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RESEARCH PAPER

Oral Delivery of Low Molecular Weight Heparin by Polyaminomethacrylate Coacervates

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ABSTRACT

Purpose Oral bioavailability of low molecular weight heparin (LMWH) can be achieved by several advanced drug delivery approaches. Here, a new preparation method for coacervates (CAs) using non-toxic polyethylene glycol derivates was developed.

Methods LMWH were coacervated with polyaminomethacrylates (Eudragit® RL or RS) using polyethylene glycol (PEG) derivatives as non-toxic solvents. CAs were analyzed for their physicochemical properties and pharmacokinetic parameters were determined for different formulations in rabbits.

Results CAs from both polymer types using various PEGs were of irregular shape and had particle sizes of around 40 μm, encapsulation efficiencies of >90%, and complete LMWH *in vitro* release was obtained within 2 h. *In vivo*, oral Absorption at doses of 300 IU/kg was rather low (F < 2.5%) while dose increase resulted in a maximum at 600 IU/kg (F_{RL}: 6.0 ± 1.2%; F_{RS}: 5.8 ± 2.5%) and 1,200 IU/kg did not result in higher bioavailability (F_{RL}: 4.6 ± 0.4%; F_{RS}: 4.1 ± 0.8%). CAs were applicable to various LMWH types where the oral availability decreased in the order fondaparinux>enoxaparin>nadroparin>certoparin depending mainly on the molecular weight.

Conclusions CAs prepared by an organic solvent-free method allowed the oral delivery of LMWHs. The therapeutic efficiency and the simple and solvent-free manufacturing process underlines the high potential of this new preparation method.

KEY WORDS coacervates · glycofurol · LMWH · oral drug delivery · polyethylene glycol

ABBREVIATIONS

%	percent
°C	degree Celsius
μl	microlitre
μm	micrometer
AUC	area under the curve
$BaSO_4$	barium sulphate
CAs	coacervates
c_{max}	maximum concentration
Da/kDa	dalton/kilodalton
EE	encapsulation efficiency
F	bioavailability
g	acceleration of gravity
GLY	glycofurol
IU	international units
LMWHs	low molecular weight heparins
min	minutes
ml	millilitre
mM	millimolar
MPs	microparticles
nm	nanometer
NPs	nanoparticles
PEG	polyethylene glycol
PLGA	poly(lactic-co-glycolic acid)
RL	Eudragit® RL
rpm	rounds per minute
RS	Eudragit® RS
s	seconds
SD	standard deviation
t_{max}	maximum concentration at this time

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INTRODUCTION

Low molecular weight heparins (LMWHs) are the standard therapy for the prophylaxis and treatment of deep vein thrombosis, pulmonary embolism and other thrombotic disease forms (1). Unfortunately, they still have to be administered subcutaneously and the parenteral administration causes many drawbacks such as pain, danger of infections, inflammations at the puncture and therefore a bad compliance is the consequence, especially for patients with long term therapies (2). Thus, an oral formulation for these polysaccharides would be highly desirable.

Diverse approaches have been investigated for the oral delivery of LMWHs. Bile salts such as deoxycholic acid which was chemically conjugated to LMWHs (3–6) or sodium cholate which was mixed with a LMWH solution (7) were used to achieve an interaction with bile salt transporters in the intestine and thereby an oral uptake. Furthermore, different enhancers were tested for oral drug delivery such as labrasol (8), sodium caprate (9) or glycyrrhetinic acid (10) or pellets containing LMWHs were administered to rabbits (11). Alternatively, nano- (NPs) and microparticles (MPs) seem to have a very high potential to achieve oral drug delivery of LMWHs. Different kinds of particles have already been studied such as chitosan NPs (12–15) and MPs prepared by complex coacervation with gelatin type A and B (16). Moreover, such particles prepared by multiple emulsion technique mainly using poly(lactic-co-glycolic acid) (PLGA), poly-(ε-caprolactone) and/or polyaminomethacrylate copolymers (17–21) achieved promising results *in vitro* and *in vivo*. Despite the fact that this technique is approved and common, it still has several disadvantages such as the use of harmful organic solvents that are standards for this preparation process. Thus, an additional step to omit these solvents after particle solidification is required to ensure that residual solvent amounts are lower than the requested specific parts per million limits (*e.g.* lyophilisation). Besides, the quality control for such dosage forms proving the fulfillment of the requested limits is time consuming and expensive (22,23).

Here we suggest an easy and fast encapsulation process for LMWH loaded particles which is based on a coacervation/desolvation step. By now, all previous described similar processes used organic solvents as acetone or ethanol which had to be removed afterwards (24). Liquid polyethylene glycol (PEG) derivatives as glycofurol (GLY), PEG 200, 300 and 400 emerged as potential non-toxic solvents for the preparation due to the fact that they are generally recognized as safe (25), water-miscible and can dissolve certain polymers (26). Polyaminomethacrylates, namely Eudragit® RS and RL (RL, RS), were selected for particle preparation due to their mucoadhesive properties as reported earlier (27).

In the following, we report the particle preparation method on the basis of PEG derivatives as polymer solvent. The particles were characterized *in vitro* and a pharmacokinetic study in rabbits revealed the influence by the polymer type, the LMWH type and dose. Finally, the gastrointestinal passage studied by x-ray was correlated with the pharmacokinetic observations.

MATERIALS AND METHODS

Materials

All LMWHs were obtained commercially from the respective marketed products. Glycofurol, PEG 200 and 300 were purchased from Sigma Aldrich (Steinheim, Germany). Polyaminomethacrylates (RL and RS) were kindly supplied by Evonik Röhm GmbH (Darmstadt, Germany). PEG 400, polysorbate 20 and 80 (Tween® 20 or 80) were obtained from Caelo (Hilden, Germany). Cetylpyridinium chloride was provided from Roth (Karlsruhe, Germany). Barium sulphate (Micropaque®) was purchased from Guerbet (Sulzbach, Germany).

Preparation of Particles

Particles were prepared by dissolving RL or RS overnight in glycofurol, PEG 200, PEG 300, PEG 400 (P200–400) (100 mg/ml). The respective polymer solution was either mixed with 1.0 ml LMWH solution (10,000 IU/ml) (LMWH particles) or 1.0 ml water (blank) by magnetic stirring for 30 min. This mixture was again mixed with 0.01% polysorbate 20 (8.0 ml) under magnetic stirring. The formulations without polymer were prepared by mixing the respective polymer solvent with 1 ml enoxaparin solution and subsequently with 0.01% polysorbate 20 under magnetic stirring for 60 min. CAs were separated from residual PEG and unentrapped LMWH by centrifugation (500 g for 5 min) and resuspended in distilled water before being dried for 24 h on a Petri dish at room temperature.

If not stated otherwise, enoxaparin was used as LMWH for all preparations except for the influence study of different LMWH types with variable molecular weight where fondaparinux, nadroparin, and certoparin were encapsulated instead.

Morphology, Particle Size, LMWH Encapsulation Efficiency and *in Vitro* Drug Release

Dry particle samples were coated with gold by Edwards Sputter Coater S150B (Crawley, UK) and subsequently, the morphology of the particles was analysed by scanning electron microscopy (S-2460 N Hitachi, Tokyo, Japan) at 15 kV.

The particle size of the formed LMWH coacervates (CAs) was measured by laser light diffraction (Helos, Sympatec®, Clausthal-Zellerfeld, Germany) whereat particles were dispersed in 40 ml of 0.2% polysorbate 80.

CAs suspensions were centrifuged for 10 min at 18,000 g (Hermle Z 233 M-2, Wehingen, Germany) and the clear supernatants were analyzed for free LMWH by a turbidimetric assay (28) as follows: 500 µl samples were mixed with 500 µl acetate buffer (pH 5, 1 M) and 2 ml of an aqueous sodium chloride solution with cetylpyridinium chloride (168.8 mM). This mixture was incubated for 1 h at 37°C and afterwards assayed at 500 nm with a micro plate reader (Wallac 1420, PerkinElmer, Turku, Finland).

The LMWH release was studied *in vitro* in phosphate buffer pH 7.4 in a closed Erlenmeyer flask on a water shaking bath (50 ml, 37°C, 75 rpm). At predetermined times 1 ml of sample was removed and replaced with fresh buffer. CAs were separated by centrifugation for 10 min at 18,000 g and the released LMWH was quantified by turbidimetry as described. Each sample was analyzed in triplicate.

In Vivo Experiments

All animal experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, US).

Female white New Zealand rabbits (3.8 to 5.6 kg) were used in this study and housed in separated cages. They were fasted for 4 h with water *ad libitum* before the experiment started, except for studies where the influence of food on oral absorption of LMWH was analysed; then all animals had free access to food. Formulations were administered orally *via* gavage at variable enoxaparin doses (300, 600, or 1200 IU/kg). Control experiments with enoxaparin solutions + PEGs (without polymer) were performed accordingly at a dose of 600 IU/kg. Enoxaparin solution and also CAs were administered subcutaneously at a dose of 150 IU/kg as reference dose. When comparing different LMWHs, fondaparinux, nadroparin, and certoparin trapping CAs were given orally at a dose of 600 IU/kg following exactly the same procedure described for enoxaparin.

Blood samples (200 µl) were withdrawn from the ear vein of the rabbits at predetermined time points and mixed with 20 µl of sodium citrate (0.129 M). The recovery of the plasma was carried out by centrifugation at 2,000 g for 10 min. The biological assay of the anti-factor Xa activity of the plasma samples was performed with ACTICHROME® Heparin (anti-Xa) (american diagnostica GmbH, Pfungstadt, Germany) according to the suppliers instructions. The area under the curve (AUC) of the concentration-time profile was calculated with the linear trapezoidal method. All described *in vivo* experiments were performed in triplicate or quadruplicate.

X-ray Analyses

The intestinal transit of RL/GLY-CAs was analyzed by $BaSO_4$ (Micropaque®, Guerbet, Sulzbach, Germany). $BaSO_4$ was concentrated by centrifugation (20 min at 4,000 g (Hermle Z 206 A, Wehingen, Germany) before use and association with CAs. After oral administration of the mixture by gavage animals were x-rayed *via* a C-arm X-ray unit (Siemens, Munich, Germany) after 15 min, 90 min and 4 h. Controls received $BaSO_4$ suspension alone. The rabbits were not anaesthetized during x-raying to avoid an influence on the intestinal transit. Thus, variable positions of stomach, gut, spinal column and further organs on the pictures were caused by changed positions of the rabbits.

Statistical Analysis

The statistical analysis was carried out with the software Sigmastat 2.0. All analyses of statistical significance were examined by Kruskal-Wallis ANOVA on Ranks followed by multiple comparisons with Student Newman-Keuls test. The *in vivo* analyses were carried out in comparison to oral enoxaparin solution and blank formulations. All data were expressed as mean ± SD. In all cases, $P<0.05$ was considered to be significant.

RESULTS

The solubility of RL and RS in the different PEG derivatives was determined in preliminary tests exhibiting sufficient solubility of both polymers in different PEG derivatives in concentrations of <20% (w/v; data not shown). Based on these solubility data, blank and LMWH CAs were successfully prepared with different non-toxic PEGs by the described one step preparation method. Microscopic studies showed that the morphology of CAs was of irregular shape and revealed that CAs were formed from smaller particles obtained from the desolvation step followed by aggregation (Fig. 1). The particle sizes varied around 40 µm and were comparable between the different formulations (Table I). Surprisingly, neither polymer nor PEG type influenced the CAs diameter. The encapsulation efficiency of all formulations was relatively high and varied between 85 and 100%. Drug release from all CAs was immediate and nearly completed within the first 30 min (Fig. 2).

Therapeutically relevant anti-Xa concentrations reaching c_{max} values above 0.1 IU/ml (5) were obtained basically with all formulations (Tables II, III and IV). The t_{max} values indicated a fast onset of the anti-Xa effect after approximative

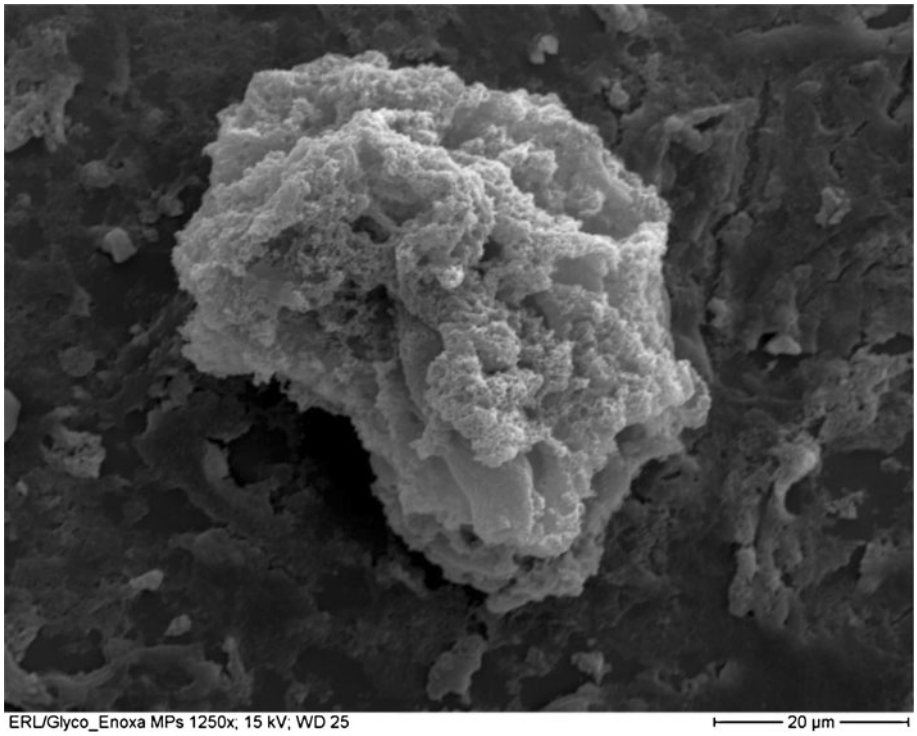

Fig. 1 Scanning electron micrograph of the RL/GLY formulation exemplarily chosen to illustrate the formation coacervates (magnification: 1250×).

1 or 2 h. Exemplarily, subcutaneous administration of RL/GLY and RS/P200 formulations maintained full activity of the entrapped LMWH (F_{RL} = 106.9 ± 40.4%, F_{RS} = 105.0 ± 26.0%) while oral controls, such as blank particles and LMWH solution mixed with the various PEGs resulted in no therapeutic effect (Fig. 3). Surprisingly, general pharmacokinetic parameters for oral administered CAs were relatively similar for either polymer. Different doses ranging from 300 to 1200 IU/kg exhibited a dose dependent LMWH availability with an optimum at 600 IU/kg and lower relative bioavailability at higher doses (Fig. 4). Highest values were achieved when using low viscosity PEGs and the influence of the polymer type was limited (F_{RL} = 6.0 ± 1.2% and F_{RS} = 5.8 ± 2.5%), respectively (Table II).

Table I Particle Size and Encapsulation Efficiency (EE) of Enoxaparin CAs Prepared with Different PEGs (A) and CAs Prepared with Different Commercially Available LMWH Types (B) (mean ± SD, n = 3)

Formulation	d50 [μm]	EE [%]
A		
RL/GLY CAs	35.3 ± 3.9	99.6 ± 5.4
RL/P200 CAs	38.9 ± 2.7	95.7 ± 3.3
RL/P300 CAs	35.6 ± 1.3	100.2 ± 1.3
RL/P400 CAs	41.1 ± 2.4	99.4 ± 4.4
RS/GLY CAs	37.8 ± 3.7	98.7 ± 3.1
RS/P200 CAs	34.8 ± 6.6	99.5 ± 1.1
RS/P300 CAs	43.7 ± 4.2	98.7 ± 4.1
RS/P400 CAs	39.4 ± 6.9	95.1 ± 4.4
B		
RL/GLY-fonda CAs	33.2 ± 3.6	88.7 ± 6.4
RL/GLY-nadro CAs	34.2 ± 5.4	84.4 ± 3.6
RL/GLY-enoxa CAs	35.3 ± 3.9	99.6 ± 5.4
RL/GLY-certo CAs	44.6 ± 7.1	91.0 ± 7.7
RS/P200-fonda CAs	28.0 ± 1.9	87.1 ± 7.7
RS/P200-nadro CAs	31.6 ± 2.7	92.2 ± 4.8
RS/P200-enoxa CAs	34.8 ± 6.6	99.5 ± 1.1
RS/P200-certo CAs	43.8 ± 5.4	86.6 ± 8.6

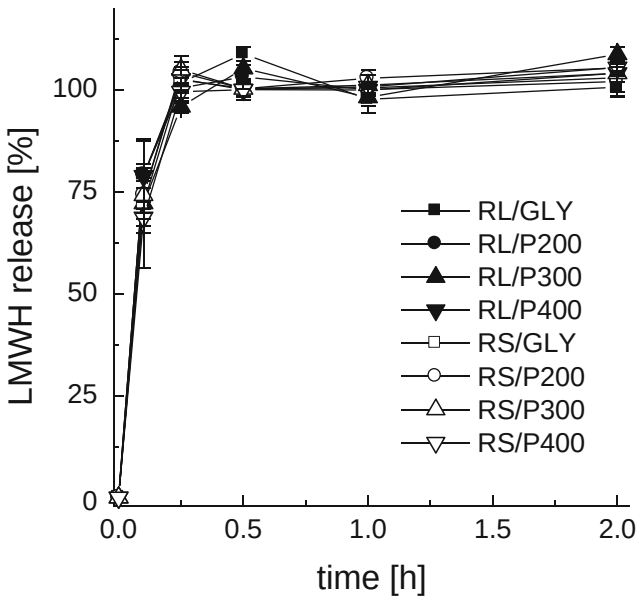

Fig. 2 Illustration of the nearly complete release profiles of CAs prepared with different polymer solvents (mean ± SD, n = 3).

Table II Pharmacokinetic Parameters of Enoxaparin CAs After Oral Administration in Rabbits Prepared with PEGs at 600 IU/kg

Formulation	C_{max} [IU/ml]	t_{max} [h]	AUC (0–6 h) [IU*h/ml]	F [%]
RL/GLY CAs	0.19 ± 0.04	1	0.37 ± 0.08*	6.0 ± 1.2*
RL/P200 CAs	0.07 ± 0.06	1	0.01 ± 0.05	0.1 ± 0.9
RL/P300 CAs	0.14 ± 0.08	1	0.13 ± 0.17	2.1 ± 2.8
RL/P400 CAs	0.11 ± 0.10	1	0.03 ± 0.09	0.5 ± 1.4
RS/GLY CAs	0.18 ± 0.15	1	0.11 ± 0.16	1.8 ± 2.6
RS/P200 CAs	0.20 ± 0.07	1	0.35 ± 0.15*	5.8 ± 2.5*
RS/P300 CAs	0.11 ± 0.12	1	0.10 ± 0.18	1.6 ± 3.0
RS/P400 CAs	0.06 ± 0.10	1	0.04 ± 0.08	0.7 ± 1.3

mean ± SD, $n = 3–4$; *$P < 0.05$ Compared to Oral Enoxaparin Solution

Moreover, the LMWH type influenced the oral absorption as the bioavailability was decreasing in the order fondaparinux>enoxaparin>nadroparin>certoparin depending mainly on the average molecular weight of the heparins (Fig. 5). The access to food influenced significantly the oral bioavailability of oral LMWHs, in the case of RL/GLY-CAs it was lowered from 6.0 ± 1.2% to 2.3 ± 2.8% and for the formulation RS/P200 from 5.8 ± 2.5% to 2.3 ± 0.2%.

X-ray studies revealed that $BaSO_4$ was located in the stomach 15 min after oral administration (Fig. 6) while 90 min after administration the CAs could be located in the jejunum and ileum by the $BaSO_4$ signal. After 4 h no $BaSO_4$ could be observed in the small intestine which correlated well with the pharmacokinetic data.

DISCUSSION

The oral bioavailability of macromolecules such as heparin is challenging and successful delivery strategies are still rare. Especially, the high potential of particulate carriers for oral drug delivery of LMWHs has already been mentioned in recent years (17,20). However, the particles with promising results *in vivo* were prepared by multiple emulsion technique involving a relatively complex preparation technique and the major drawback of using organic solvents with elevated toxicological potential. Although solvent removal is usually part of the preparation process, a residual solvent content often requires additional purifications steps. Thus, regulatory problems occur which is obviously one reason for the lack of commercially available oral LMWH formulation by now.

Rather than using standard organic solvents with their known toxicity potential, we suggested the use of short chain PEG derivatives which have proven their suitability as water miscible solvents for pharmaceutical polymers (26). PEGs are non-toxic which is emphasized by their use in parenteral products in concentrations up to 50% (25,29). Besides, it arises from their non-toxicity that solvents can remain in the formulation and the manufacturing process can be simplified to one mixing step. The resulting one step method allowed the preparation of LMWH loaded CAs without a solvent removal step. The robustness of the developed process was underlined by comparable results between the different screened polymers and polymer solvents concerning particle size, encapsulation efficiency and dissolution profile. The CA formation is based on a microencapsulation method based on a quasi-emulsion extraction with a hydrophilic external phase (26). However, LMWH-polymer interactions increased coherence of the particle matrix and obtaining microparticulates was possible without increased viscosity of the external aqueous phase reported from earlier experiments when small molecules were encapsulated.

The different CAs achieved nearly a complete drug release (> 80%) *in vitro* while other studies *e.g.* with NPs or MPs often reported limited LMWH release (17,19,30–32). Since encapsulation efficiencies for CAs were similar compared to microcarriers in the related literature, this distinct difference in release kinetic is surely related to the different structural arrangement of the particles. While standard entrapment methods by double emulsion retain the encapsulated heparins inside the internal aqueous phase of the particle in our case heparins are attached to the matrix polymer potentially by electrostatic interactions and are easily released from the particle matrix upon contact with the dissolution medium.

Table III Pharmacokinetic Parameters of Enoxaparin CAs After Oral Administration in Rabbits Prepared with Different LMWH Doses, 300, 600, or 1,200 IU/kg

Formulation	C_{max} [IU/ml]	t_{max} [h]	AUC (0–6 h) [mg*h/l]	F (0–6 h) [%]
RL/GLY [300 IU/kg]	0.01 ± 0.02	1	0.07 ± 0.02	0.2 ± 0.6
RL/GLY [600 IU/kg]	0.19 ± 0.04	1	0.37 ± 0.08*	6.0 ± 1.2*
RL/GLY [1200 IU/kg]	0.25 ± 0.05	1	0.56 ± 0.05*	4.6 ± 0.4*
RS/P200 [300 IU/kg]	0.08 ± 0.08	1	0.08 ± 0.07	2.6 ± 2.3
RS/P200 [600 IU/kg]	0.20 ± 0.07	2	0.35 ± 0.15*	5.8 ± 2.5*
RS/P200 [1,200 IU/kg]	0.26 ± 0.03	1	0.50 ± 0.09*	4.1 ± 0.8*

mean ± SD, n = 3–4; *P < 0.05 Compared to Oral Enoxaparin Solution

Table IV Pharmacokinetic Parameters of CAs Prepared with Different LMWH Types After Oral Administration in Rabbits at 600 IU/kg

Formulation	C_{max} [IU/ml]	t_{max} [h]	AUC (0–6 h) [IU*h/ml]	F [%]
RL/GLY-fonda CAs	0.15 ± 0.04	2	0.37 ± 0.08*	6.0 ± 1.2*
RL/GLY-enoxa CAs	0.19 ± 0.04	1	0.35 ± 0.08*	5.0 ± 1.2*
RL/GLY-nadro CAs	0.13 ± 0.02	1	0.19 ± 0.17*	3.2 ± 2.8*
RL/GLY-certo CAs	0.09 ± 0.11	1	0.09 ± 0.15*	0.9 ± 1.5
RS/P200-fonda CAs	0.19 ± 0.02	1	0.50 ± 0.10*	7.1 ± 1.5*
RS/P200-enoxa CAs	0.20 ± 0.07	2	0.35 ± 0.15*	5.8 ± 2.5*
RS/P200-nadro CAs	0.13 ± 0.02	2	0.27 ± 0.09*	4.4 ± 1.5*
RS/P200-certo CAs	0.11 ± 0.14	2	0.20 ± 0.17*	2.1 ± 1.7

Mean ± SD, n = 3–4; *P < 0.05 Compared to Oral Enoxaparin Solution

Although *in vitro* results of the different CAs were similar in all cases significant differences between the formulations were observable *in vivo*. Surely, a simple dissolution test is unable mimic complex conditions of the gastrointestinal tract, however it allows excluding that the major differences in bioavailability are related to simple release phenomena.

All tested formulations were able to reach an effect on anti-Xa blood levels after oral administration, the most prominent results were observed with the combinations RL/GLY and RS/P200. Here, variable interactions between LMWH and polymer seemed to play an important factor for an oral LMWH effect. Enoxaparin as all heparins is characterized by a large portion of negative charged sulphate groups. In contrast, the poylaminomethacrylates RL and RS consist of 10% or respectively 5% quaternary ammonium groups and are therefore positively charged. Based on the different charges of polymer and drug ionic interactions occur (30). As mentioned above the strong negative charge of heparin prevents it to cross intestinal barrier, whereas ionic interaction with the opposite charged polymer may mask its negative charge and enhance intestinal absorption. However, this fact is valid for all tested preparation *in vivo* and the explanation for the different results seems to be much more complex. It is known that RL and RS do not induce paracellular drug absorption, however significant mucoadhesion of LMWH loaded RS nanoparticles was reported from cultured cells *in vitro* earlier (27). This is in contradiction to observations in the x-ray study *in vivo* here, but might be related to significantly smaller size of the drug delivery system in the earlier study as well as the absence of motility. This mechanism surely requires further in-depth analyses. PEGs with higher molecular weight reduced the ability of the CAs to provide oral LMWH absorption. The reason for this behaviour is not clear. Although a significant residual amount of PEGs was still located in the CA matrix, *in vitro* dissolution exhibited similar results.

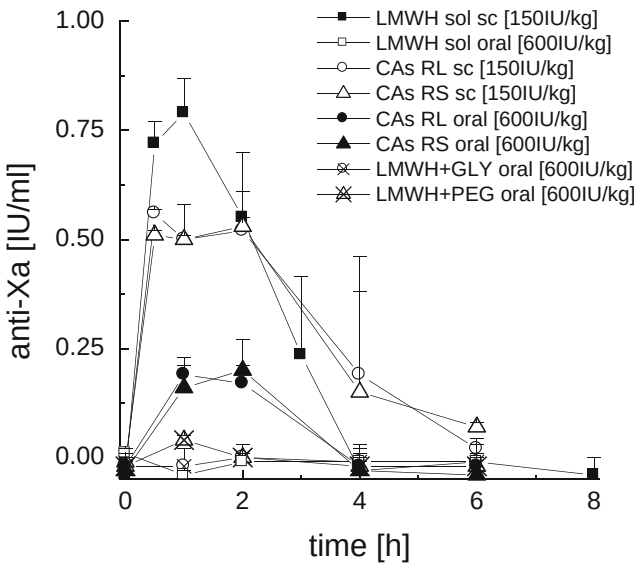

Fig. 3 Bioavailability of enoxaparin CAs compared to controls of oral and subcutaneous enoxaparin solution, and LMWH+PEGs without polymer (mean ± SD, $n = 3–4$, $^*P < 0.05$ compared to oral LMWH solution).

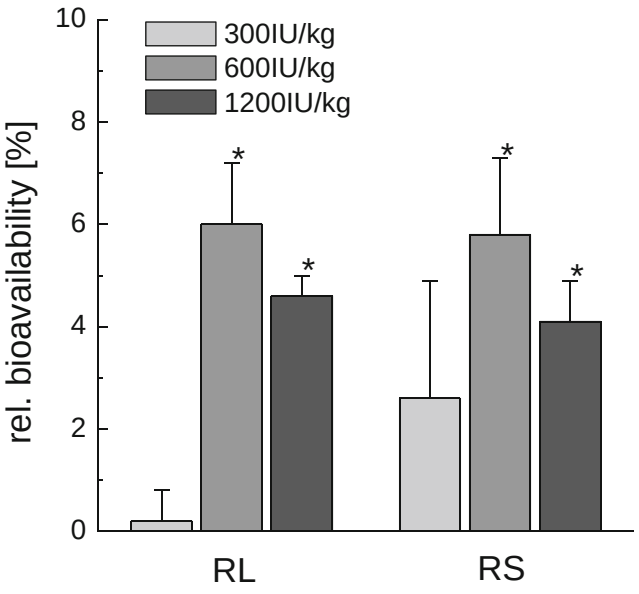

Fig. 4 Dose dependent bioavailability of enoxaparin CAs prepared with RL/GLY and RS/P200 (mean ± SD, $n = 4$, $^*P < 0.05$ compared to oral LMWH solution and blank CAs).

While the non-linear pharmacokinetics were in line with reports on other drug delivery systems (17), it is noticeable that the onset and the maxima of the pharmacological effect with CAs were earlier compared to other LMWH delivery approaches (17,30,32). This might be considered as a therapeutic advantage over existing delivery systems. X-ray analyses elucidated that CAs were mainly located in the small intestine during the maximum anti-Xa effect. These observations suggest duodenum and jejunum as main LMWH absorption sites.

The hypothesis on the appropriate mechanism of LMWH absorption involves various possible pathways. Although paracellular transport of macromolecular drugs has been reported previously for other cationic polymers (33) it is not probable with CAs and the polymers used in this study. Earlier data did not reveal any effect on the tight junctions in cultured cells covered with mucin (27). Also, a transcellular transport of the entire CAs might be excluded due to their size but also the simple fact of sticking of the CAs to the mucus as described recently even for smaller particles (34). Thus, the most plausible mechanism is the non-specific particle adhesion to the epithelial barrier followed by a replacement of the drug on the particle surface by mucin. The free drug is then diffusing through the mucus layer and actively either taken up into or transported through the absorption barrier. This mechanism is in line with a transport phenomenon described for enhanced particle deposition close to biological barriers leading to a highly increased concentration of the entrapped drug at the apical side being responsible for enhanced absorption by a drug gradient concentration toward the systemic blood flow.

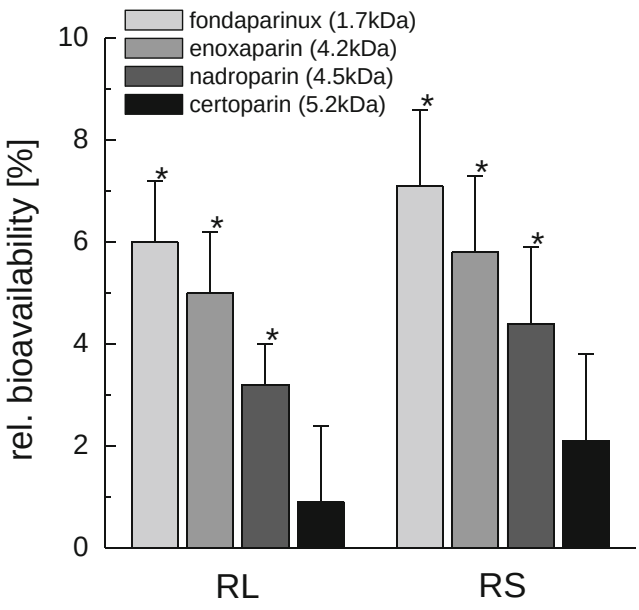

Fig. 5 Choice of the LMWH type influenced the bioavailability as seen with fondaparinux, enoxaparin, nadroparin, and certoparin (mean ± SD, $n = 3–4$, $^*P < 0.05$ compared to oral LMWH solution).

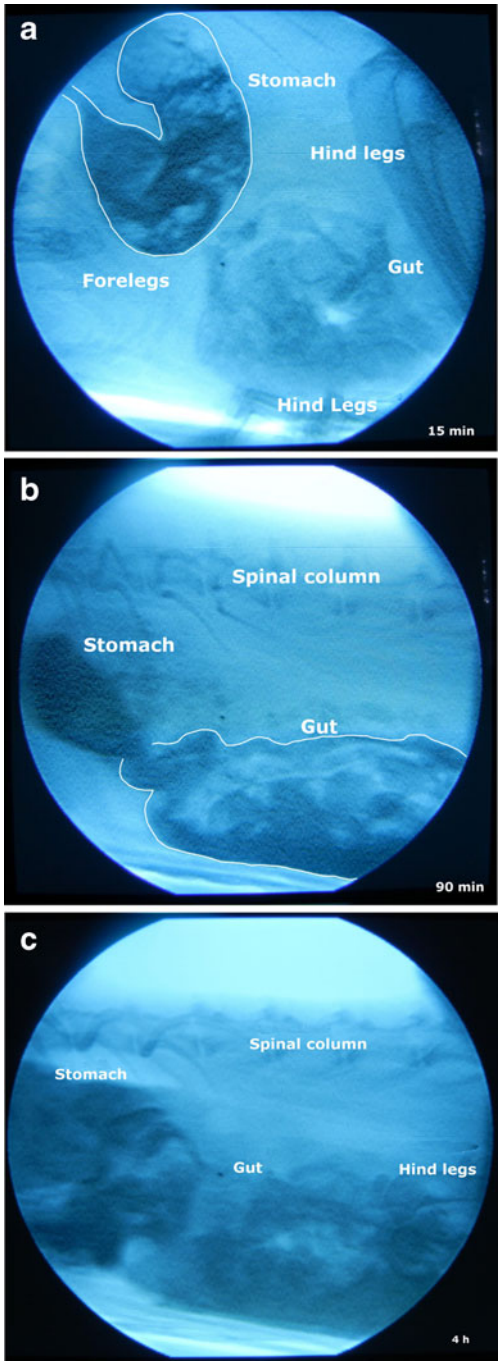

Fig. 6 Gastrointestinal passage of CAs analyzed by x-ray: 15 min (**a**) or 90 min (**b**) after oral administration of $BaSO_4$ associated CAs show their passage in the small intestine, 4 h after oral administration of CAs no $BaSO_4$ signal is observed (**c**).

We believe that the mucoadhesive properties of polyaminomethacrylates that have been demonstrated earlier (27) are responsible for a prolonged and intensified contact of the drug carrier with the epithelial barrier resulting in an increased absorption. Obviously, a number of additional factors can influence the extent of absorption *in vivo* such as

particle translocation across the Peyer's patches (35) which seems to be without importance due to the diameter of the CAs. Moreover, relative bioavailabilities of around 7% will probably not be reached by such a mechanism.

With a view to properties of the active principle it is well known that LMWHs are complex mixtures of oligosaccharides. Due to the high encapsulation efficiency throughout all formulations we reason that actually all components of the oligosaccharide mixtures bind to the polyaminomethacrylates and are therefore encapsulated into the CAs. However, one has to admit that it is not clear which part of the oligosaccharide mixtures is finally passing the absorption barrier. It may be concluded that smaller molecules undergo more efficient absorption which is in line with the observations from experiments analyzing the influence of the LMWH type (and molecular weight) on their oral absorption.

CONCLUSION

LMWH CAs were successfully prepared with different non-toxic PEG derivatives as polymer solvents. The use of these non-toxic solvents allowed to establish a new one step preparation method without the necessity of solvent removal. Such CAs provided significant oral bioavailability of LMWHs in rabbits. The absorption of LMWH essentially took place in the small intestine, was depending on the LMWH type and non-linear pharmacokinetics were observed. Based on the *in vivo* outcome we believe that this method is a promising approach for oral LMWH delivery, especially since the preparation process is simple and fast and shows a great potential for manufacturing at industrial scale.

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REFERENCES

1. Arbit E, Goldberg M, Gomez-Orellana I, Majuru S. Oral heparin: status review. Thromb J. 2006;4:6.
2. Javot L, Lecompte T, Rabiskova M, Maincent P. Encapsulation of low molecular weight heparins: influence on the anti-Xa/anti-IIa ratio. J Control Release. 2009;139:8–14.
3. Kim SK, Huh J, Kim SY, Byun Y, Lee DY, Moon HT. Physicochemical conjugation with deoxycholic acid and dimethylsulfoxide for heparin oral delivery. Bioconjug Chem. 2011;22:1451–8.
4. Kim SK, Lee DY, Kim CY, Moon HT, Byun Y. Prevention effect of orally active heparin derivative on deep vein thrombosis. Thromb Haemost. 2006;96:149–53.
5. Kim SK, Lee DY, Kim CY, Nam JH, Moon HT, Byun Y. A newly developed oral heparin derivative for deep vein thrombosis: non-human primate study. J Control Release. 2007;123:155–63.
6. Lee Y, Nam JH, Shin HC, Byun Y. Conjugation of low-molecular-weight heparin and deoxycholic acid for the development of a new oral anticoagulant agent. Circulation. 2001;104:3116–20.
7. Nissan A, Ziv E, Kidron M, Bar-On H, Friedman G, Hyam E, *et al.* Intestinal absorption of low molecular weight heparin in animals and human subjects. Haemostasis. 2000;30:225–32.
8. Rama Prasad YV, Minamimoto T, Yoshikawa Y, Shibata N, Mori S, Matsuura A, *et al. In situ* intestinal absorption studies on low molecular weight heparin in rats using labrasol as absorption enhancer. Int J Pharm. 2004;271:225–32.
9. Motlekar NA, Srivenugopal KS, Wachtel MS, Youan BB. Oral delivery of low-molecular-weight heparin using sodium caprate as absorption enhancer reaches therapeutic levels. J Drug Target. 2005;13:573–83.
10. Motlekar NA, Srivenugopal KS, Wachtel MS, Youan BB. Evaluation of the oral bioavailability of low molecular weight heparin formulated with glycyrrhetinic acid as permeation enhancer. Drug Dev Res. 2006;67:166–74.
11. Scala-Bertola J, Gajdziok J, Rabiskova M, Bonneaux F, Lecompte T, Sapin A, *et al.* Pellets for oral administration of low-molecular-weight heparin. Drug Dev Ind Pharm. 2009;35:1503–10.
12. Paliwal R, Paliwal SR, Agrawal GP, Vyas SP. Biomimetic solid lipid nanoparticles for oral bioavailability enhancement of low molecular weight heparin and its lipid conjugates: *in vitro* and *in vivo* evaluation. Mol Pharm. 2011;8:1314–21.
13. Paliwal R, Paliwal SR, Agrawal GP, Vyas SP. Chitosan nanoconstructs for improved oral delivery of low molecular weight heparin: *in vitro* and *in vivo* evaluation. Int J Pharm. 2012;422:179–84.
14. Sun W, Mao S, Mei D, Kissel T. Self-assembled polyelectrolyte nanocomplexes between chitosan derivatives and enoxaparin. Eur J Pharm Biopharm. 2008;69:417–25.
15. Sun W, Mao S, Wang Y, Junyaprasert VB, Zhang T, Na L, *et al.* Bioadhesion and oral absorption of enoxaparin nanocomplexes. Int J Pharm. 2010;386:275–81.
16. Lamprecht A, Ubrich N, Maincent P. Oral low molecular weight heparin delivery by microparticles from complex coacervation. Eur J Pharm Biopharm. 2007;67:632–8.
17. Hoffart V, Lamprecht A, Maincent P, Lecompte T, Vigneron C, Ubrich N. Oral bioavailability of a low molecular weight heparin using a polymeric delivery system. J Control Release. 2006;113:38–42.
18. Hoffart V, Ubrich N, Lamprecht A, Bachelier K, Vigneron C, Lecompte T, *et al.* Microencapsulation of low molecular weight heparin into polymeric particles designed with biodegradable and nonbiodegradable polycationic polymers. Drug Deliv. 2003;10:1–7.
19. Hoffart V, Ubrich N, Simonin C, Babak V, Vigneron C, Hoffman M, *et al.* Low molecular weight heparin-loaded polymeric nanoparticles: formulation, characterization, and release characteristics. Drug Dev Ind Pharm. 2002;28:1091–9.
20. Javot L, Sapin A, Scala-Bertola J, Vigneron C, Lecompte T, Maincent P. Oral administration of a microencapsulated low-molecular-weight heparin to rabbits: anti-Xa and anti-IIa profiles. Thromb Haemost. 2010;103:1254–67.
21. Meissner Y, Ubrich N, El Ghazouani F, Maincent P, Lamprecht A. Low molecular weight heparin loaded pH-sensitive microparticles. Int J Pharm. 2007;335:147–53.

22. Bitzand C, Doelker E. Influence of the preparation method on residual solvents in biodegradable microspheres. Int J Pharm. 1996;131:171–81.
23. Witschiand C, Doelker E. Residual solvents in pharmaceutical products: acceptable limits, influences on physicochemical properties, analytical methods and documented values. Eur J Pharm Biopharm. 1997;43:215–42.
24. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm. 1989;55:R1–4.
25. Rowe RC, Sheskey PJ, Owen SC. (ed.). Handbook of pharmaceutical excipients, polyethylene glycol, Pharmaceutical Press, London, 2006.
26. Allhenn D, Lamprecht A. Microsphere preparation using the untoxic solvent glycofurol. Pharm Res. 2011;28:563–71.
27. Lamprecht A, Koenig P, Ubrich N, Maincent P, Neumann D. Low molecular weight heparin nanoparticles: mucoadhesion and behaviour in Caco-2 cells. Nanotechnology. 2006;17:3673–80.
28. Demoré B, Benoit E, Maincent P, Hoffman M, Bessière J. Determination of heparin in aqueous solutions. J Clin Pharm Ther. 1998;23:381–4.
29. Bonacucinaand G, Palmieri GF. Acrylic polymers as thickening agents for tetraglycol cosolvent. J Pharm Sci. 2006;95:726–36.
30. Jiao Y, Ubrich N, Hoffart V, Marchand-Arvier M, Vigneron C, Hoffman M, *et al.* Anticoagulant activity of heparin following oral administration of heparin-loaded microparticles in rabbits. J Pharm Sci. 2002;91:760–8.
31. Jiao Y, Ubrich N, Marchand-Arvier M, Vigneron C, Hoffman M, Lecompte T, *et al. In vitro* and *in vivo* evaluation of oral heparin-loaded polymeric nanoparticles in rabbits. Circulation. 2002;105:230–5.
32. Jiao YY, Ubrich N, Hoffart V, Marchand-Arvier M, Vigneron C, Hoffman M, *et al.* Preparation and characterization of heparin-loaded polymeric microparticles. Drug Dev Ind Pharm. 2002;28:1033–41.
33. Thanou M, Florea BI, Langemeyer MW, Verhoef JC, Junginger HE. N-trimethylated chitosan chloride (TMC) improves the intestinal permeation of the peptide drug buserelin *in vitro* (Caco-2 cells) and *in vivo* (rats). Pharm Res. 2000;17:27–31.
34. Niebel W, Walkenbach K, Béduneau A, Pellequer Y, Lamprecht A. Nanoparticle-based clodronate delivery mitigates murine experimental colitis. J Control Release. 2012;160:659–65.
35. O'Hagan DT. The intestinal uptake of particles and the implications for drug and antigen delivery. J Anat. 1996; 189:477–82.