Pharmaceutical Sciences, 2023, 29(1), 84-89
doi:10.34172/PS.2021.77
https://ps.tbzmed.ac.ir/

Research Article

Cell-Penetrating Peptide-Surface Modified Liposomes to Enhance the Intestinal Absorption of Enoxaparin

Hessam Rostamian[1], Hadi Valizadeh[2], Mohammad Mahmoudian[1], Ziba Islambulchilar[1], Parvin Zakeri-Milani[3*]

[1]*Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*
[2]*Drug Applied Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*
[3]*Liver and Gastrointestinal Diseases Research Center and Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*

Article Info

Article History:
Received: 23 May 2021
Accepted: 6 Dec 2021
ePublished: 24 Dec 2021

Keywords:
-Cell-penetrating peptide
-Enoxaparin
-Intestinal absorption
-Liposome

Abstract

Background: Enoxaparin is low-molecular-weight heparin administered by subcutaneous/intravenous injection. The oral bioavailability of enoxaparin is restricted by its low absorption through the intestine. In this study cell-penetrating peptide-surface functionalized liposomes (CPPs-L) were prepared to improve the intestinal absorption of enoxaparin.

Methods: Liposomal formulations were prepared by the ethanol injection method and the intestinal absorption of the formulation was evaluated using the single-pass intestinal perfusion (SPIP) technique in rats. Meanwhile, the human fraction dose absorbed value ($F_{a\ (human)}$) of the formulations was predicted based on the calculated effective intestinal permeability ($P_{effect\ (rat)}$) values obtained from the SPIP study.

Results: Liposomal enoxaparin revealed an increased intestinal absorption by ten-time compared with the free drug solution. Meanwhile, CPPs-L formulation revealed an enhanced intestinal absorption compared with the un-modified liposomal formulation. Regarding $F_{a\ (human)}$, it is predicted that liposomal formulations could have the potential to improve the fraction dose absorbed of enoxaparin from low to intermediate levels.

Conclusion: Overall, the liposomal formulation can be considered as a mighty drug carrier for the oral delivery of enoxaparin.

Introduction

Enoxaparin is low-molecular-weight heparin used as an anticoagulant drug used for the prevention and treatment of venous thrombo-embolism by subcutaneous/intravenous injection. Low intestinal absorption is the main obstacle for oral delivery of enoxaparin, which is attributed to its physicochemical properties such as large molecular weight, highly negative charged structure, and hydrophilicity. Meanwhile, the structure of enoxaparin is vulnerable to degradation in the acidic fluid of the stomach. To improve the oral bioavailability of enoxaparin, a variety of strategies, e.g., co-delivery with permeation enhancers, tight junction modifiers, and lyophilization of the drug have been evaluated.[1-4] Besides, nano-drug delivery systems (NDDSs) have been considered as drug carriers for THE oral delivery of enoxaparin. NDDSs own the capability to protect enoxaparin from *in vivo* acid and enzymatic degradation in the gastrointestinal (GI) tract and to increase the intestinal absorption of the drug.

Different types of NDDSs have been studied to improve the oral bioavailability of enoxaparin. For example, the study of Zupančič and colleagues[3] revealed a boosted-therapeutic activity for enoxaparin-introduced self-emulsifying drug delivery system compared to the aqueous drug solution. In another study, NE. Eleraky *et al.*[5] incorporated enoxaparin-loaded poly (lactic-co-glycolic acid) nanoparticles (PLGA NPs). Based on the *in vitro* permeation study enoxaparin-loaded PLGA NPs revealed three-fold increased cell permeability in comparison with free enoxaparin solution.

Taking the advantages of NDDSs to improve the oral bioavailability of enoxaparin, it is proved that the surface chemistry of NDDSs may potentially impact the bioavailability aspect of the drug.[6,7] Recently, the study of Lavanya and co-workers[8] revealed that Eudragit-coated liposomes significantly improve the oral bioavailability of enoxaparin in rats compared to the plain drug solution. In addition, *ex vivo* permeation studies showed a five-time increased intestinal absorption of enoxaparin with Eudragit-coated liposomes compared to uncoated liposomes.

Recently cell-penetrating peptides (CPPs) are considered as a mighty strategy for peptide-drug conjugated delivery strategy or as a functional moiety for surface modification of NDDSs.[9-11] In general, CPPs are short peptide sequences of usually less than 30 amino acids, which can be classified

*Corresponding Author: Parvin Zakeri-Milani, E-mail: pzakeri@tbzmed.ac.ir
©2023 The Author(s). This is an open access article and applies the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/). Non-commercial uses of the work are permitted, provided the original work is properly cited.

as polycationic or amphipathic peptides. In addition, CPPs may be subdivided as naturally occurring fragments, synthetic peptides, or chimeras ones.[12,13] By chemically hybridizing of CPPs with drugs or surface functionalizing of drug-loaded nanoparticles, CPPs demonstrated a potential to promote the oral absorption of different types of therapeutics. Firstly, in 2005 the potential of CPPs to increase the intestinal permeability of therapeutics was demonstrated followed by covalent conjugation of Tat to the insulin. Since then, novel drug delivery systems are emerged using CPPs to improve the oral bioavailability of medicines.[14-16] For instance, Chen *et al.*[17] investigated the intestinal absorption of CPP-coated tripterine-loaded nanostructured-lipid carriers (CPP-T-NLCs) using *in situ* intestinal technique (SPIP) in rats. The data indicated significantly higher duodenal and jejunal absorption levels for CPP-surface functionalized NLCs compared to un-coated NLCs and the free drug solution.

Taking into consideration of the mentioned details about the impact of NDDSs and CPPs on the intestinal absorption of the therapeutics, this study aimed to investigate the impact of the different synthetic moieties of CPPs on the intestinal absorption of enoxaparin-loaded liposomes. In this study, liposomal enoxaparin was prepared using the ethanol injection method and then the surface of the liposomal formulation was modified with two types of CPPs; W-K (WKWKWKWK) and W-R (WRWRWRWR), which W, K, and R represent tryptophan, lysine, and arginine amino asides. The intestinal absorption of the formulations was evaluated using the SPIP technique in rats.

Materials and Methods

Animals

Male Wistar rats weighing 180-250 g were used for *in vivo* intestinal permeability studies. The animal study was directed according to the "Guide to the care and use of experimental animals" approved by Canadian the Council on Animal Care.[18]

Materials

Enoxaparin sodium was provided from Merck (Darmstadt, Germany). Phenol red was obtained from Sigma-Aldrich (St.Louis, MO, USA). Sodium pentobarbital was obtained from Kela (Hoogstraten, Belgium). Azure II was obtained from Elder Pharmaceutical LTD (India). FMOC-Rink-Amide AM resin and amino acid derivatives were purchased from AAPPTec (Louisville, KY). Coupling agents (TBTU and DIEPA), scavengers (ethanedithiol, phenol, and TIPS), and cleavage reagents (piperidine and TFA) were provided from Sigma (St. Louis, MO). Other reagents including HPLC-grade ethanol, egg lecithin, cholesterol, NaCl, $NaH_2PO_4.2H_2O$, and Na_2HPO_4 were supplied from Merck (Darmstadt, Germany). Double distilled water was used during the study.

CPPs synthesis

Solid-phase peptide synthesis method was used for preparing the CPPs as reported recently.[19] At first, Rink-Amide AM resin was swelled in a fritted glass vessel in anhydrous DMF for 30 min under nitrogen flow. Then FMOC deprotection of the resin was done using two milliliters of piperidine solution (20% v/v, DMF) for 30 min. In the next step, FMOC-Trp (Pbf)-OH (0.14 mmol) was coupled to the resin in the presence of TBTU (0.12 mmol) and DIPEA (50 µL) in DMF for two hours. After that, the reaction solution was removed and the resin was washed with DMF (four times) and then with DCM (four times). Following the coupling of all amino acids, the resin was dried using a vacuum desiccator for 24 h. In the end, three milliliters of the cleavage cocktail reagent B (TFA/TIPS/phenol/water 88:2:5:5 v/v/v/v), was added to the resin for side-chain deprotection and the final cleavage of the synthesized CPPs from the solid support follow shaking for two hours at room temperature. Finally, the resin was collected by filtration and washed with two milliliters of cleavage cocktail. The crude CPPs was precipitated by adding diethyl ether (100 mL) and centrifuged at 4000 rpm for 5 min and then lyophilized for the next studies.

Preparation of plain/CPP-surface modified liposomal enoxaparin

Liposomal enoxaparin was prepared as reported recently.[20] Briefly, 300 mg egg lecithin and 40 mg cholesterol were dissolved in 10 mL ethanol and heated at 60°C while stirring magnetically and considered as the organic phase. The drug aqueous phase was prepared by dissolving 20 mg enoxaparin in 40 mL distilled water (70°C) and then the organic phase was added by a syringe rapidly into the aqueous phase while stirring magnetically)1200 rpm (at 70°C for 5 min. After that, ethanol was extracted completely using the rotary evaporator (Laborta 4010, Heidolph, Germany) at 40°C and followed 5 min sonicated using prob sonication (CT-chromtech, Taiwan). Then the sample was passed through a syringe filter (Millipore, cutoff 0.2 µm) five-time and then put in an ice bath to obtain the liposomal drug solution. To separate unloaded drugs, the sample was centrifuged at 60000 rpm for 1 h using OPTIMA PLX-ultracentrifuge instrument (Bechman, USA) and the final sample was considered as plain liposomes.

To surface modification of liposomes, 50 µg of each type of CPPs was added to the aqueous phase with the same preparation procedure of the plain liposomes.

Entrapment efficacy (EE)

To evaluate the EE (%) of the formulations, the unloaded enoxaparin was separated using ultracentrifugation technique at 60000 rpm and then the free drug concentration was assessed using spectrophotometry (UV-1800, Shimadzu, Japan) at 513 nm. The EE (%) value was calculated using the following equation:

$$EE\,(\%) = \frac{Total\ amount\ of\ entrapped\ drug\ (mg)}{Total\ amount\ of\ added\ drug\ (mg)} \times 100 \qquad (1)$$

Particle size and zeta potential analysis

Mean diameter and zeta potential values of the liposomes were characterized by Malvern dynamic light scattering instruments (Worcestershire, UK) without any dilution.

Preparation of perfusion solutions

At first, the perfusion buffer (PBS, pH 7.2) was prepared by using NaCl (7 g/L), $NaH_2PO_4.2H_2O$ (4.08 g/L), and Na_2HPO_4 (5.77 g/L) as reagents.[21] Then perfusion solutions of free/liposomal enoxaparin with a final concentration of 10 mg/mL were prepared by diluting the drug stock solution (100 mg/mL) and liposome solution into the perfusion buffer. In this study, phenol red with a final concentration of 50 µg/mL was added to all perfusion solutions as a non-absorbable marker to correct the effect of water flux on the outlet concentration of the drug.

In situ single-pass intestinal perfusion (SPIP)

In vivo intestinal absorption of enoxaparin was evaluated by the SPIP technique as reported previously.[22] Briefly, male Wistar rats fasted 12 h before the experiment with free access to water. Animals were anesthetized following the intraperitoneal injection of sodium pentobarbital (60 mg/kg) and placed over the heating pad. The abdomen of animals was opened following creating a middle incision and a region of proximal jejunum with a length of ~10 cm was selected by considering the Trietz ligament and cecum as landmarks. The start point of the selected segment was connected to the syringe pump (SP-500, Palmer, UK) by a plastic tube (3 mm o.d.) and the endpoint was calculated to the plastic tube, which was used to collect the outlet solution. Then, the jejunal segment was washed with the perfusion solution (37°C) at a flow rate of 12 mL/h for 10 min to take away the debris and provide a steady state of drug concentration through the intestinal wall. Afterward the outlet solution was collected over 10 min intervals. The surgical area was covered with a wetted-gauze and parafilm to keep the moisture of the area and finally, the length of the selected segment was measured at the end of the experiment.

Determination of the rat intestinal absorption of free/liposomal enoxaparin

The outlet concentration of the drug can be affected by water absorption/secretion in the selected intestinal segment. For this reason, phenol red as a non-absorbable marker was added to the perfusion solution to correct the effect of water flux which was done using the following equation:

$$C\,out\,(corrected) = C\,out\left(\frac{C\,phenol\,red\,(in)}{C\,phenol\,red\,(out)}\right) \quad (2)$$

The effective jejunal permeability ($P_{eff\,(rat),}$ cm/sec) of the drug was calculated based on the inlet and corrected-outlet concentration of the perfused drug using the following equation:

$$P_{eff\,(rat)} = -Q\,\ln\frac{(Cout\,(corrected)\,/\,Cin)}{2\pi rL} \quad (3)$$

The perfusion rate (Q) was considered as 0.2 mL/min, R represents the radius of the rat intestine (0.18 cm), and L is the length of the intestinal segment (cm).

The intestinal net water flux (NWF)

NWF (µL/h/cm) was calculated according to the following equation:

$$NWF = Q\,in\,\frac{1-(Ph.red\,out\,-\,Ph.red\,in)}{L} \quad (4)$$

where Ph.red (in) and Ph.red (out) are the inlet and outlet concentrations of the non-absorbable water flux marker phenol red, Q is the perfusion rate (0.2 mL/min), and L is the length of the intestinal segment (cm). A negative net water flux indicates loss of fluid from the mucosal side (lumen) to the serosal side (blood). A positive net water flux indicates the secretion of fluid into the segment.[23]

Predicting fraction of dose absorbed of enoxaparin and liposomal enoxaparin in humans

The fraction of dose absorbed of enoxaparin and the liposomal formulation in humans ($F_{a(human)}$, %) were predicted using the following equation, which shows the correlation between $F_{a(human)}$ and $P_{eff(rat)}$ values obtained from SPIP studies:[21]

$$F_{a(human)} = 1-e^{-38450\,P_{eff\,(rat)}} \quad (5)$$

Spectrophotometry analysis

The drug concentration in the perfused solutions was quantified using the AzurrII method. At first, a working solution of AzurrII with a final concentration of 10 µg/mL was prepared in distilled water. Then the perfused solutions were diluted 100 times and a volume of 50 µL of each sample was added to one milliliter of the working solution of AzurrII. After that, the absorbance was analyzed using Shimadzu spectrophotometry (UV-1800, Japan) at the wavelength of 513 nm. In the case of liposomal enoxaparin, firstly the liposomes were decomposed by adding an equal volume of chloroform to the samples then the water was extracted. Afterward the samples were analyzed as explained above. In order to quantify the concentration of phenol red in the samples, the absorbance was analyzed at a wavelength of 560 nm by spectrophotometry.

Data analysis

A one-way ANOVA test was used to statistically analysis of the data. All data were obtained from n = 4 repetitions for animal study and n = 3 repetitions for the rest experiments.

Results and Discussion

In this study, enoxaparin-loaded liposomes were prepared using the ethanol injection method. The mean particle size of the liposomes was obtained 50 ± 0.3 (±PDI) and a surface charge of -31 ± 7 (±SD) mV was determined

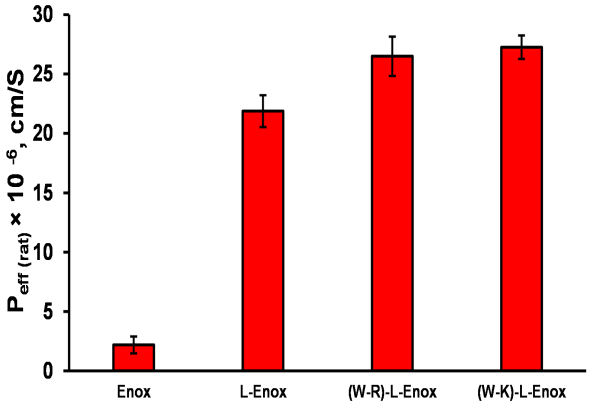

Figure 1. Effective intestinal permeability of enoxaparin-loaded liposomes compared to the free drug solution. A P value of <0.05 was considered statistically significant.

Table 1. The mean water fluxes of the perfused samples.

Sample	Average	SD
Enoxaparin (free drug)	-0.025	0.06
Liposome	0.042	0.23
Liposome + CPP (W-R)	-0.191	0.55
Liposome + CPP (W-K)	0.019	0.60

CPP: cell penetrating peptide; W-K: WKWKWKWK and W-R: WRWRWRWR, which W, K, and R represent tryptophan, lysine, and arginine amino asides.

for the liposomes. The EE (%) value of the formulation was obtained 25% ± 4. The determined NWF values for the perfused samples are summarized in Table 1 which indicates the water flux can impact the outlet concentration of the samples. Therefore, the outlet concentrations were corrected based on the outlet and inlet concentrations on phenol red. Figure 1 compares the effective intestinal absorption of free/liposomal-enoxaparin obtained from SPIP studies in rats. It can clearly be seen that liposomal formulations significantly increased the intestinal absorption of enoxaparin. Besides, CPPs-modified liposomal formulations showed higher P_{eff} values compared to plain-liposomal formulations.

Table 2 shows the $F_{a\ (human)}$ values of free/liposomal enoxaparin, which were predicted based on the $P_{eff\ (rat)}$ values of the formulations. The main idea extracted from the table is that liposomal formulations remarkably boosted the F_a value of the drug.

Enoxaparin is a highly sulfated and acidic glycosaminoglycan composed of chains of alternating residues of d-glucosamine and uronic acid. The oral bioavailability of enoxaparin is restricted mainly due to its low intestinal absorption because of; a) acidic or enzymatic degradation of enoxaparin, b) a limited absorption across the mucus of the gastrointestinal tract because of its large size and high negative charge density, and c) restricted transition through the epithelial wall as a consequence of its hydrophilicity and ionic repulsion from negatively charged epithelial tissue.[1-3] In 1982, Ueno *et al.*[24] prepared a liposomal formulation of heparin using egg lecithin and stearyl amine for oral administration to beagle dogs. In comparison with the free heparin solution, the liposomal formulation revealed an increased heparin activity in the blood. This finding could be due to the drug protection from digestive degradation or the enhanced intestinal absorption of liposomes from the gastrointestinal tract. In this study, we evaluated the impact of liposomes on the intestinal absorption of enoxaparin using the SPIP technique. The result of our study revealed a dramatically increased intestinal absorption for the liposome-entrapped enoxaparin compared with the solution of free drug. The absorption enhancing effect of liposomal formulation could be explained by; a) the decreased electrostatic interaction of enoxaparin with the mucin may be as a result

Table 2. The $F_{a\ (human)}$ value of free/liposomal enoxaparin predicted based on the $P_{eff\ (rat)}$ values of the formulations.

Formulation	$P_{eff\ (rat)}$ (cm/sec, ×10^{-6})	$F_{a\ (human)}$ (%)
Enoxaparin (free drug)	2.2	8
L-Enoxaparin	21.9	57
(W-R)-L-Enoxaparin	26.5	64
(W-K)-L-Enoxaparin	27.3	65

W-K: WKWKWKWK and W-R: WRWRWRWR, which W, K, and R represent tryptophan, lysine, and arginine amino asides.

of the shielding effect of liposomes. the mucus layer is a hydrogel composed of glycoproteins posing a net negative charge, mucin being its main component. To reach the epithelium, enoxaparin must overcome the mucus barrier to ensure sufficient drug levels in the systemic circulation. Due to the lipophilic nature of liposomes, their interaction with the mucus layer is presumably low,[2,3] b) a promoted intestinal absorption of entrapped heparin into liposomes by membrane fusion between the intestinal cell membrane and liposomal membrane,[24] c) decreased ionic repulsion between the negatively charged epithelial tissue and the surface of enoxaparin.

In contrast to our finding, Chia-Ming *et al.*[25] reported that liposomal entrapment of drugs fails to facilitate the transport of non-absorbable drugs and prevents the transport of absorbable ones.[25] They evaluated the impact of L-α-cy-phosphatidylcholine distearoyl/cholesterol liposomes on the intestinal absorption of nano-absorbable markers using two *in vitro* methods; the diffusion cell and intestinal everted-sac experiments, and *in situ* permeation study using the modified Doluisio technique. The data indicated that the intestinal absorption of nano-absorbable markers had no changes compared with the liposomal formulations. This difference may be attributed to the composition of liposomes. As Kimura *et al.*[26] reported that egg lecithin liposomes are endocytosed by epithelial cells while L-cy-phosphatidylcholine distearoyl liposomes are not.

In regards to the CPPs-modified liposomes (CPP-L), our results revealed a slightly higher absorption for CPPs-L compared with unmodified liposomes. The improved absorption of CPPs-L formulation compared to the un-modified liposomes could be due to the internalization efficiency of the CPPs due to the facilitates endocytosis and direct penetration into the epithelium via an energy-dependent pathway.[17] Similarly, an improved intestinal absorption was reported for several CPP-surface modified NPs such as chitosan nanoparticles,[27] PLGA NPs,[28] NLCs,[17] and porous silicon NPs,[29] which induce a better pharmacokinetic property, an improved oral bioavailability, and boost the therapeutic efficacy of medicines.

From the point of the fraction of dose absorbed of enoxaparin in humans, $F_{a\ (human)}$ values of 8%, 57%, and near 65% were predicted for the free drug solution, plain liposomes, and CPP-surface modified liposomes, respectively. Three different classes of *in vivo* absorption is defined in man; (a) poor (0-30%), (b) intermediate (30-90%), and (c) complete (90-100%).[21,30] Therefore, liposomal formulations are predicted to have a mighty potential to boost the fraction of dose absorbed of enoxaparin in humans.

Conclusion

In this work, liposomal formulations were developed to enhance the intestinal absorption of enoxaparin. All liposomal formulations revealed a dramatically improved intestinal absorption compared with free enoxaparin solution. CPPs-modified liposomes showed a slightly increased intestinal absorption compared with un-modified liposomes. In conclusion, liposomes showed a mighty potential for the oral delivery of enoxaparin.

Ethical Issues

The animal study was received a permission from the local ethics committee of Tabriz University of Medical Sciences (Tabriz, Iran). All animal experiments were performed according to the "Guide to the care and use of experimental animals" approved by Canadian the Council on Animal Care.[18]

Acknowledgements

This paper was founded by the National Institute for Medical Research (NIMAD, grant NO. 963499).

Author Contributions

Study concept and design: PZM and HV; Acquisition of data: HR; Analysis and interpretation of data: PZM, HV, ZI, and HR; Administrative, technical and material supports: HV and PZM; Drafting of the manuscript: MM and PZM; Writing of manuscript: MM; Critical revision of the article for important intellectual content: PZM and ZI.

Conflict of Interest

The authors have no conflict of interest to report.

References

1. Scala-Bertola J, Rabiskova M, Lecompte T, Bonneaux F, Maincent P. Granules in the improvement of oral heparin bioavailability. Int J Pharm. 2009;374(1-2):12-6. doi:10.1016/j.ijpharm.2009.02.020
2. 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(1-2):275-81. doi:10.1016/j.ijpharm.2009.11.025
3. Zupančič O, Grieβinger JA, Rohrer J, de Sousa IP, Danninger L, Partenhauser A, et al. Development, in vitro and in vivo evaluation of a self-emulsifying drug delivery system (SEDDS) for oral enoxaparin administration. Eur J Pharm Biopharm. 2016;109:113-21. doi:10.1016/j.ejpb.2016.09.013
4. Motlekar NA, Srivenugopal KS, Wachtel MS, Youan BBC. Evaluation of the oral bioavailability of low molecular weight heparin formulated with glycyrrhetinic acid as permeation enhancer. Drug Dev Res. 2006;67(2):166-174. doi:10.1002/ddr.20087
5. Eleraky NE, Swarnakar NK, Mohamed DF, Attia MA, Pauletti GM. Permeation-enhancing nanoparticle formulation to enable oral absorption of enoxaparin. AAPS PharmSciTech. 2020;21(3):88. doi:10.1208/s12249-020-1618-2
6. Yazdi JR, Tafaghodi M, Sadri K, Mashreghi M, Nikpoor AR, Nikoofal-Sahlabadi S, et al. Folate targeted PEGylated liposomes for the oral delivery of insulin: In vitro and in vivo studies. Colloids Surf B Biointerfaces.

2020;194:111203. doi:10.1016/j.colsurfb.2020.111203
7. Yamazoe E, Fang J-Y, Tahara K. Oral mucus-penetrating PEGylated liposomes to improve drug absorption: Differences in the interaction mechanisms of a mucoadhesive liposome. Int J Pharm. 2021;593:120148. doi:10.1016/j.ijpharm.2020.120148
8. Lavanya N, Muzib YI, Aukunuru J, Balekari U. Preparation and evaluation of a novel oral delivery system for low molecular weight heparin. Int J Pharm Investig. 2016;6(3):148. doi:10.4103/2230-973X.187351
9. Xu Y, Zhang X, Wang N, Pei X, Guo Y, Wang J, et al. Cell-penetrating peptide enhanced insulin buccal absorption. Int J Pharm. 2020;584:119469. doi:10.1016/j.ijpharm.2020.119469
10. Kristensen M, Kucharz K, Felipe Alves Fernandes E, Strømgaard K, Schallburg Nielsen M, Cederberg Helms HC, et al. Conjugation of therapeutic psd-95 inhibitors to the cell-penetrating peptide tat affects blood–brain barrier adherence, uptake, and permeation. Pharmaceutics. 2020;12(7):661. doi:10.3390/pharmaceutics12070661
11. Danielsen EM. Intestinal permeation enhancers: Lessons learned from studies using an organ culture model. Biochim Biophys Acta Biomembr. 2021;1863(1):183474. doi:10.1016/j.bbamem.2020.183474
12. Foged C, Nielsen HM. Cell-penetrating peptides for drug delivery across membrane barriers. Expert Opin Drug Deliv 2008;5(1):105-17. doi:10.1517/17425247.5.1.105
13. Xu J, Khan AR, Fu M, Wang R, Ji J, Zhai G. Cell-penetrating peptide: A means of breaking through the physiological barriers of different tissues and organs. J Control Release. 2019;309:106-24. doi:10.1016/j.jconrel.2019.07.020
14. Nielsen EJB, Kamei N, Takeda-Morishita M. Safety of the cell-penetrating peptide penetratin as an oral absorption enhancer. Biol Pharm Bull. 2015;38(1):144-6. doi:10.1248/bpb.b14-00517.
15. Bashyal S, Noh G, Keum T, Choi YW, Lee S. Cell penetrating peptides as an innovative approach for drug delivery; then, present and the future. Int J Pharm Investig. 2016;46(3):205-20. doi:10.1007/s40005-016-0253-0
16. Yin N, Brimble MA, Harris PW, Wen J. Enhancing the oral bioavailability of peptide drugs by using chemical modification and other approaches. Med Chem. 2014;4(12):763-9. doi:10.4172/2161-0444.1000227
17. Chen Y, Yuan L, Zhou L, Zhang Z-h, Cao W, Wu Q. Effect of cell-penetrating peptide-coated nanostructured lipid carriers on the oral absorption of tripterine. Int J Nanomedicine 2012;7:4581. doi:10.2147/IJN.S34991
18. Olfert ED, Cross BM, McWilliam AA. Guide to the care and use of experimental animals. Ottawa: Canadian Council on Animal Care Ottawa; 1993.
19. Mussa Farkhani S, Asoudeh Fard A, Zakeri-Milani P, Shahbazi Mojarrad J, Valizadeh H. Enhancing antitumor activity of silver nanoparticles by modification with cell-penetrating peptides. Artif Cells Nanomed Biotechnol. 2017;45(5):1029-35. doi:10.1080/21691401.2016.1200059
20. Palassi S, Valizadeh H, Allahyari S, Zakeri-Milani P. Preparation and In-vitro characterization of enoxaparin nano-liposomes through different methods. Adv Pharm Bull. 2021;11(2):295-300. doi:10.34172/apb.2021.042
21. Mahmoudian M, Zakeri-Milani P, Valizadeh H. Investigating intestinal permeability of bortezomib using a validated HPLC-UV method. Drug Res. 2019;69(3):130-5. doi:10.1055/s-0043-115648
22. Mahmoudian M, Valizadeh H, Löbenberg R, Zakeri-Milani P. Enhancement of the intestinal absorption of bortezomib by self-nanoemulsifying drug delivery system. Pharm Dev Technol. 2020;25(3):351-8. doi:10.1080/10837450.2019.1699109
23. Zakeri-Milani P, Valizadeh H, Tajerzadeh H, Azarmi Y, Islambolchilar Z, Barzegar S, et al. Predicting human intestinal permeability using single-pass intestinal perfusion to rat. J Pharm Pharm Sci. 2007;10(3):368-79.
24. Ueno M, Nnakasaki T, Horihoshi I, Sakurswawa N. Oral administration of liposomally-entrapped heparin to beagle dogs. Chem Pharm Bull. 1982;30(6):2245-7. doi:10.1248/cpb.30.2245
25. Chia-Ming C, Weiner N. Gastrointestinal uptake of liposomes. I. In vitro and in situ studies. Int J Pharm 1987;37(1-2):75-85. doi:10.1016/0378-5173(87)90011-1
26. Kimura T, Higaki K, Sezaki H. Transmucosal passage of liposomally-entrapped drugs in rat small intestine. Pharm Res. 1984;1(5):221-4. doi:10.1023/A:1016373330348
27. Li L, Yang L, Li M, Zhang L. A cell-penetrating peptide mediated chitosan nanocarriers for improving intestinal insulin delivery. Carbohydr Polym. 2017;174:182-9. doi:10.1016/j.carbpol.2017.06.061
28. Bu X, Zhu T, Ma Y, Shen Q. Co-administration with cell penetrating peptide enhances the oral bioavailability of docetaxel-loaded nanoparticles. Drug Dev Ind Pharm. 2015;41(5):764-71. doi:10.3109/03639045.2014.902465
29. Shrestha N, Araújo F, Shahbazi MA, Mäkilä E, Gomes MJ, Herranz-Blanco B, et al. Thiolation and cell-penetrating peptide surface functionalization of porous silicon nanoparticles for oral delivery of insulin. Adv Funct Mater. 2016;26(20):3405-16. doi:10.1002/adfm.201505252
30. Fagerholm U, Johansson M, Lennernäs H. Comparison between permeability coefficients in rat and human jejunum. Pharm Res. 1996;13(9):1336-42. doi:10.1023/a:1016065715308