Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 317 (2006) 114–119
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Preparation and evaluation of oral solid heparin using emulsifier and adsorbent for in vitro and in vivo studies

Yukako Ito *, Tomohiro Kusawake, Y.V. Rama Prasad, Nobuyuki Sugioka, Nobuhito Shibata, Kanji Takada [1]

Department of Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan

Received 6 October 2005; received in revised form 22 February 2006; accepted 24 February 2006
Available online 14 March 2006

Abstract

Oral anticoagulant therapy with heparin has been challenged by formulating heparin in oral solid preparation. As heparin, low molecular weight heparin (LMWH) was used. LMWH was dispersed with a surfactant used for the self-microemulsifying drug delivery system (SMEDDS), PEG-8 caprylic/capric glycerides (Labrasol), and the mixture was solidified with three kinds of adsorbents, microporous calcium silicate (Florite^{TM} RE), magnesium alminometa silicate (Neusilin^{TM} US$_2$) and silicon dioxide (Sylysia^{TM} 320). The in vitro release study showed that the T50%s were 3.2 ± 0.1 min for Sylysia 320, 4.6 ± 0.2 min for Florite RE, 13.7 ± 0.1 min for Neusilin US$_2$. The in vivo rat absorption study showed that Florite RE system had the highest C_{max}, 0.42 ± 0.01 IU/mL and AUC, 0.59 ± 0.06 IU h/mL, where plasma LMWH levels were measured as anti-Xa activity. Other preparations had the C_{max} and AUC, 0.12 ± 0.01 IU/mL and 0.15 ± 0.02 IU h/mL for Neusilin US$_2$ and 0.25 ± 0.02 IU/mL and 0.40 ± 0.03 IU h/mL for Sylysia 320, respectively. The bioavailability (BA) of LMWH from the microporous calcium silicate preparation, Florite RE, was 18.8% in rats by comparing the AUC obtained after i.v. injection of LMWH, 40 IU/kg to another group of rats. Florite RE system was evaluated in dogs after oral administration in an enteric capsule made of Eudragit S100 at the LMWH dose of 200 IU/kg. High plasma anti-Xa activity levels were obtained, i.e., the C_{max} was 0.48 ± 0.11 IU/mL and AUC was 1.64 ± 0.32 IU h/mL. These results suggest that adsorbent system is useful as an oral solid delivery system of poorly absorbable drugs such as LMWH.

Keywords: Low molecular weight heparin; Labrasol; Adsorbent; Microporous calcium silicate; Magnesium alminometa silicate; Silicon dioxide; Absorption enhancement

1. Introduction

As heparin preparation, unfractionated heparin (UH) and low molecular weight heparin (LMWH) exist as anticoagulant for the treatment of deep vein thrombosis and pulmonary embolism (Agnelli and Sonaglia, 2000; Ageno, 2000). UH is a naturally occurring glycosaminoglycan that exists as a heterogenous mixture of oligosaccharides composed of alternating chains of D-glucosamine and uronic acid (Hirsh et al., 1992). Heparin is sulfated, highly acidic and has a negative ionic charge. In many countries, LMWHs have replaced UH for the prevention and treatment of venous thrombo-embolism (Boneu, 2000), because UH has a short systemic half-life and has a side effect, i.e. bleeding for a given antithrombotic effect compared to UH. However, LMWH also has a disadvantage. LMWH is clinically available only through parenteral route. Patients are usually switched from intravenous LMWH to oral warfarin upon hospital discharge. In contrast warfarin has a slow onset, is predominantly protein bound, is subject to drug–drug interactions and requires careful monitoring. Although both agents are effective anticoagulants, LMWH is pharmacologically superior to warfarin. An oral LMWH formulation is the preferred warfarin replacement option for out-patient therapy because monitoring can be carried out by the simple and rapid activated partial thromboplastin time (APTT) assay (Leone-Bay et al., 1998). If oral LMWH preparation is available, the clinical use of LMWH will increase dramatically.

Different researches have been carried out to improve the intestinal absorption of LMWH by using *N*-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC) and *N*-[8-(2-hydroxybenzoyl)amino] decanoate (SNAD) (Leone-Bay et al., 1998) or

* Corresponding author. Tel.: +81 75 595 4626; fax: +81 75 595 6311.
E-mail addresses: yukako@mb.kyoto-phu.ac.jp (Y. Ito), takada@mb.kyoto-phu.ac.jp (K. Takada).
[1] Tel.: +81 75 595 4625; fax: +81 75 595 4751.

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.02.056

Carbopol® 934P (Thanou et al., 2001) as a carrier of heparin is reported. However, the development of the oral formulation of LMWH has not succeeded yet. We have been studying the oral heparin delivery system and found out that a surfactant, Labrasol, has a strong absorption enhancing effect on the absorption of LMWH from the rat small intestine (Prasad et al., 2004). Labrasol is PEG-8 caprylic/capric glycerides where C_8 is 58.1% and C_{10} is 39.8% and has been used as a main component of a self-microemulsifying drug delivery system (SMEDDS). SMEDDS is expected to self-emulsify rapidly in the aqueous environment in the small intestine, thereby presenting the drug in solution in small droplets of oil. The gentle agitation required for the emulsification is provided by the digestive motility of the small intestine. By formulating LMWH in Labrasol SMEDDS, the plasma anti-Xa activity obtained with 50 mg/kg dose of Labrasol after administration to the rat small intestine was above therapeutic level, 0.2 IU/mL, and sustained for about 160 min (Prasad et al., 2004). The dosage form that result from SMEDDS is usually either a liquid or liquid filled into soft gelatin capsules. However, from the standpoint of pharmaceutics, solid dosage form is preferred than liquid preparation because of the convenience for the manufacturing process. The formulated amount of such adsorbent differs in each preparation because of their capacities to adsorb liquid preparation.

Solid dispersion is a pharmaceutical system for improving the solubility of poorly water-soluble drugs. In this system, the solubility of a drug is improved due to its amorphization. Currently, spray drying and layering on core particles are generally used to manufacture solid dispersions on a large scale (Kinoshita et al., 2002). However, these methods require a large amount of organic solvent to dissolve the drug and a hydrophilic polymer, which serves as the matrix in most cases. Organic solvents cause several problems including environmental pollution and toxicity due to the residual solvents. Sylysia 320 is light anhydrous silicic acid and was qualified as a pharmaceutical additive. It is used to pulverize liquid formulation and also is used in tablet. Neusilin US_2, is a porous magnesium aluminometasilicate and has an antacid effect. Florite RE is calcium silicate and is used for the solidification of oily preparation. These materials have a pore structure in themselves and work as an adsorbent. We found that the effectiveness of solid dosage form composed of adsorbents, drug and surfactant (Ito et al., 2005a,b). Therefore, alternative dosage form that can incorporate SMEDDS into a microparticles to produce a solid dosage form has been challenged and the prepared solid dosage forms have been evaluated both in rats and dogs. In this study, by applying this system to LMWH, the BA improvement of LMWH was enabled, and the possibility of the oral formulation in using the adsorbent, was examined.

2. Materials and methods

2.1. Materials

LMWH (Parnaparin sodium, anti-Xa factor activity: 85.4 IU/mg) was a gift from Shimizu Pharmaceutical Co., Ltd. (Shizuoka, Japan). PEG-8 caprylic/capric glycerides (Labrasol) (Gattefösse, Lyon, France) was a gift from Chugai Boyeki Co., Ltd. (Tokyo, Japan). Eudragit® S100 (Röhm Pharm, Darmstadt, Germany) was obtained through Higuchi Inc. (Tokyo, Japan). Polysorbate 80 (commonly called Tween 80) was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Magnesium aluminometa silicates (Neusilin™ US_2) were obtained from Fuji Chemical Industry Co., Ltd. (Toyama, Japan). Porous silicon dioxide (Sylysia™ 320) was obtained from Fuji Silysia Co., Ltd. (Aichi, Japan). Porous calcium silicate (Florite™ RE) was obtained from Eisai Co., Ltd. (Tokyo, Japan). All other reagents were of analytical-reagent grade and were used as received. Male Wistar rats used in the study and standard solid meal of commercial food (LabDiet®) were obtained from Nippon SLC Company (Hamamatsu, Japan). Male beagle dogs (10.0–12.5 kg) used in this study and standard solid meal of commercial food (Labo D stock®) were obtained from Nippon Nousan Co., Ltd. (Yokohama, Japan).

2.2. LMWH preparations

LMWH, 25 mg, was well suspended with 0.5 mL of Labrasol and thereafter 100 mg of Florite RE, 250 mg of Neusilin US_2 or 150 mg of Sylysia 320 was added. By kneading well, solid preparation was obtained as shown in Table 1. In the dog experiment, the film of Eudragit S100 of 40 μm in thickness was rolled in the capsule of size #000 and made to be an enteric capsule. The edge of the film was sealed with the highly concentrated solution of Eudragit S100. The solid preparation was then filled in a #000 enteric capsule and finally the end was also sealed with glue.

2.3. Dissolution experiment

In vitro dissolution studies were carried out in reduced scale with the test solid preparations listed in Table 1. The dissolution medium was 200 mL of 0.067 M phosphate buffer, pH 7.4, under the rotation speed of 150 rpm at 37 °C. The dissolution medium was degassed by sonication at room temperature and maintained at 37 °C throughout the test period. To determine the amount of LMWH released from the test preparations, 0.5 mL of the medium was removed for analysis at the predetermined time and replaced with fresh dissolution medium. The collected samples were filtered through a Millex-LG filter (0.2 μm, Milipore Corp., MA, USA) and was used for the assay. Factor Xa inhibition activity was measured by the Hemos IL™ (Instrumentaition Laboratory, Italy). The cumulative amount of released LMWH from the preparation is defined by the following equation:

$$\text{cumulative amount released} = \left(\frac{\sum_{t=0}^{t} M_t}{M_{\text{actual}}} \right) \times 100\%$$

Table 1
Test formulation of LMWH solid dosage form

Formulation name	LMWH[a] (mg)	Labrasol (mL)	Adsorbent (mg)
Florite RE	2.5	0.05	10
Neusilin US_2	2.5	0.05	25
Sylysia 320	2.5	0.05	15

[a] One milligram of LMWH = 80 IU of Parnaparin sodium.

where M_t shows the amount of LMWH released at time t, and M_{actual} is the total released amount of LMWH. The recovery rate was determined by dissolving each test preparation in the dissolution medium.

2.4. Absorption experiment in rats

Male Wistar rats (351–383 g) fasted overnight for at least 12 h were used in the study. The rats were anaesthetized by an intraperitoneal administration of sodium pentobarbital solution (50 mg/kg). Before the abdominal incision, blank blood sample, 0.5 mL, was obtained at 5 min prior to the administration of the test preparations from the right jugular vein by making a small incision. Next, small intestine was isolated and small abdominal incision was made in the duodenum, and the test solid preparation was administered at a dose of 200 IU/kg of LMWH and 50 mg/kg of Labrasol. The incision was sutured with surgical tread. The pore was sealed with synthetic glue. The same formulation at the same dose of LMWH and Labrasol was also administered to the jejunum (30 cm from ileo-caecal junction) and ileum (15 cm from ileo-caecal junction) of the rats to find out the best site of LMWH absorption in the small intestine. Blood samples of 0.5 mL each were collected from the right jugular vein at 0.5, 1, 1.5, 2, 3 and 4 h after administration. The blood samples were collected into syringes containing 0.05 mL of 3.2% w/v trisodium citrate dihydrate solution as anticoagulant. The samples were mixed well and immediately cooled on an ice bath. Plasma was obtained from whole blood by centrifugation at 5000 rpm for 20 min at 4 °C using Kubota 1720 centrifuge (Tokyo, Japan), and then stored at −80 °C until analysis. Factor Xa inhibition activity was measured in plasma samples by the Hemos IL™ (Instrumentaition Laboratory, Italy).

2.5. Intravenous administration experiment in rats

Male Wistar rats (340–378 g) fasted overnight for at least 12 h were used in the study. The rats were anaesthetized by an intraperitoneal administration of sodium pentobarbital solution, 50 mg/kg. LMWH solution was intravenously injected, 40 IU/kg, and blood samples, 0.5 mL, were obtained at 10, 20, 30, 45 min, 1, 1.5 and 2 h. Plasma fraction was obtained and stored as noted above.

2.6. Absorption experiment in dogs

Three adult male beagle dogs (weighing 10.0–12.1 kg) were fasted overnight for at least 12 h, although free access to water was allowed. However, during the course of the experiment, water was not given until 4 h after the test preparation was administered. The enteric LMWH capsule was orally administered to the dogs. At 4 h after administration, a solid meal of commercial food, 450 g, and water were given. No additional food was given during the study. Cross-over study was performed with a washing out period of one week. Each dog received an oral administration of one test capsule in all studies. All experiments were carried out at the same time of the day to exclude the influences by circadian rhythm. Drug administration was carried out at 10 a.m. with 50 mL of water. At 30 min before drug administration, a control blood sample (1 mL) was removed from the jugular vein. After oral administration of the test preparation, 0.5 mL blood samples were collected from the jugular vein at 0, 1, 2, 3, 4, 5, 6 and 8 h. The plasma fraction used for LMWH assay was obtained by centrifuging the blood samples at 12,000 rpm for 5 min. These plasma samples were immediately frozen and stored at −80 °C until analysis. All experiments using rats and beagle dogs were approved by the animal care and use committee of Kyoto Pharmaceutical University and were performed in accordance with the standards listed in the "Guideline for Animal Experimentation (1987)", published by the Japanese Association for Laboratory Animal Science.

2.7. Pharmacokinetic analysis

The time to reach maximum plasma anti-Xa activity (T_{max}) and the maximum plasma anti-Xa activity (C_{max}) were determined from the authentic plasma anti-Xa activity versus time data. The area under the plasma anti-Xa activity versus time curve up to last sampling time, T_{last}, 4 h for rats and 8 h for dog experiment ($AUC_{0-T_{last}}$) and the area under the first-moment curve up to T_{last} ($AUMC_{0-T_{last}}$) after administration of the test preparations were calculated using the linear trapezoidal rule up to the last measured anti-Xa activity. The mean residence time (MRT) was calculated by $AUMC_{0-T_{last}}/AUC_{0-T_{last}}$.

2.8. Statistical analysis

All values are expressed as their mean ± S.E. Means of two groups were compared using non-paired Student's t-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

At first, the dissolution study of LMWH was performed with an in vitro release experiment and the results are shown in Fig. 1. Sylysia 320 system showed the fastest release rate of LMWH.

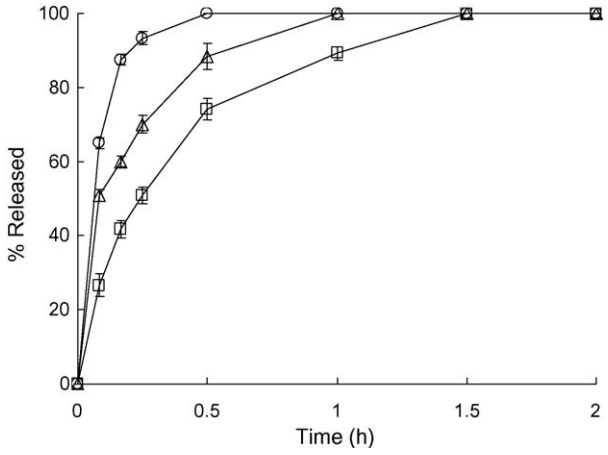

Fig. 1. Dissolution profiles of LMWH from different solid preparations (△): Florite RE 10 mg, (□): Neusilin US$_2$, (◯): Sylysia 320. Each point shows the mean ± S.E. of four experiments.

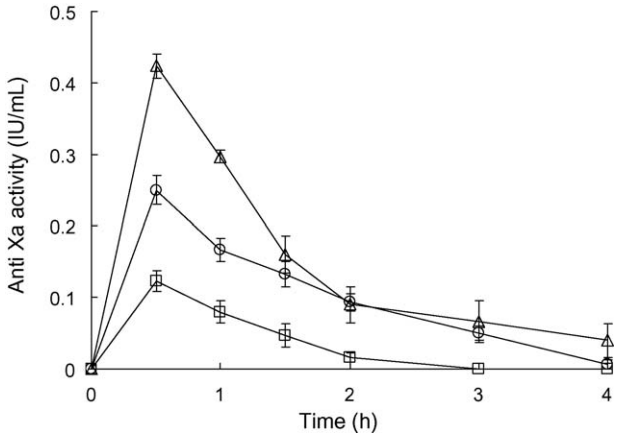

Fig. 2. Plasma anti-Xa activity-time profiles after an intrajejunal administration of LMWH solid preparations to rats, 200 IU/kg (△): Florite RE 10 mg, (□): Neusilin US_2, (◯): Sylysia 320. Each point shows the mean ± S.E. of four rats.

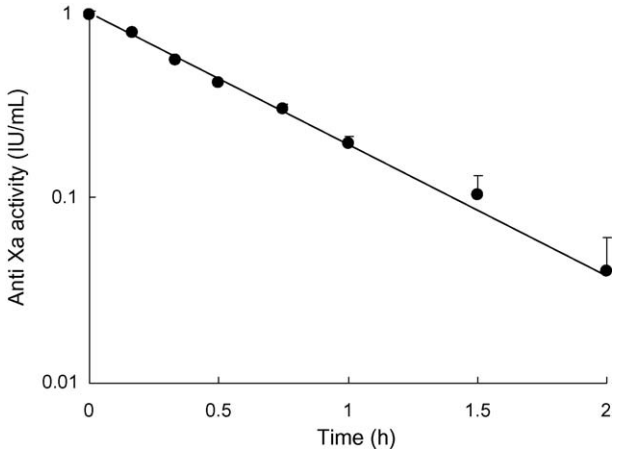

Fig. 3. Plasma anti-Xa activity-time profiles after an intravenous administration of LMWH solution to rats, 40 IU/kg. Each point shows the mean ± S.E. of four rats.

After the start of the experiment, the solid preparation disintegrated within 2 min and more than 90% of the formulated LMWH was released from the preparation at 15 min after the start of the release experiment. Florite RE system disintegrated gradually for 30 min after the start of the experiment and all the LMWH was released from the system at 1 h after the start of the experiment. Neusilin US_2 system showed the slowest release rate of LMWH, and it took about 1.5 h for LMHW to be completely released from the system. T50%s were 3.2 ± 0.1 min for Sylysia 320, 4.6 ± 0.2 min for Florite RE, 13.7 ± 0.1 min for Neusilin US_2, respectively. Based on these in vitro experiment, in vivo absorption experiment was performed in rats. In our previous study on the absorption of LMWH liquid preparation where Labrasol was used as an absorption enhancer for LMWH, jejunum showed the highest absorption efficiency of LMWH in rats. Therefore, three-test solid preparations were also administered into the rat jejunum at the dose level of 200 IU/kg and the plasma anti-Xa activity was measured as an index of plasma LMWH concentration. Fig. 2 shows the plasma anti-Xa activity versus time profiles of LMHW from three test preparations. Florite RE system showed the highest plasma anti-Xa activity, C_{max} was 0.42 ± 0.01 IU/mL, among the three test preparations. Sylysia 320 and Neusilin US_2 systems showed lower plasma anti-Xa activities than Florite RE system. From these data, non-compartmental pharmacokinetic analysis was performed and the parameters such as T_{max}, C_{max}, MRT and $AUC_{0-T_{last}}$ were obtained as shown in Table 2. The AUC obtained from Florite RE system was significantly higher than that obtained from Neusilin US_2, i.e. 0.59 ± 0.06 IU h/mL. The AUCs of Sylysia 320 and Neusilin US_2 were 0.40 ± 0.03 IU h/mL and 0.15 ± 0.02 IU h/mL, respectively.

To evaluate the bioavailability (BA) of LMWH from the test preparations, LMWH solution was administered to another group of rats by an i.v. injection to the jugular vein, 40 IU/kg. Fig. 3 shows the plasma anti-Xa activity versus time profiles of LMHW after i.v. injection of LMWH. The calculated AUC was 0.63 ± 0.03 IU h/mL. By comparing the mean AUC value obtained after the intra-jejunum administration of LMWH solid preparations with thus obtained value, the BA of LMWH from Florite RE system was 18.8%. Other two preparations, Sylysia 320 and Neusilin US_2 systems showed BA values of 12.7 and 4.9%, respectively.

In addition, the Florite RE system was administered orally to beagle dogs as shown in Fig. 4. After oral administration of the Florite RE preparation in an enteric capsule made of Eudragit S100, plasma anti-Xa activity level started to increase after an absorption lag-time of 1.0 h, and showed its C_{max}, 0.48 ± 0.11 IU/mL at 3.0 h after oral administration. As the minimum effective level of LMWH was expected to be 0.2 IU/mL (Bianchini et al., 1995), the effective concentration of LMWH was maintained for 3.8 h. The mean AUC was 1.64 ± 0.32 IU h/mL.

Table 2
Pharmacokinetic parameters of LMWH after administration of test preparations to rats

Formulation name	Route of administration	Dose (IU/kg)	C_{max} (IU/mL)	$AUC_{0-4\,h}$ (IU h/mL)	MRT (h)	BA (%)
Saline	Intravenous	40	0.96 ± 0.01	0.63 ± 0.03	0.71 ± 0.04	100
Florite RE	Jejunum	200	0.42 ± 0.01[a,b]	0.59 ± 0.06[a,b]	1.21 ± 0.15	18.8
Neusilin US_2	Jejunum	200	0.12 ± 0.01	0.15 ± 0.02	1.17 ± 0.02	4.9
Sylysia 320	Jejunum	200	0.25 ± 0.02	0.40 ± 0.03	1.38 ± 0.05	12.7

Values are the mean ± S.E.
[a] Significantly different from Neusilin US2.
[b] Significantly different from Sylysia 320.

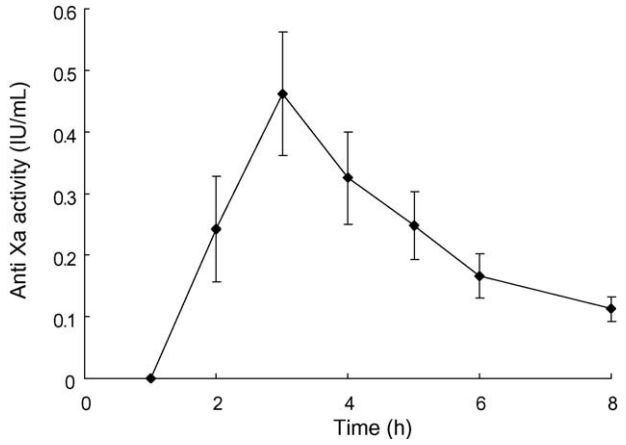

Fig. 4. Plasma anti-Xa activity-time profiles after an intravenous administration of LMWH solid preparation to dogs, 200 IU/kg. Each point shows the mean ± S.E. of three dogs.

4. Discussion

LMWH is a hydrophilic macromolecular drug and has a negative charge in itself. Such a physicochemical property of LMWH limits the membrane permeability of LMWH resulting low BA after oral administration. Several factors, i.e. low partition coefficient and three-dimensional structure including much hydrogen bonds and charge of the functional groups etc., are pointed out to explain the low membrane permeability of hydrophilic macromolecular drugs (Aungst and Saitoh, 1996). As an absorption mechanism of drugs, both transcellular route via active or passive transports, and paracellular route have been proposed. The hydrophobic drugs having the low molecular weight is absorbed by the transcellular route. On the other hand, hydrophilic macromolecular drugs are absorbed through the paracellular route. Under the normal physiological condition, tight junction works as a strong barrier against drugs being absorbed through a paracellular route. Some of the surfactants have been reported to work as an absorption enhancer for the drugs absorbed through the paracellular route (Hashimoto et al., 1995, 1998; Shimizu et al., 1997). Labrasol is a representative surfactant having a strong absorption enhancing effect on poorly absorbed drugs like gentamicin and vancomicin (Hu et al., 2001; Prasad et al., 2003). In addition, intestinal absorptions of LMWH have been reported by Labrasol in rats (Nissan et al., 2000). The absorption enhancing effect of the surfactants with high lipophilicity are weak and the partition of hydrophilic surfactants to the lipid bilayers is low Swenson and Curatolo (1992). However, surfactants having middle length alkyl chain are thought to have affinity to the lipid bilayers and show strong absorption enhancing effect. We already showed that Labrasol containing saturated polyglycolysed C_6–C_{14} glycerides, where C_8 is 58.1% and C_{10} is 39.8%, showed a strong absorption enhancing effect on gentamicin (Hu et al., 2001). Recently Sha et al. (2005) showed that Labrasol enhanced the paracellular transport of mannitol across Caco-2 cell monolayers involving the change of F-actin-related and the redistribution of ZO-1, membrane-associated guanine kinase. Generally, the effect of absorption enhancer is correlated with its concentration at the absorption site of drugs. Therefore, both the drug and absorption enhancer must be delivered concomitantly to the absorption site to make enhancer show its effectiveness maximally. Recently, good absorption enhancing effect has been observed in dogs from the solid preparation made of microparticles, Labrasol and gentamicin (Ito et al., 2005b).

In the in vitro dissolution study, Sylysia 320 system showed the fastest release rate of LMWH among the used three microparticles due to the smallest mean diameter of the particle. Each particle size is 3.2 μm (Sylysis 320), 26.1 μm (Florite RE) and 75.0 μm (Neusilin US_2), respectively. The dissolution rate was decreased dependent on the increase of the particle size. Because the smaller particle has larger contact area with the dissolution medium. In the case of Neusilin US_2 system, the lowest value of AUC was showed due to the slowest dissolution rate. Sylysis 320 has the property of the cross-linking of microparticles and Florite RE adsorbs the drug by the capillary attraction (Takashima et al., 2003). Generally, the adsorption mechanism of the microparticles differs because of the structure of the microparticles. Florite RE system showed higher BA, 18.8%, than Sylysia 320, though the release rate of LMWH from Sylysia 320 was faster than that from Florite RE system. Because of the smaller particle size than that of Florite RE, Sylysia 320 system was easily dispersed in the gastrointestinal tract. As a result, the residence time of LMWH in the gastrointestinal tract decreased and the amount of the absorbed LMWH was decreased. Therefore, the long residence of both drug and enhancer at the absorption site is an important factor for the absorption of LMWH. The release rate of LMWH from the microparticle system is thought to be dependent on both the physicochemical property and formulated amount of the microparticles. LMWH used in this study belongs to the BCS class-III compounds (hydrophilic and low permeability) (Amidon et al., 1995). Bianchini et al. (1995) have reported that the clinical target level of plasma anti-Xa activity was 0.2 IU/mL to show an anti-coagulant effect. In this study, plasma anti-Xa activity beyond 0.2 IU/mL was maintained for 1 h in the rat experiment and for 3.7 h in the dog experiment.

In conclusion, microparticulate system consisted of Florite RE, Labrasol and LMWH has been suggested to work well for the absorption of LMWH from the small intestine in both rats and dogs. These results suggest the usefulness of microparticulate system for the oral delivery of LMWH.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research provided by Ministry of Education, Science and Culture of Japan. This research was also supported in part by the Bioventure Developing Program of the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Venture SME-University Research Promotion Program of Japan Society for the Promotion of Science.

References

Ageno, W., 2000. Treatment of venous thromboembolism. Thromb. Res. 97, V63–V72.

Agnelli, G., Sonaglia, F., 2000. Prevention of venous thromboembolism. Thromb. Res. 97, V49–V62.

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Aungst, B.J., Saitoh, H., 1996. Intestinal absorption barriers and transport mechanisms, including secretary transport, for a cyclic peptide, fibrinogen antagonist. Pharm. Res. 13, 114–119.

Bianchini, P., Bergonzini, G.L., Parma, B., Osima, B., 1995. Relationship between plasma antifactor Xa activity and the antithromtic activity of heparins of different molecular mass. Haemostasis 25, 288–298.

Boneu, B., 2000. Low molecular weight heparins: are they superior to unfractionated heparins to prevent and to treat deep vein thrombosis? Thromb. Res. 100, V113–V120.

Hashimoto, K., Takada, K., Nakayama, T., Shimizu, M., 1995. Stabilization of the tight junction of the intestinal Caco-2-cell monolayer by milk whey proteins. Biosci. Biotech. Biochem. 59, 1951–1952.

Hashimoto, K., Nakayama, T., Shimizu, M., 1998. Effect of β-lactoglobulin on the tight-junctional stability of Caco-2-SF monolayer. Biosci. Biotechnol. Biochem. 62, 1819–1821.

Hirsh, J., Dalen, J.E., Poller, L., 1992. Heparin: mechanism of action, pharmacokinetics, dosing considerations, monitoring, efficacy and safety. Chest 4, 337s–351s.

Hu, Z., Tawa, R., Konishi, T., Shibata, N., Takada, K., 2001. A novel emulsifier, Labrasol, enhances gastrointestinal absorption of gentamicin by inhibiting transporter. Life Sci. 69, 2899–2910.

Ito, Y., Arai, H., Uchino, K., Iwasaki, K., Shibata, N., Takada, K., 2005a. Effect of adsorbents on the absorption of lansoprazole with surfactant. Int. J. Pharm. 289, 69–77.

Ito, Y., Kusawake, T., Ishida, M., Tawa, R., Shibata, N., Takada, K., 2005b. Oral solid gentamicin preparation using emulsifier and adsorbent. J. Control. Rel. 105, 23–31.

Kinoshita, M., Baba, K., Nagayasu, A., Yamabe, K., Shimooka, T., Takeichi, T., Azuma, M., Houchi, H., Minakuchi, K., 2002. Improvement of solubility and oral bioavailability of a poorly water-soluble drug, TAS-301, by its melt-adsorption on a porous calcium silicate. J. Pharm. Sci. 91, 362–370.

Leone-Bay, A., Paton, D.R., Freeman, J., Lercara, C., O'Toole, Doris, Gschneidner, D., Wang, E., Harris, E., Rosado, C., Rivera, T., Baughman, R.A., 1998. Synthesis and evaluation of compounds that facilitate the gastrointestinal absorption of heparin. J. Med. Chem. 41, 1163–1171.

Nissan, E.Z., Kidron, M., Bar-On, H., Friedman, G., Hyam, G., Eldor, A., 2000. Intestinal absorption of low molecular weight heparin in animals and human subjects. Haemostasis 30, 225–232.

Prasad, Y.V.R., Eaimtrakarn, S., Ishida, M., Kusawake, Y., Tawa, R., Yoshikawa, Y., Shibata, N., Takada, K., 2003. Evaluation of oral formulations of gentamicin containing Labrasol in beagle dogs. Int. J. Pharm. 268, 13–21.

Prasad, Y.V.R., Minamimoto, T., Yoshikawa, Y., Shibata, N., Mori, S., Matsumura, A., Takada, A., 2004. In situ intestinal absorption studies on low molecular weight heparin in rats using Labrasol as absorption enhancer. Int. J. Pharm. 271, 225–232.

Sha, X., Yan, G., Wu, Y., Li, J., Fang, X., 2005. Effect of self-microemulsifying drug delivery systems containing Labrasol on tight junctions in Caco-2 cells. Eur. J. Pharm. Sci. 24, 477–486.

Shimizu, M., Tsunogai, M., Arai, S., 1997. Transepithelial transport of oligopeptides in the human intestinal cell, Caco-2. Peptides 18, 681–687.

Swenson, E.S., Curatolo, W.J., 1992. Means to enhance penetration (2) intestinal permeability enhancement for proteins, peptides and other drugs: mechanisms and potential toxicity. Adv. Drug Del. Rev. 8, 39–92.

Takashima, Y., Yuasa, H., Kanaya, Y., Okada, H., 2003. Application of a pharmaceutical excipient, porous calcium silicate, for preparing higher functional solid dosage forms. Pharm. Technol. Jpn. 19, 135–146.

Thanou, M., Verhoef, J.C., Nihot, M.-T., Verheijden, J.H.M., Junginger, H.E., 2001. Enhancement of the intestinal absorption of low molecular weight heparin (LMWH) in rats and pigs using Carbopol® 934P. Pharm. Res. 18, 1638–1641.