry Schmitz,[1] Florian Föger,[1] and Andreas Bernkop-Schnürch[1,2]

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Purpose. The purpose of this study was to evaluate an effect of the proteolytic enzyme papain on permeation of low molecular weight heparin (LMWH) *in vitro* and *in vivo*.
Materials and Methods. *In vitro* permeation studies were performed using rat small intestine as permeation barrier. In order to determine the ratio of papain to heparin resulting in the highest heparin permeation rate, molar ratios 1:1, 1:2 and 2:1 of papain to heparin were tested. Interactions of heparin with papain were investigated spectro-photometrically. For *in vivo* studies, 15 mg tablets containing heparin (13%), papain (64%) and hydroxyethylcellulose (22%) were orally administered to rats.
Results. Since molar ratio papain to heparin 1:1 resulted in the highest permeation rate, it was used for *in vivo* studies. The results of binding studies of papain with heparin indicated a strong interaction between papain and heparin. Oral administration of tablets containing LMWH/papain/HEC resulted in sevenfold improvement of plasma anti-Xa activity in comparison to control. For tablets based on heparin/papain/HEC, a relative bioavailability of 9.1% vs. subcutaneous injection was obtained, whereas the relative bioavailability of control was 2.4%.
Conclusion. The co-administration of papain with heparin represents a new approach in improvement of absorption and bioavailability of orally administered heparin.

KEY WORDS: heparin; intestinal; oral; permeation enhancer; proteolytic enzymes.

INTRODUCTION

Oral administration of low molecular weight heparin (LMWH) would represent the most convenient way of dosing for patients, as it neither causes pain nor requires patientś hospitalization. For that reason, several research groups have meanwhile tried to find suitable strategies to facilitate the gastrointestinal absorption of orally applied LMWHs. Although LMWHs are smaller and less charged than unfractionated heparins (UFH), their permeation through the intestinal epithelium is limited and they are not able to reach the blood circulation without support of auxiliary agents. Different approaches have been proposed to increase heparin absorption across intestinal tract such as use of absorption enhancers (1,2) and different carrier systems (3) The reason for diminished or lacking absorption of heparin can be characterized by two biological barriers namely mucus gel layer on the surface of epithelial cells and extracellular matrix (4). On the one hand, mucus gel layer covering epithelia is mainly based on glycoproteins having a linear protein core and cysteine-rich subdomains (4) building up a three-dimensional network which has to be overcome by LMWH in order to reach the absorption membrane. On the other hand, tight junctions, as part of the extracellular matrix, represent a major regulatory unit for paracellular absorption pathway of LMWHs (5). The function of tight junctions is regulated by different transmembrane and intracellular proteins (6).

Bromelain, trypsin (7) and papain (8) are regarded to as strong mucolytic agents. Its mucolytic activity is based upon splitting the bond within the amino acid sequence of mucus glycoproteins (4). Moreover, proteolytic enzymes are known to affect the tightness of intercellular junctions and to open the paracellular route across the epithelium for non-absorbable compounds (8,9). Proteolytic enzymes are widely used in cell isolation. Due to their ability to degrade extracellular matrix components (10,11), including proteins of tight junctions. Within some tissues, papain has proved to be less damaging and more effective than other proteases (12). In previous studies, a permeation enhancing effect of papain on low molecular size compounds such as sodium fluorescein and flourescein isothiocyanate-dextran (MW 4.4 kDa) through the small intestinal mucosa could be shown (13). According to previously reported findings (14–16) papain is also well-absorbed from the gastrointestinal tract.

In comparison to oral absorption enhancers, which are absorbed into the systemic circulation, often leading to systemic toxic side effects, papain is present in fruits like papaya, in many Over the Counter products as well as in drugs such as Wobenzym N (MucosPharma GmbH &Co, Gretsried, Germany), its intake can be considered as safe. It

[1] Institute of Pharmacy, Department of Pharmaceutical Technology, University of Innsbruck, Innrain 52, 6020 Innsbruck, Austria.
[2] To whom correspondence should be addressed. (e-mail: andreas.bernkop@uibk.ac.at)

is claimed to support wound healing, inflammatory diseases, reduces the appearance of swollen legs, etc. The Food and Drug Administration even approved orphan drug status for oral prescriptions products containing papain.

The aim of this study was to investigate the ability of proteolytic enzyme papain on the permeation of heparin across rat intestine. Permeation studies *in vitro* were performed with Ussing-type chambers using freshly excised rat small intestine. In order to determine the most efficient ratio of papain to heparin leading to the highest heparin permeation through rat small intestinal mucosa, the molar ratios 1:1, 1:2 and 2:1 of papain to heparin were tested. The ratio resulting in the highest uptake of heparin was then used in an oral dosage form for *in vivo* studies.

MATERIALS AND METHODS

Materials

Low molecular weight heparin (LMWH, 105 IU/mg, average MW 4.125 kDa), purchased from ICN Biomedicals; papain (EC 3.4.22.2) (14 units/mg protein), heparin-acrylic beads saline suspension, N-(2-hydroxyethyl)piperazine-N′-(2-ethane-sulfonic acid) (HEPES) and N-acetyl-L-cysteine were purchased from Sigma-Aldrich, Austria. Hydroxyethylcellulose (MW 250 Da) was purchased from Gatt-Koller, Vienna, Austria.

Permeation Studies *In Vitro*

Studies were performed in Ussing type chambers measuring 1 ml volume in both, donor and acceptor chamber and permeation area of 0.64 cm^2. As a permeation barrier, rat small intestine was excised immediately after dispatching the rat, its duodenum was excised and mounted in the Ussing-type chambers and displaced in incubation medium mimicking intestinal fluid. The medium contained 250 mM NaCl, 2.6 mM $MgSO_4$, 10 mM KCl, 40 mM glucose and 50 mM $NaHCO_3$ and was buffered with 40 mM HEPES, maintaining pH 6.8 during the whole experiment. In order to activate the enzyme, cysteine was added in a final concentration of 5 mM (14).

After 10–15 min of pre-incubation of small intestine with pure incubation medium, the incubation media in donor compartments were substituted by solutions containing 50 IU/ml heparin and papain in following concentrations: 0.24, 0.48 and 0.96 mg/ml, corresponding heparin to papain concentration ratios 2:1, 1:1 and 1:2. Samples of 100 µl were withdrawn from the acceptor compartments every 30 min over a period of 150 min. Each time, the removed quantity was replaced by fresh artificial intestinal fluid equilibrated at 37°C. The sink conditions were maintained throughout the experiment. All experiments were performed at least five times in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. Anti-Xa activity of permeated heparin was determined by COAMATIC® LMWH (CoaChrom, Diagnostica, Vienna, Austria) test kit using microplate method and detected by absorbance/fluorescence microplate reader Fluostar Galaxy (BMG Labtechnologies, Offenburg, Germany) at 405 nm. Anti-factor Xa activity is a measure for antithrombotic activity of Low Molecular Weight heparins, expressed in IU/ml plasma. The detection limit of heparin was 0.01 IU/ml. Results have been reported in IU/ml. Cumulative corrections were made for the previously removed samples. The apparent permeability coefficients (P_{app}) for different papain concentrations were calculated according to the following equation:

$$P_{app} = Q/(A*c*t)$$

P_{app} being the apparent permeability coefficient (cm/s), Q is the total amount permeated within the incubation time (µg), A is the diffusion area of the Ussing chamber (cm^2), c is the initial concentration of heparin within the donor compartment (µg/cm^3) and t is the total time of the experiment (s). Transport enhancement ratios (R) were calculated from P_{app} values as follows.

$$R = P_{app}(\text{sample})/P_{app}(\text{control})$$

Papain–heparin Binding Studies

Spectrophotometric Analysis

First, absorption maxima of increasing concentrations of papain (0–1.8 mg/ml) in 50 mM acetate buffer pH 5.5 were determined using spectrophotometer (Beckman DU® 650). Thereafter, to each of these papain solutions 940 mg of previously washed heparin-acrylic beads were added. The samples were incubated for 1 h at 37°C in oscillating thermomixer at 1,100 rpm (Comfort, Eppendorf). After removing the heparin acrylic beads, absorption maxima of papain in the supernatant were determined. The amount of papain being bound to heparin-acrylic beads was calculated via a standard curve.

Preparation of LMWH Dosage Forms

To homogenize hydroxyethylcellulose (HEC) with heparin and papain, 50 mg of heparin, 242.5 mg of papain, and 82.5 mg of HEC were hydrated in 8 ml of demineralized water. The mixture was frozen at −20°C and lyophilized. Thereafter the lyophilized homogenate was compressed into 15 mg tablets (diameter 2.5 mm; height 1.2 mm). In order to facilitate the swallowing the tablets were coated with hard fat by immersing them three times into the coating material being melted at 40°C. After being left to air-dry at room temperature, tablets stored at 4°C until use.

In Vitro Evaluation of the Drug Release From Tablets

The dosage forms were placed into 2.2 ml reaction tubes containing 1 ml of 80 mM HCl, pH 2. Closed tubes were placed into the water bath provided with orbital shaker (GLF 1083, 66 rpm) at 37°C. Every 30 min 100 µl of the release medium were withdrawn from the tubes and replaced by the same quantity of 80 mM HCl. The amount of the LMWH was evaluated using the COAMATIC® LMWH (CoaChrom, Diagnostica,Vienna, Austria) test kit. Cumulative corrections were done for previously removed samples. The sink conditions were maintained throughout the experiment.

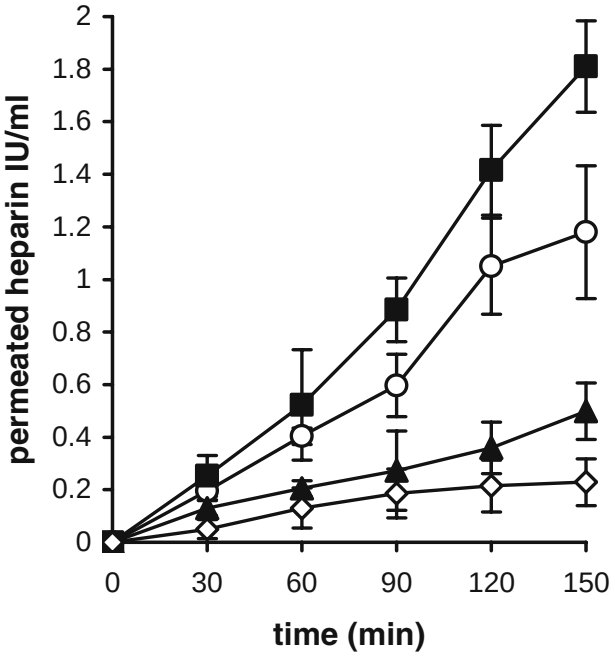

Fig. 1. Comparison of the permeation of heparin in presence of papain in increasing concentrations *in vitro*. Donor chambers contained 50 IU/ml heparin and papain in molar ratios papain to heparin 1:1 (*filled square*); 2:1 (*empty circle*) and 1:2 (*filled triangle*). Control chambers contained 50 IU/ml heparin (*empty diamond*). Data represent the transport of heparin through intestinal mucosa of means ± S.D. of at least five experiments.

Oral Heparin Delivery

In Vivo Evaluation of the Delivery System

The protocol for the animal studies was approved by the Ethical Committee of Austria. Male Norwegian rats (*Rattus norvegicus*) with average weight 270±20 g were obtained from the Institut für Labortierkunde und Genetik, University of Vienna. Prior to applying the delivery system, 180 µl of blood samples were taken from tail vein. The samples were collected in 1.5 ml tubes containing 20 µl of 3.8% aqueous sodium citrate solution. These values served as reference and the time of their withdrawal was noted as zero. Thereafter, rats were dosed with tablets containing different formulations (Table II). Tablets were orally administered to non-anaesthetized animals by placing the tablet deep into the throat in order to initiate a swallowing reflex. To ensure the swallowing reflex, immediately after the administration of the tablet, 200 µl of 2% of aqueous ascorbic acid solution was given. In order to determine the relative bioavailability of the oral formulation vs. subcutaneous injection, five rats were given a subcutaneous injection containing 200 µl of sterile LMWH solution (50 IU in sterile 0.9% NaCl solution).

During the study, dosed rats were kept in restraining cages and supplied only with drinking water. Blood samples of 180 µl of blood were collected from the tail vein 60, 120, 240, 360 and 480 min after administration. Blood samples of rats given the subcutaneous heparin injections, were withdrawn from the tail vein at the same time intervals.

Samples were centrifuged (4,000× g for 5 min), plasma was collected and stored at −20°C until analysis. The amount of heparin in each plasma sample was determined in duplicate using the chromogenic assay as described above and calculated by using a standard curve.

Pharmacokinetic Analysis

c_{max} and t_{max} were determined from the pharmacokinetic profiles generated by plotting the concentration of heparin in plasma (IU/ml) vs. time. The areas under the concentration time curves (AUC) were calculated according to the linear trapezoidal rule. The relative bioavailability was calculated from the dose and corrected areas under the curves for oral vs. subcutaneous administration.

Statistical Data Analysis

Statistical data analysis was performed using the Student *t* test with $p<0.05$ as the minimal level of significance unless indicated otherwise.

RESULTS

In Vitro Permeation Studies: Influence of Papain Concentration on the Permeation of Heparin

The evaluation of permeation enhancing effect of papain from *Carica papaya* was performed in Ussing-type chamber using rat small intestine as an absorption barrier. The permeation of heparin was tested in presence of three different papain concentrations. Results show that presence of papain exerts a dramatic enhancing effect. Permeated amount of heparin was plotted against time (Fig. 1). The highest increment in permeation rate showed the experimental setup with a molar ratio papain to heparin of 1:1. After 150 min, for the ratio 1:1, an average concentration of heparin measured in acceptor chamber was 1.81 IU/ml representing an enhancement ratio (R) of 7.9, while the concentration determined in acceptor chamber of the control setup was merely 0.23 IU/ml. The apparent permeability coefficients (P_{app}) and corresponding enhancement ratios (R) are shown in Table I. Although, concentration ratios of papain to heparin 2:1 and 1:2 provided a significantly lower enhancing effect than 1:1, they were nevertheless significantly more effective than the control. On the one hand, a lower permeation of heparin in the presence of twofold higher concentration of papain might be explained by possible competition of papain with heparin–papain complex for the

Table I. Influence of Papain on the Apparent Permeability Coefficient (P_{app}) for Heparin

Ratio Papain: Heparin	Apparent Permeability Coefficient [$P_{app}\times10^{-6}$ (cm/s)], means ± SD; n=5	Enhancement Ratio (P_{app} Sample/ P_{app} Control)
2:1	4.0±0.8	5.1
1:1	6.2±0.6	7.9
1:2	1.6±0.2	2.1
Control	0.8±0.2	1

paracellular route of uptake. On the other hand, the reason for weaker enhancing effect of the papain to heparin ratio 1:2 could be insufficient quantity of papain facilitating the uptake of heparin.

Spectro-photometrical Analysis

The amount of papain bound to 940 mg heparin-acrylic beads in acetate puffer at pH 5.5 was plotted against the equilibrium concentration (Fig. 2). When papain concentration was below the binding capacity of the heparin acrylic beads a linear relationship was found. The average percentage of papain bound to heparin acrylic beads amounted 76.8±1.9%. Meldrum et al. reported pH profiles in the human small intestine from ~4 to ~7, therefore a pH of 5.5 used in this experiment could be expected in the upper part of the small intestine (17).

In Vitro Release of Heparin From Tablets

In Fig. 3 heparin release rates from tablets based on heparin/papain/HEC and heparin/HEC are shown. In comparison to formulations comprising only heparin and HEC, papain containing formulations showed a significantly higher drug release rate. After 2 h, a difference in released heparin between these two formulations remained constant resulting in 9.5% more released heparin from test tablets than from control tablets. The reason for higher release rate from test tablets might be the lower share of the release controlling polymer HEC in test tablets being 22% in comparison to that in control tablets being 87%.

In Vivo Study

The effectiveness of papain as an intestinal absorption enhancer for LMWH was evaluated by determining heparin concentration in plasma after oral administration. The composition of tablets used for *in vivo* studies is shown in Table II. Administration of heparin with papain and HEC resulted in significantly higher plasma levels of LMWH compared to plasma levels achieved by administration of control tablets comprising only LMWH and HEC. Maximal plasma concentration (c_{max}) of heparin in plasma was, for both formulations, reached after 2 h (Fig. 4). C_{max} of control tablets was 0.05±0.03 IU/ml, whereas c_{max} of papain containing tablets was 0.35±0.06 IU/ml. In addition, a significant ($p<0.05$) increment in relative bioavailability of heparin, administered via tablets containing papain, in comparison to control tablets was observed (Table III). The relative bioavailability of heparin in control tablets was 2.4%, whereas the relative bioavailability of heparin in tablets containing papain was 3.8-fold higher than of the control tablets. Furthermore, oral formulations containing papain exhibited prolonged heparin plasma level.

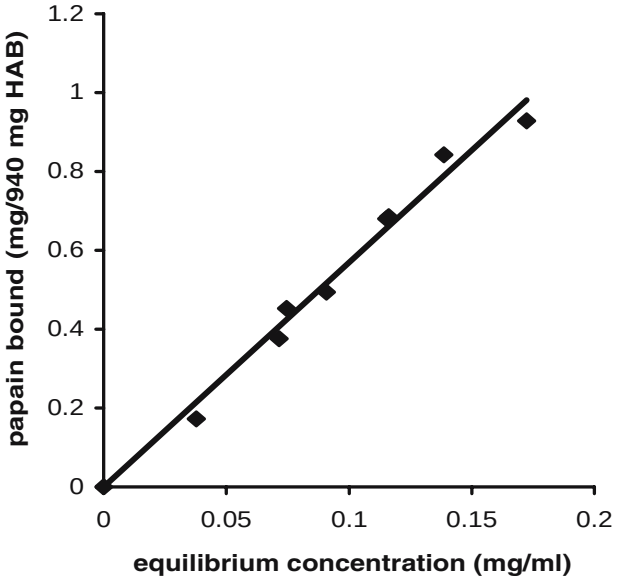

Fig. 2. Binding affinity of papain to heparin acrylic beads in 50 mM acetate buffer at pH 5.5.

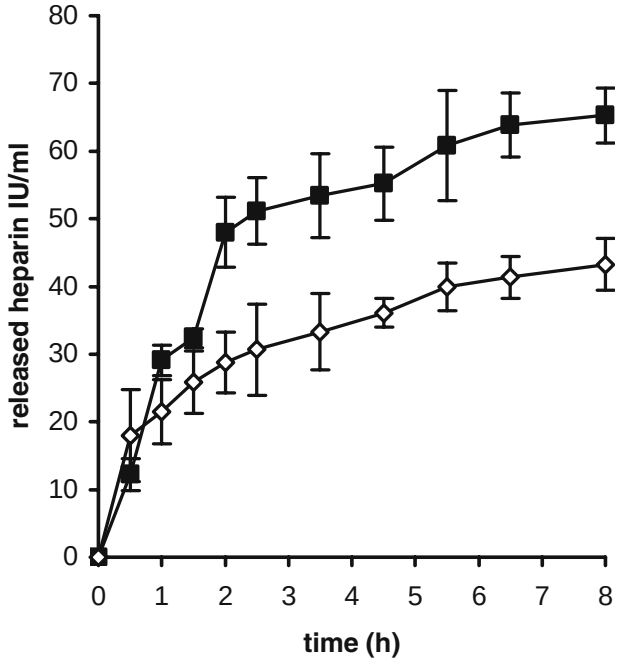

Fig. 3. *In Vitro* release profile of heparin from 15 mg tablets containing HEC and 210 IU of heparin (13/2 mg) (*filled diamond*) and tablets containing HEC, papain and 210 IU of hepain (3.3/9.7/2 mg) (*filled square*). Studies were performed in demineralized water. Indicated values are means ± S.D. of at least five experiments.

Table II. Characteristics of Different Delivery Systems Used for *In Vivo* Studies

	Heparin	HEC	Papain	Additional Information
oral formulation with papain	2 mg (=210 IU)	3.3 mg	9.7 mg	15 mg coated with hard fat
oral formulation without papain	2 mg (=210 IU)	13 mg	–	15 mg tablet coated with hard fat
subcutaneous solution	0.508 mg (=53.3 IU)	–	–	

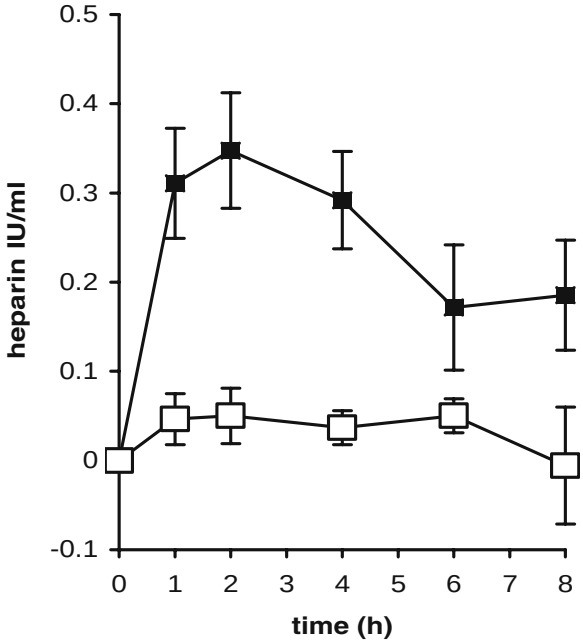

Fig. 4. Comparison of the concentration profiles of heparin in plasma obtained after oral administration of 210 IU of heparin embedded in 13 mg of HEC matrix (*empty square*) and in matrix containing 3.3 mg HEC and 9.7 mg papain (*filled square*). Data represent means ± S.D. of at least three experiments.

DISCUSSION

Hydrophilic macromolecular drugs are poorly absorbed from mucosal membranes of the gastrointestinal tract. The reason for low oral bioavailability of this type of therapeutic agents is mainly based on their poor absorption, due to the incapability of these drugs to overcome the intestinal absorption barriers including mucus layer (4) and tight junctions (18). A reduction of these barriers seems to be a key issue in order to improve the absorption and bioavailability of these therapeutic agents.

Findings of several studies suggest that papain can bind to anionic polysaccharides like heparin, due to its positively charged protein structure (19,20). Moreover, the interaction of heparin with papain seems to alter the function of this enzyme. According to Almeida *et al*. (21), the presence of heparin significantly increases the α-helix content of papain and promotes a powerful increase in the affinity of the enzyme for the substrate. The advantage of these synergistic effects could be demonstrated within this study.

Table III. Main Pharmacokinetic Parameters Obtained by Orally Administered Tablets and Subcutaneous Administration of Heparin to Rats (means ± SD, n=3−5)

Pharmacokinetic Parameter	HEC Matrix	HEC+Papain Matrix	Subcutaneous Solution
c_{max}; IU/ml	0.05±0.03	0.35±0.06	2.38±0.49
t_{max};h	2	2	1
$AUC_{0\rightarrow 8}$; IU/ml * h	0.5±0.31	1.94±0.28	5.41±1.98
relative bioavailability %	2.4	9.1	100

Results of *in vivo* studies showed that papain significantly (p<0.05) enhances the intestinal absorption of LMWH. The relative bioavailability of heparin being embedded in HEC/papain matrix was at least 9.12% versus subcutaneous administration (Table III) (Fig. 5). In order to avoid life threatening bleeding complications for animals, the amount of subcutaneously injected heparin was only one-fourth of the orally applied dose. Limiting the duration of the experiment to 8 h seemed reasonable considering the motility of the gastrointestinal tract and the simple formulated drug delivery system. In addition, as previously reported by our research group, molecular weight of heparin has a significant impact on its mucosal uptake. Heparins with 3 kDa showed higher c_{max}, higher bioavailability and lagged t_{max} in comparison to 6 kDa heparins. According to these findings the pharmacokinetic values of 4.125 kDa heparins used in this study are, as expected, positioned in between (22).

The levels found in blood after oral applications of 210 IU heparin (Fig. 4) were, on the one hand, sufficient to prevent the thrombi formation, since according to Bianchini *et al.* (23) the anti-Xa concentration in plasma exceeding 0.2 IU/ml always result in evident antithrombotic effect. On the other hand, administration of the same dose of LMWH comprised in formulation consisting of only HEC as matrix, resulted in plasma concentration insufficient to induce antithrombotic effect. When N-[8-(2-hydroxybenzoyl)amino]caprylate was used as absorption enhancer for orally administered heparin an effectiveness for in maximum 3 h was achieved (1). In another study Prasad *et al.* (24) used a surfactant Lubrasol® as permeation enhancer. The blood levels necessary to

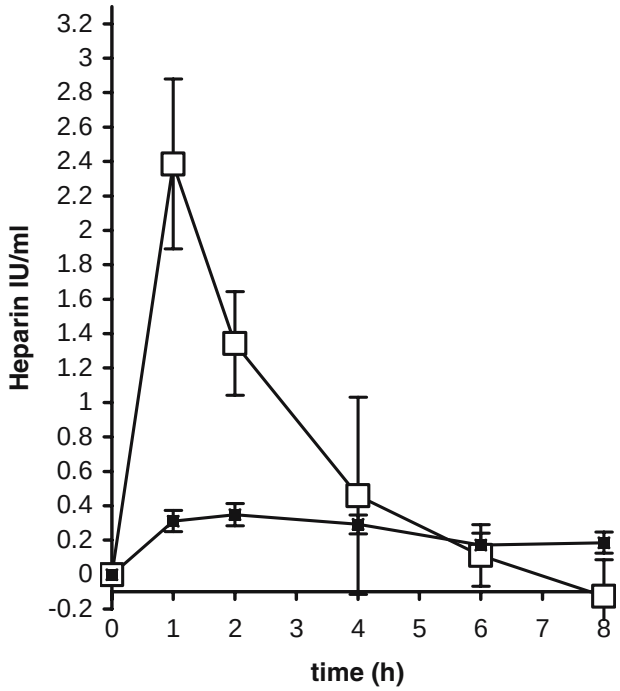

Fig. 5. Comparison of the concentration profiles of heparin in plasma obtained after subcutaneous administration of 53.3 IU heparin (*empty square*) and oral administration of mini-tablets containing 210 IU heparin, HEC and papain (2/3.3/9.7 mg) (*filled square*). Data represent means ± S.D. of at least three experiments for oral and five experiments for subcutaneous administration.

induce the antithrombotic effect were maintained for about 160 min. However, due to variations in different experimental setups such as animal species, analytic methods and administration pathway, a direct comparison with these results is unfortunately not feasible.

In order to achieve an effective bioavailability, it is not only important to find an effective permeation enhancer, but also to develop drug delivery systems that are able to provide a constant heparin blood level over a prolonged period of time. Strategies to improve corresponding delivery system include enlargements of the contact area between the drug and intestinal mucosa by incorporation in micro-(3) and nanoparticles (25), as well as prolongation of contact time by taking advantage of the phenomenon of mucoadhesion (26). For example, Jiao *et al.* (3) used heparin loaded nanoparticles, achieving a bioavailability of 22.7% in comparison to the control. Further on, the results of experiments performed by our research group showed that PCP-cysteine/gluthation, as a mucoadhesive matrix, provides both, significantly increased LMWH absorption and prolonged duration of effectiveness (26). Since such mucoadhesive formulation ensured the maintenance of the heparin blood level over 24 h, the strategy of combining papain as a permeation enhancer and thiomer matrix as a device to prolong the drug residence in the gastrointestinal tract could lead to a substantial progress in efforts to increase both, heparin bioavailability and duration of effectiveness.

CONCLUSION

Papain was identified as an effective intestinal permeation enhancer for heparin *in vitro*. The permeation enhancing effect was most pronounced when papain was added to heparin in a molar ratio of 1:1. This study provides the proof of concept that papain with its cationic moiety interacts with the negatively charged heparin forming a stable complex. Further on, a considerable improvement of absorption rate of heparin in rats was obtained by the administration of oral formulations comprising papain and heparin embedded in an inert carrier matrix. Co-administration of papain with heparin showed encouraging results and therefore might represent a promising new strategy to increase the bioavailability of orally administered heparins.

REFERENCES

1. T. Rivera, A. Leone-Bay, D. R. Paton, H. Leipold, and R. A. Baughman. Oral delivery of heparin in combination with N-[8-(2-hydroxybenzoyl)amino]caprylate: pharmacological considerations. *Pharm. Res.* **14**:1830–1834 (1997).
2. I. Caramazza, G. D'Atri, M. Bossi, F. DePonti, L. D'Angelo, and A. Crema. Intraduodenal absorption of the new UF-heparin salt ITF 1057 in the conscious dog. *Thromb. Res.* **62**:785–789 (1991).
3. Y. Jiao, N. Ubrich, V. Hoffart, M. Marchand-Arvier, C. Vigneron, M. Hoffman, and P. Maincent. Anticoagulant activity of heparin following oral administration of heparin-loaded microparticles in rabbits. *Pharm. Sci.* **91**:760–768 (2002).
4. A. Bernkop-Schnürch, A. E. Clausen, and D. Guggi. The use of auxilliary agents to improve the mucosal uptake of peptides. *Med. Chem.* **1**:1–10 (2004).
5. J. L. Madara and J. R. Pappenheimer. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J. Membr. Biol.* **100**:149–164 (1987).
6. N. F. H. Ho, T. J Raub, and P. S. Burton. *Transport Processes in Pharmaceutical Systems*, Marcel Dekker, New York, 2004.
7. A. Bernkop-Schnürch, R. Giovanelli, and C. Valenta. Peroral administration of enzymes: strategies to improve the galenic of dosage forms for trypsin and bromelain. *Drug Dev. Ind. Pharm.* **26**:115–121 (2000).
8. U. Bock, C. Kolac, G. Borchard, K. Koch, R. Fuchs, P. Streichhahn, and C.-M. Lehr. Transport of proteolytic enzymes across caco-2 cell monolayers. *Pharm. Res.* **15**:1393–1400 (1998).
9. A. Fasano, S. Uzzau, C. Fiore, and K. Margaretten. The enterotoxic effect of zonula occludens toxin on rabbit small intestine involves the paracellular pathway. *Gastroenterology* **112**:839–846 (1997).
10. V. Dalet-Fumeron, L. Boudjennah, and M. Pagano. Binding of the cysteine proteinases papain and cathepsin B-like to coated laminin: use of synthetic peptides from laminin and from the laminin binding region of the beta 1 integrin subunit to characterize the binding site. *Arch. Biochem. Biophys.* **358**(2):283–290 (1998).
11. K. Moin, L. Cao, N. A. Day, J. E. Koblinski, and B. F. Sloane. Tumor cell membrane cathepsin B. *Biol. Chem.* **8–9**:1093–1099 (1998).
12. D. M. K. Lam. Biosynthesis of acetylcholine in turtle photoreceptors. *Proc. Natl. Acad. Sci. USA*. **69**:1987–1991 (1974).
13. D. Guggi and A. Bernkop-Schnürch. Improved paracellular uptake by the combination of permeation enhancers acting in different ways. *Int. J. Pharm.* **288**:141–150 (2005).
14. G. J. Martin, R. Brendel, and J. M. Beiler. Absorption of enzymes from the intestinal tract. *Am. J. Pharm.* **127**:194–197 (1957).
15. J. L. Ambrus, H. B. Lassman, and J. J. DeMarchi. Absorption of exogenous and endogenous proteolytic enzymes. *Clin. Phamacol. Ther.* **8**:362–368 (2004).
16. L. A. Sluyterman. The activation reaction of papain. *Biochim. Biophys. Acta* **139**:430–438 (1966).
17. B. W. Watson, S. J. Meldrum, H. C. Riddle, R. L. Brown, and G. E. Sladen. pH profile of gut as measured by radiotelemetry capsule. *Br. Med. J.* **2** (5805):104–106 (1972).
18. A. Bernkop-Schnürch, C. Valenta, and S. M. Daee. Peroral polypeptide delivery. A comparative *in vitro* study of mucolytic agents. *Drug Res.* **49**:799–803 (1999).
19. S. Solis-Mendiola, A. Arroyo-Reyna, and A. Hernandez-Arana. Circular dichroism of cysteine proteinases from papaya latex. Evidence of differences in the folding of their polypeptide chains. *Biochim. Biophys. Acta* **1118**:288–292 (1992).
20. R. Menard, H. E. Khouri, C. Plouffe, R. Dupras, D. Ripoll, T. Vernet, D. C. Tessier, F. Lalberte, D. Y. Thomas, and A. C Storer. A protein engineering study of the role of aspartate 158 in the catalytic mechanism of papain. *Biochemistry* **29**:6706–6713 (1990).
21. P. C. Almeida, I. L. Nantes, C. C. A. Rizzi, W. A. S. Júdice, J. R. Chagas, L. Juliano, H. B. Nader, and I. L. S. Tersariol. Cysteine Proteinase Activity Regulation: A possible role of heparin and heparin-like glycosaminoglycans. *J. Biol. Chem.* **274**:30433–30438 (1999).
22. T. Schmitz, V. Leitner, and A. Bernkop-Schnürch. Evaluation of the stomach-targeted uptake of different low molecular weight heparins in rats from tablets based on a mucoadhesive polycarbophil-cysteine matrix. *Int. J. Pharm.* **3**:343–345 (2004).
23. P. Bianchini, G. L. Berngonzini, B. Parma, and B. Osima. Relationship between plasma antifactor Xa activity and the antithrombotic activity of heparins of different molecular mass. *Haemostasis* **25**(6):288–298 (1995).
24. R. Y. V. Prasad, T. Minamaimoto, Y. Yoshikawa, N. Shibata, S. Mori, A. Matsuura, and K. Takada. *In situ* intestinal absorption studies on low molecular weight heparin in rats using Labrasol as absorption enhancer. *Int. J. Pharm.* **271**:225–232 (2004).
25. Y. Jiao, N. Ubrich, M. Marchand-Arvier, C. Vigneron, M. Hoffman, T. Lecompte, and P. Maincent. *In vitro* and *in vivo* evaluation of oral heparin-loaded polymeric nanoparticles in rabbits. *Circulation* **105**:230–235 (2002).
26. C.E. Kast, D. Guggi, N. Langoth, and A. Bernkop-Schnürch. Development and *in vivo* evaluation of an oral delivery system for low molecular weight heparin based on thiolated polycarbophil. *Pharm. Res.* **20**:931–936 (2003).