Development and *in Vivo* Evaluation of an Oral Delivery System for Low Molecular Weight Heparin Based on Thiolated Polycarbophil

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Purpose. It was the purpose of this study to develop a new oral drug delivery system for low molecular weight heparin (LMWH) providing an improved bioavailability and a prolonged therapeutic effect.

Methods. The permeation enhancing polycarbophil–cysteine conjugate (PCP–Cys) used in this study displayed 111.4 ± 6.4 μM thiol groups per gram polymer. Permeation studies on freshly excised intestinal mucosa were performed in Ussing chambers demonstrating a 2-fold improved uptake of heparin as a result of the addition of 0.5% (w/v) PCP–Cys and the permeation mediator glutathione (GSH).

Results. Tablets containing PCP–Cys, GSH, and 279 IU of LMWH showed a sustained drug release over 4 h. To guarantee the swelling of the polymeric carrier matrix in the small intestine tablets were enteric coated. They were orally given to rats. For tablets being based on the thiomer/GSH system an absolute bioavailability of 19.9 ± 9.3% (means ± SD; n = 5) vs. intravenous injection could be achieved, whereas tablets comprising unmodified PCP did not lead to a significant ($p < 0.01$) heparin concentration in plasma. The permeation enhancing effect and subsequently a therapeutic heparin level was maintained for 24 h after a single dose.

Conclusions. Because of the strong and prolonged lasting permeation enhancing effect of the thiomer/GSH system, the oral bioavailability of LMWH could be significantly improved. This new delivery system represents therefore a promising tool for the oral administration of heparin.

KEY WORDS: heparin; oral drug delivery system; bioavailability; thiomer; reduced glutathione.

INTRODUCTION

Heparin is a glycosaminoglycan used mainly as an anticoagulant substance for the prevention of venous thrombosis and pulmonary embolism in patients undergoing surgery and postsurgical complications (1,2). Because of its relatively large size with an average molecular weight of about 20 kDa and numerous negative charges, the gastrointestinal absorption after peroral administration is very poor (3). Consequently it has to be administered by the parenteral route, which requires careful patient monitoring. Moreover, high doses have to be given inducing the anticoagulation. Rather often, bleeding complications may occur. Numerous attempts have therefore been undertaken to overcome these shortcomings. One promising alternative is the use of low molecular weight heparins (LMWHs; Mw approx.2–8 kDa), which can be self-administered via subcutaneous injection and do not need an intensive monitoring of the patient. Furthermore, the anticoagulant response is predictable as a protein binding can be excluded (1). In addition, because of its comparatively lower molecular size, the oral administration of LMWH seems feasible (4). Because the oral administration represents the most convenient way of dosing for the patients, several research groups have meanwhile tried to find suitable strategies to facilitate the gastrointestinal absorption of orally delivered LMWHs. All these attempts are mainly based on the use of permeation-enhancing systems, such as organic acids or bases, bile salts, or liposomes (3,5–7). Most concepts, however, have failed so far because the permeation enhancers are absorbed much more rapidly from the intestine than the drug itself (8).

A new and completely different type of permeation enhancers compared with commonly used permeation enhancers can be seen in the thiolated polymers or so-called thiomers. In contrast with low molecular weight permeation enhancers, they are not absorbed from the mucosal tissue because of their larger size. Thiomers are mucoadhesive polymers displaying thiol groups, which are responsible for strongly improved mucoadhesive and permeation enhancing properties (9–12). It was shown recently that thiolated polyacrylates, such as polycarbophil-cysteine (PCP-Cys) or poly-(acrylic acid)-cysteine, enhance the permeation of hydrophilic compounds significantly (13). Promising results have already been obtained *in vivo* by the oral administration of insulin with PCP-Cys as matrix system (14). Furthermore, the addition of reduced glutathione (GSH) being very poorly absorbed from the intestinal tract (15) has a positive impact on the permeation enhancing effect of thiolated polyacrylates (16).

It was the aim of this study, to develop a new delivery system for the oral administration of LMWH. This delivery system is based on thiolated PCP and GSH providing a prolonged uptake and consequently an improved bioavailability of LMWH in comparison with recently established dosage forms. Within this study the newly created dosage form is tested *in vitro* with regard to its release of LMWH and its permeation enhancing properties across small intestinal mucosa. Furthermore, the efficacy of the new delivery system is evaluated *in vivo*.

MATERIALS AND METHODS

Preparation of the PCP–Cys Conjugate

The covalent attachment of cysteine to neutralized polycarbophil (Na–PCP; Mw = $3.5*10^9$ [g/Mol]; Noveon AA1, BF Goodrich, Brecksville, OH, USA) was achieved by the formation of amide bonds between the primary amino group of cysteine and the carboxylic acid moieties of the polymer as described previously by our research group (10). In brief, 1 g of Na–PCP was hydrated in 250 mL of demineralized water. The carboxylic acid moieties of the polymer were activated for 45 min by the addition 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Sigma, MO, USA) in a final concentration of 50 mM. The pH of the reaction mixture was adjusted to 5 with 1 M NaOH. Cysteine–hydrochloride was added to the activated Na–PCP in a weight-ratio 5:1 (poly-

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mer:cysteine). The pH of the reaction mixture was again adjusted to 5 either with 1 M HCl or 1 M NaOH. The reaction mixture was incubated for 3 h under stirring at room temperature. The resulting polymer-cysteine conjugate was isolated by dialyzing at 10°C in the dark against 0.2 mM HCl, two times against the same medium but also containing 1% NaCl—in order to quench ionic interactions between the polymer and the sulfhydryl compound—and then again two times against 0.2 mM HCl. The acidic medium was necessary to avoid the oxidation of the thiolated polymer. A sample being prepared and isolated in exactly the same way as the PCP–Cys conjugate, but without 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride during the coupling reaction, served as control. The obtained polymer solution was lyophilized by drying the frozen aqueous polymer solution at –30°C and 0.01 mbar (Christ Beta 1-8K; Osterode am Harz, Germany). Polymer–cysteine conjugate and control were stored at 4°C until further use.

Determination of the Thiol Group Content

The degree of modification, i.e., the amount of thiol groups immobilized on the polymer, was determined photometrically with Ellman's reagent quantifying free thiol groups. First, 0.5 mg each of the conjugates and the control was hydrated in 500 μL of 0.5 M phosphate buffer pH 8.0 and then 500 μL of Ellman's reagent (3 mg dissolved in 10 mL of 0.5 M phosphate buffer, pH 8.0) were added. The samples were incubated for 2 h at room temperature. Thereafter, 300 μL of each sample was transferred into a microplate and the absorbency was measured at a wavelength of 450 nm with a microplate reader (Anthos reader 2001; Salzburg, Austria). L-Cysteine standards were used to calculate the amount of thiol groups immobilized on the polymer.

Preparation of the LMWH Dosage Form

To homogenize the PCP-Cys conjugate with heparin, 50 mg of lyophilized polymer was hydrated in 4 mL of demineralized water. 30 mg of LMWH (ICN Biomedicals, 93 IU/mg) and 20 mg of reduced GSH (Sigma, St. Louis, MO, USA) were each dissolved in 500 μL of demineralized water and added to the polymer solution. Neutralized PCP (70 mg) and 30 mg of heparin dissolved in 5 mL of demineralized water were used as control. The mixtures were frozen at –80°C and lyophilized. Thereafter, the freeze-dried homogenates were divided in equal parts and compressed into 10-mg tablets (diameter: 2.5 mm, height: 1 mm). The compaction pressure was kept constant during the preparation of all tablets. The hardness of the tablets was determined with a Pharma Test PTB 311 (Hainburg, Germany). The stability of polymer tablets in 50 mM phosphate buffer pH 6.8 at 37°C was analyzed with a disintegration test apparatus according to the European Pharmacopeia with an oscillating frequency of 0.5 s^{-1}. Afterwards, tablets were enteric coated with Eudragit L 100-55 (Röhm GmbH, Chem. Fabrik, Darmstadt, Germany) to guarantee a swelling and consequently improved adhesion of the polymeric carrier matrix in the intestine. The coating was achieved by dipping the tablets five times into a 3% (w/v) acetonic Eudragit L 100-55 solution followed by air drying according to a method described previously (14).

In vitro Evaluation of the Drug Release from Tablets

The dosage forms containing either 279 IU heparin and GSH or only 279 IU heparin were each placed in a 5-mL glass vessel containing 4 mL of release medium (100 mM phosphate buffer pH 6.8). The vessels were closed, placed on an oscillating water bath (GFL 1092; 100 rev/min), and incubated at 37°C ± 0.5°C. Sink conditions were maintained throughout all studies. Aliquots of 100 μL were withdrawn in 30- and 60-min intervals, respectively, over a period of 8 h. Withdrawn samples were replaced with an equal volume of release medium equilibrated at 37°C. The amount of LMWH was evaluated using a modified method originally established by Blumenkrantz and Asboe–Hansen (17). In brief, each sample (100 μL) was mixed with 600 μl of 0.0125 M tetraborate in concentrated sulfuric acid on crushed ice and then heated for 1 min at 95°C. After cooling for 5 min on ice, 10 μL of a 0.15% solution of meta-hydroxydiphenyl in 0.5% (w/v) NaOH was added. After an incubation period of 5 min under continuos shaking absorbency was measured at 524.5 nm (Lambda-16, Perkin–Elmer, Austria). Amounts of LMWH were calculated by a standard curve. Cumulative corrections were made for previously removed samples.

In Vitro Permeation Studies

Permeation studies were performed in Ussing type chambers displaying a volume of both, donor and acceptor chamber, of 1 ml (=1 cm^3) and a permeation area of 0.64 cm^2 (11,12). To mimic the intestinal fluid an incubation medium was prepared containing 250 mM NaCl, 2.6 mM $MgSO_4$, 10 mM KCl, 40 mM glucose and 50 mM $NaHCO_3$ buffered with 40 mM HEPES, pH 7.4.

Immediately after sacrificing the guinea pig, 15 cm of the small intestine (duodenum) were excised and mounted in the Ussing chamber. All experiments were performed at least four times in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. After 15–20 min of preincubation with the artificial intestinal fluid, the incubation medium of the donor compartment was substituted by either PCP-Cys conjugate (0.5% w/v) containing 0.5% (w/v) GSH, PCP-Cys conjugate, or the unmodified polymer (0.5% w/v). Furthermore each sample contained 0.5% heparin (w/v; 465 IU/ml). As control, 0.5% heparin in buffer only was used. Aliquots of 100 μL were withdrawn from the acceptor compartment every 30 min over a time period of 3 h. Samples were immediately replaced by 100 μl of artificial intestinal fluid equilibrated at 37°C. The amount of permeated heparin was determined using a chromogenic assay (Heparin Accucolor, Methode N°CRS106, SIGMA-Diagnostics®), which measures the factor Xa activity, being inversely proportional to the amount of the polysaccharide in the sample. Cumulative corrections were made for the previously removed samples. The apparent permeability coefficients (P_{app}) for heparin were calculated according to the following equation:

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

where P_{app} is 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 the marker in 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 according the following equation:

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

In Vivo Evaluation of the Delivery System

The protocol for the animal studies was approved by the Ethical Committee of Austria. Male Wistar rats SPF (average body weight 250 g) were obtained from the Institut für Labortierkunde und Genetik, University of Vienna. Before dosing the animals 180 μL of blood samples, which were collected in vessels containing 20 μL of a 3.8% sodium-citrate solution were taken from the tail vein. Samples withdrawn at time point zero served as reference. Rats were treated separately with different delivery systems (see Table I). On the one hand, LMWH/PCP tablets or LMWH/PCP-Cys/GSH tablets, each containing 279 IU of heparin (797 IU/kg), were orally administered to nonanesthetized animals by placing the tablets deeply into the throat to initiate the swallow reflex. Additionally, 200 μL of a 0.1 M aqueous ascorbic acid solution was administered. On the other hand, LMWH was given in 200 μL of ascorbic acid solution (0.1 M; 279 IU/rat). To determine the oral bioavailability vs. intravenous injection, rats were dosed with LMWH by intravenous injection (tail vein) of 200 μL sterile LMWH solution (46.5 IU/rat; in 0.9% sterile NaCl solution).

The dosed rats were fasted during the study and kept in restraining cages with free access to water. Blood samples of 180 μL were taken at 120-min intervals for 12 h and then after 24 h from the tail vein. In case of intravenous application an additional blood sample was withdrawn after 60 min. Blood samples were centrifuged (4000 *g* for 5 min) and plasma samples were collected and stored at –20°C until analysis. The amount of heparin in plasma was determined at least two times from each sample using the chromogenic assay mentioned above.

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 were calculated according to the linear trapezoidal rule. The absolute bioavailability was calculated from the dose corrected areas under the curves for oral vs. intravenous routes of administration.

Statistical Data Analysis

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

RESULTS

Characterization of the PCP–Cys Conjugate

Cysteine was attached to PCP via an amide bond formation between the primary amino groups of cysteine and the carboxylic acid moieties of PCP as described previously (10). The amount of thiol groups attached covalently to the polymer was 111.4 ± 6.4 μM/g polymer (mean ± SD n = 3). The purification of the conjugate via dialysis was efficient as no thiol groups could be detected on the control polymer. Obtained results were in good accordance with former studies (10). The obtained polymer was white, odorless, and showed a fibrous structure. It was easy swellable in water and buffer solutions. The point of decomposition of the PCP-Cys conjugate was 250°C, whereas it was 310°C for the unmodified polymer. The polymer was easy compressible to tablets, which had a hardness of 36.3 ± 2.9 N (n = 5) and displayed a disintegration time of >12 h (n = 4).

In vitro Release of Heparin

A comparison of the release rates from tablets based on unmodified PCP and PCP–Cys/GSH is given in Fig. 1. Compared with the control, the test formulation showed a pseudo zero-order release profile until reaching a plateau phase after 5 h, when approximately 35% of the drug was released. The addition of GSH to tablets containing PCP-Cys influenced neither their disintegration behavior nor the release profile (data not shown).

In vitro Permeation Studies

The permeation enhancing effect of unmodified PCP and the corresponding thiolated polymer on the transport of heparin was evaluated on freshly excised intestinal mucosa. Permeation studies were carried out with PCP–Cys and optionally GSH. Unmodified PCP and PCP–Cys without GSH showed no significant increase in the permeation of heparin across the mucosal membrane, whereas PCP–Cys in presence of GSH displayed a strong increase in the permeation of heparin with about 1.25 IU/mL (~0.26%) compared to the control buffer solution (Fig. 2). The apparent permeability coefficient was more than 2-fold higher than that of the control (Table II).

In case of the thiomer/GSH system the permeation across the mucosa was accompanied by a relative strong decrease in the transepithelial electrical resistance (TEER), indicating the loss in tightness of the intercellular junctions (16). This in turn indicates the opening of the paracellular route across the epithelium for hydrophilic compounds. After removing the thiomer/GSH system the TEER increased again

Table I. Characteristics of the Different Delivery Systems Used for *in Vivo* Studies

	Heparin	Polymer	GSH	Additional information
Thiomer/glutathione delivery system	3 mg (=279 IU)	5 mg PCP-Cys	2 mg	10 mg tablet, coated with Eudragit 100–55 L
Unmodified polymer delivery system	3 mg (=279 IU)	7 mg PCP	—	10 mg tablet, coated with Eudragit 100–55 L
Oral solution	3 mg (=279 IU)	—	—	Heparin dissolved in 200 μl ascorbic acid
Intravenous solution	0.5 mg (=46.5 IU)	—	—	Heparin dissolved in 200 μL sterile 0.9% NaCl

Note: PCP-Cys: polycarbophil–cysteine conjugate.

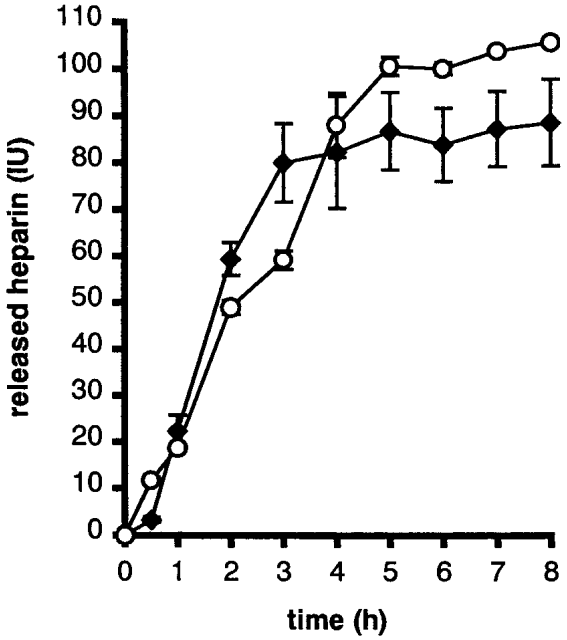

Fig. 1. *In vitro* release profiles of heparin from 10 mg tablets comprising 7 mg of unmodified PCP and 3 mg of heparin (=279 IU) (♦) and 5 mg of PCP-Cys, 2 mg of GSH and 3 mg of heparin (○), respectively. Studies were performed in 100 mM phosphate buffer pH 6.8. Data represent the release of heparin of means ± SD of three experiments.

and reached almost the same level as in the beginning of the study, indicating a reversible mechanism of opening the tight junctions (data not shown). These findings are in good agreement with previous studies focusing on the permeation enhancing effect of thiomers and their influence on the TEER (11,12,16).

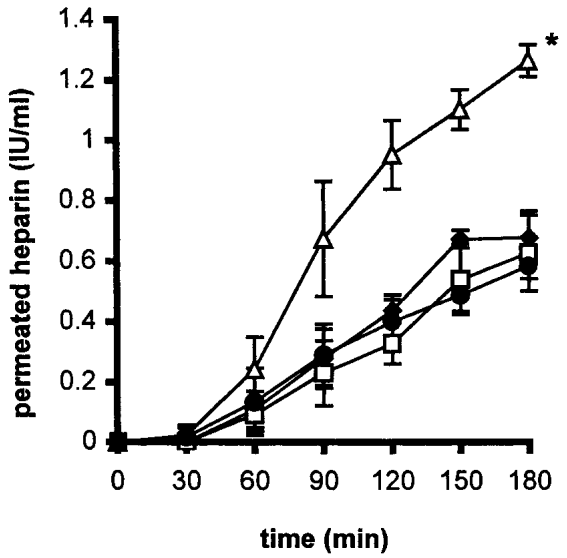

Fig. 2. Comparison of the permeation enhancing effect of unmodified PCP, PCP-Cys, and PCP-Cys/GSH, respectively. Permeation studies were carried out in Ussing type chambers with PCP-Cys/GSH (Δ), with PCP-Cys (□), unmodified PCP (♦), and buffer as control (●). Each sample contained 465 IU/mL of LMWH. Data represent the transport of heparin through intestinal mucosa of means ± SD of at least 4 experiments. * differs from control $p < 0.001$.

In Vivo Study

The different drug delivery systems tested in this study are listed in Table I. The oral administration of heparin with PCP–Cys as carrier matrix and GSH as permeation mediator (thiomer/GSH delivery system) resulted in a significantly increased absorption of LMWH compared with control tablets comprising unmodified PCP (unmodified polymer delivery system) or to an orally given aqueous heparin solution (Figs. 3 and 4, Table III). An absolute bioavailability of 19.9 ± 9.3% compared to intravenous application was thereby obtained. Control tablets with heparin showed a slight increase in the bioavailability determined to be 5.8 ± 1.4% compared to the oral heparin solution (2.3 ± 2.8%). Furthermore, the thiomer/GSH delivery system displayed a prolonged efficacy of heparin compared with the other formulations, as the maximum with 0.4 ± 0.16 IU/mL was reached after 12 h and the efficacy seemed to maintain for at least additional 12 h (Fig. 4).

DISCUSSION

Because of a molecular weight in the range of 2–8 kDa and the relatively high charge density, the permeation of LMWH across mucosal membranes is rather low (18). To achieve therapeutic blood levels of heparin by oral delivery, permeation enhancers are in great demand. Investigations on numerous permeation enhancers revealed that local and/or systemic side effects are limiting factors for their application (8,19). One of the most recently reported approaches to improve the oral uptake of LMWH is based on the use of new delivery agents, the so-called *N*-[8-(2-hydroxybenzoyl)amino] caprylate and *N*-[8-(2-hydroxybenzoyl)amino]decanoate (20,21). It is suggested that these compounds can form complexes with heparins, which are able to pass the intestinal barrier (18,20,21). The use of thiolated polymers and the addition of GSH seems to be a new alternative strategy for permeation enhancement (16). The advantage of using PCP-Cys in combination with GSH is the large molecular weight of PCP (>700 kDa). Because of its size it is not absorbed from the mucosa (22). Hence, systemic toxic side effects caused by the carrier matrix can be excluded. Native GSH is present on the apical side of the mucosa and is involved in detoxification processes (23). As shown by Yoshimura *et al.* orally given GSH is only poorly absorbed from the intestinal tract exhibiting poor ability to permeate the membrane (15).

Permeation studies were performed with unmodified PCP, PCP–Cys, and PCP-Cys with GSH, respectively. Obtained results showed that a strongly enhanced permeation of heparin was achieved by the combination of PCP–Cys and GSH. The apparent permeation coefficient of heparin in buffer only as well as in the presence of PCP or PCP-Cys was almost the same (Table II). In contrast, the amount of permeated heparin increased by more than 100% in the presence of PCP–Cys/GSH (Table II). The lag time within the first 30 min can be explained by the size of heparin. LMWH, as a relatively large molecule, needs some time to diffuse through the membrane in sufficient quantities. The following strongly increased permeation of the drug in the presence of the thiomer/GSH system can be explained by the interaction of the mediator GSH with thiol groups like the thiol group of the enzyme protein tyrosine phosphatase (PTP). PTP is able to dephosphorylate tyrosine residues of occludin, which is believed to play an essential role in the opening of the tight

Table II. Influence of the PCP-Cys/GSH System on the Apparent Permeability Coefficient (P_{app}) for Heparin in Comparison to that of PCP-Cys or the Unmodified Polymer

Test compound	GSH	Apparent permeability coefficient [$P_{app} \times 10^{-7}$ (cm/sec)], means ± SD; n = 4–7	Enhancement ratio (P_{app} sample/P_{app} control)
0.5% (m/v) PCP–Cys	16 mM	3.9* ± 0.2	2.2
0.5% (m/v) PCP-Cys	—	1.9 ± 0.4	1.1
0.5% (m/v) PCP	—	2.1 ± 0.3	1.2
Buffer without polymer	—	1.8 ± 0.1	1

GSH: glutathione; PCP–cys: polycarbophil–cystine conjugate.
*Differs from control $p < 0.001$.

junctions. This dephosphorylation results in the closing of the tight junctions, leading consequently to a decreased permeation of hydrophilic macromolecules. According to this theory, the inhibition of PTP must lead to an opening of the tight junctions and further to an increase in permeability. The presence of the thiolated polymer is essential as it prevents the oxidation of GSH on the surface of the mucosa. The ability of PCP-Cys and other thiolated polyacrylates displaying a molecular weight ≥ 450 kDa to reduce oxidized glutathione has already been shown by our research group (16). By the use of these polymers in combination with GSH the permeation of hydrophilic compounds through small intestinal mucosa was enhanced significantly. Corresponding permeation studies with GSH but without a thiolated polymer showed only a slight enhancement in the permeation of hydrophilic compounds (16).

Evidence for the potential of the PCP–Cys/GSH system is given by the significant increase in the absorption of heparin *in vivo*. The bioavailability (Table III) of the drug embedded in tablets comprising PCP-Cys/GSH was at least 19.9 ± 9.3% vs. intravenous administration. The amount of injected heparin was just 1/6 of the orally given dose, as an intravenous administration of 279 IU/rat would have been dangerous for the animals probably resulting in bleeding complications (24,25). Furthermore, also the therapeutic effect of heparin was prolonged significantly. As depicted in Fig. 4 even 24 h after administration of heparin a significant ($p < 0.01$ compared to the oral solution) concentration of 0.31 ± 0.16 IU/ml blood was determined. This indicates a prolonged efficacy of heparin for more than 24 h contributing to this high bioavailability. Jiao *et al.* described a comparatively shorter duration of effectiveness for about 8 h by the administration of heparin loaded nanoparticles with a resulting bioavailability of 22.7% (4). Rivera *et al.* reported about a duration of effectiveness of maximum three hours for an oral administered solution of heparin (24). In view of these data obtained by other research groups the results achieved with the PCP-Cys/GSH delivery system are encouraging. It was the first time that the efficacy of the thiomer/GSH permeation enhancing system could be verified *in vivo*.

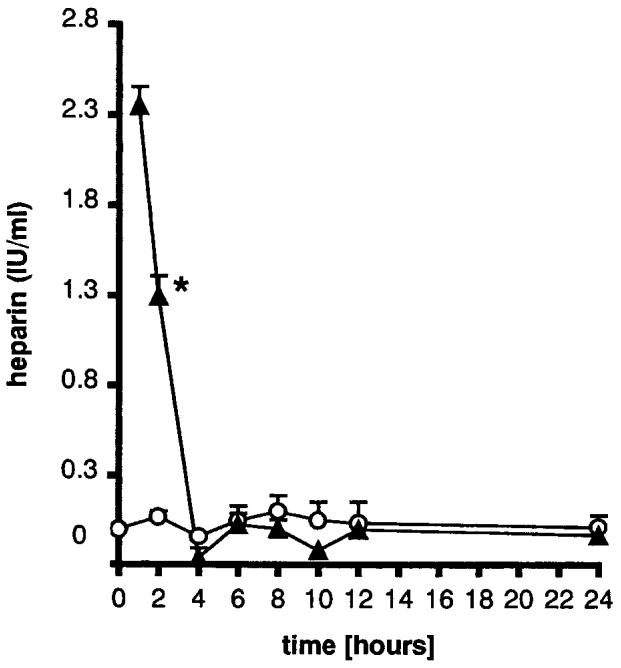

Fig. 3. Comparison of the concentration profiles of heparin in plasma obtained after intravenous (46.5 IU; ▲) and peroral (aqueous solution; 279 IU; ○) administration of heparin to rats. Data represent the mean ± SD of n = 3 for the intravenous administration and n = 4 for the oral solution. * differs from peroral administration (aqueous solution) $p < 0.001$.

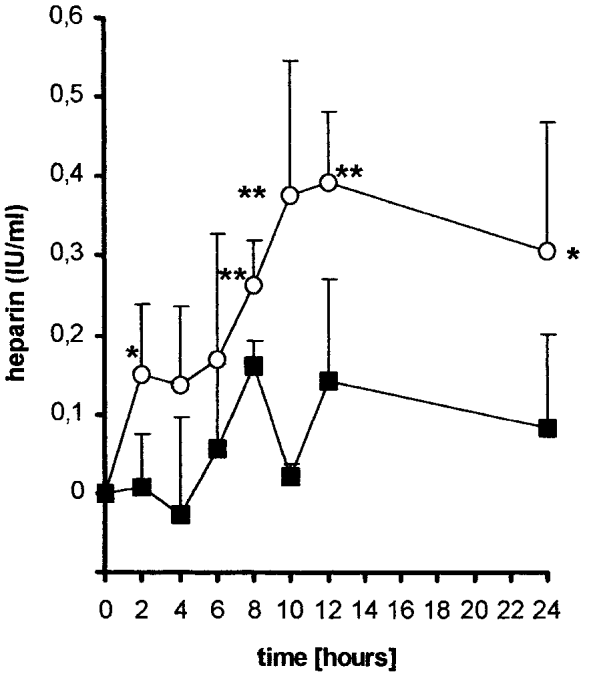

Fig. 4. Comparison of the concentration profiles of heparin in plasma obtained after peroral administration of 279 IU of heparin incorporated in the thiomer/GSH delivery system (○) and in the unmodified polymer delivery system (■) in rats. Data represent the mean ± SD of n = 5 for both delivery systems. * differs from control $p < 0.05$; ** differs from control $p < 0.01$.

Table III. Main Pharmacokinetic Parameters after Oral Administration of the Thiomer/GSH Delivery System, the Unmodified Polymer Delivery System, and the Oral Solution, Respectively, as Well as after Intravenous Administration of Heparin to Rats (means ± SD, n = 3–5)

Formulation	Thiomer/GSH delivery system	Unmodified polymer delivery system	Oral solution	Intravenous solution*
C_{max}; IU/mL	0.4 ± 0.2	0.2 ± 0.0	0.0 ± 0.1	—
t_{max}; h	12	6	—	—
$AUC_{0\rightarrow24}$/rat; IU/mL ∗ h	6.8 ± 3.2	2.0 ± 0.5	0.8 ± 1.0	5.7 ± 1.0
Absolute bioavailability; %	19.9 ± 9.3	5.8 ± 1.4	2.3 ± 2.8	100

Note for intravenous solution: AUC has to be multiplied by 6 because the dose administered intravenously was exactly 1/6 of the dose given orally.
GSH: glutathione.

CONCLUSION

The strong permeation enhancing effect of the thiomer/GSH system for hydrophilic compounds has already been shown in various *in vitro* studies. As thiomers and GSH are both not absorbed from the intestine in significant quantities a prolonged permeation enhancing effect could be expected. Within this study both theories could be verified *in vivo*, as a comparatively high oral bioavailability and the so far longest lasting therapeutic effect of orally administered heparin was achieved by the use of the thiomer/GSH delivery system. The thiomer/GSH system represents therefore a promising novel tool for the oral administration of heparin.

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