Original Article

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Improved systemic delivery of insulin by condensed drug loading in a dimpled suppository

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Summary **The development of peptide therapeutics owing to the advances in biotechnology has overcome some unmet medical needs; however, the route of administration is still limited to injections. Systemic delivery of insulin *via* an enteral route remains a great challenge due to its instability and low mucosal permeability. In this study, we investigated the effect of drug condensation in a suppository on the efficacy of insulin after rectal administration. Suppositories with dimples are prepared by a mold method using a hard fat (Suppocire® AM). Insulin or fluorescein isothiocyanate-dextran (molecular weight: 3,000-5,000) (FD4) as a model of a hydrophilic macromolecule was loaded in the dimples, and sealed with other lipids with different melting points. The *in vitro* release test showed that the time to 50% drug release depends on the melting point of the lipid for sealing but not on the number of dimples. The suppositories with one-, or three-dimple containing insulin and caprylocaproyl macrogol-8 glyceride (Labrasol®) were administered to rats at 0.5 U/head. The reduction in plasma glucose level was more significant for the one-dimple-type suppository than for the three-dimple-type although the one-dimple-type suppository contained less amount of Labrasol by one-third compared to the three-dimple-type. These results suggest that condensation of an insulin dose in a limited surface area of a suppository improves systemic availability *via* the rectal route with a reduced amount of an absorption enhancer.**

Keywords: Dimple type suppository, insulin, peptide, rectal delivery, Labrasol

1. Introduction

The recent progress in biotechnology has overcome some of the problems related to an unestablished field of therapeutics (*1*). The novel highly functional peptide therapeutics, such as agents for cancer, diabetes mellitus, cardiovascular diseases, and rheumatoid arthritis, are clinically accessible and were found to have a favorable clinical effect (*2-4*). Although these peptides drastically improve the symptoms, their administration method is limited to injections, which lead to patient inconvenience due to pain, risks of infection, and the need for continuous ambulant use. In addition, polypeptides are scarcely absorbed in the gastrointestinal tract due to their high susceptibility to the digestive and mucosal enzymes (*5-7*) in addition to their poor membrane permeability (*8*). To overcome these drawbacks of peptide therapeutics, alternative routes of administration have been investigated by many researchers. Nasal (*9*) and pulmonary (*10,11*) routes are the representative alternatives. The bioavailability of insulin *via* pulmonary route is 10-46% (*12*). Exubera®, the inhalation formulation of insulin, was marketed, although the product is withdrawn.

Oral route is the most attractive route of administration but tends to produce significant variability in absorption rate and absolute availability because of various factors, *e.g.*, gastric emptying and gut motility, digestive enzymes, pH, luminal contents, among others. Rectal route is another alternative route of administration for peptides (*13,14*). Although some proteolytic enzymes exist in the rectal mucosa (*15*), the peptides administered rectally are less degraded than those ingested orally, owing to the presence of few secretory digestive enzymes in the rectum. In addition, it has been reported that the proteolytic enzyme activities of the rectal epithelial cells are lower than those of rat nose, lung,

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and Caco-2 cells (*15*).

Besides the investigation of alternative routes, improvement of intestinal absorption of polypeptide drugs has also been investigated by many researchers, such as by suppressing enzymatic degradation *via* co-administration of protease inhibitors (*16*) and enhancing the penetration across the mucosal epithelium through use of an absorption enhancer (*17-19*). There are two distinctive approaches to improve the intestinal absorption of peptides: reducing pH at the limited site of drug administration, where the activities of peptidases are suppressed (*20,21*) and using concentration gradient as a driving force by dosing with a highly concentrated drug at the limited site (*22,23*). In the case of drug absorption through passive diffusion, the rate of absorption essentially depends on the concentration in the lumen when the blood drug concentration is low enough, *i.e.* a sink condition can be assumed.

In the present study, to improve the enteral delivery for poorly absorbable drugs such as peptides, we designed a suppository with dimples where a drug is located only in a limited area and released at a higher rate when administered in the rectum (Figure 1). Insulin, was chosen as a model peptide drug and the effect of drug condensation in a dimple of a suppository on rectal delivery was evaluated in terms of plasm glucose level. Labrasol, which is composed of polyethylene glycol (PEG) esters, a small glyceride fraction, and free PEG, is a non-ionic water dispersible surfactant. We selected Labrasol as a nontoxic absorption enhancer for insulin, which is not only not irritable to the intestinal mucosa but also does not interact with other lipoid pharmaceutical additives such as hard fat.

Sinko *et al*. reported that a bolus intestinal administration of salmon calcitonin as a higher concentration solution provided higher bioavailability than the continuous administration of a lower concentration solution (*20*). We assumed that a rapid drug release, *i.e*. a rapid melting of the sealing material of the dimples, is required for improving the rectal absorption of insulin. In addition to the selection of the sealing material suitable for the rapid release, setup of the suitable evaluation method is also important. To evaluate *in vitro* release from a suppository, basket or paddle method (*24*), dialysis membrane method (*24,25*), and flow-through method (*26*) are used commonly. However, very few of the *in vitro* tests for suppositories are for the fast release-type suppository. For the fast release-type suppository, the drug release rate through a semipermeable membrane in the dialysis membrane method is not negligible in comparison with the release rate from a suppository. The European Pharmacopoeia suggests that *in vitro* release test should be adopted as the property of suppository, although it introduces flow-through method for the *in vitro* release test of suppository (*24*). In this study, we used the system illustrated in Figure 2. By placing a suppository fixed by mesh with the dimple side up, the drug phase composed of low-gravity oil can float in medium as soon as the sealing melts. Thereafter, the drug in the dimple can be released. To select an appropriate sealing material for rapid release, we examined drug release from suppositories using the above described method with fluorescein isothiocyanate-dextran (molecular weight of 3,000-5,000 (FD4)) as a marker.

The goal of this study was to clarify if a dimple-type suppository is useful for improving rectal insulin delivery and to suggest the feasibility of the dimple-type suppository for the rectal absorption of biomedicines including peptides as well as oligonucleotides.

2. Materials and Methods

2.1. *Materials*

Fluorescein isothiocyanate-dextran with a molecular weight of 3,000-5,000 (FD4) was purchased from Sigma-Aldrich Co., Ltd. (USA). Bovine insulin (from bovine pancreas, 27 USP-U/mg) was purchased from Sigma-Aldrich (St. Louis, USA). Suppocire® AM pastilles and Labrasol® were donated by Gattefossé (Lyon, France). Soybean oil was purchased from Wako Pure Chemicals (Osaka, Japan). A glucose test kit (Glucose C-test Wako) was purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents were of reagent grade.

2.2. *Preparation of dimple-type suppository*

Suppocire AM pastilles molten at 60°C were cast into an aluminum template (0.4 mL) and solidified at 4°C to form the molded sample (ϕ: ca.4 mm × height: ca.30 mm). One, three, or five dimples (diameter: 2.5-3.0 mm)

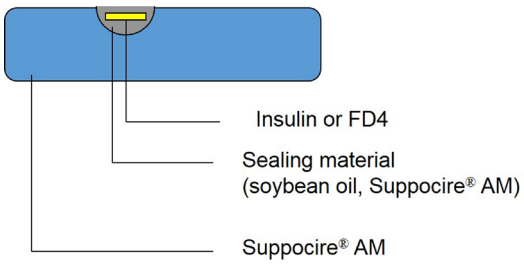

Figure 1. Illustration of a dimple type suppository.

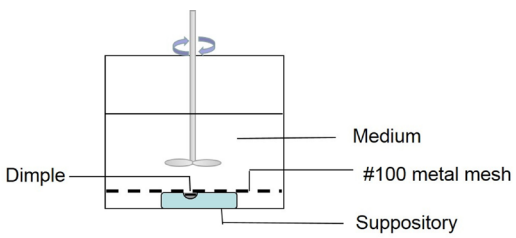

Figure 2. Scheme of the apparatus for the dissolution test.

were created on the lateral face of sample; 5 μL of 100°C glycerin was poured onto the lateral face to melt the surface and vacuum off the molten surface.

2.3. *Drug loading to the dimples of suppository*

Fifty percent (w/v) of Labrasol aqueous solution in which insulin or FD4 was dissolved was used as a loading solution. The solution was loaded onto the dimples of a suppository *via* the following procedure; 2 μL of a molten sealing material (soybean oil, a mixture of Suppocire AM pastilles and soybean oil (10:14, w/w) or Suppocire AM alone was loaded onto each dimple and solidified, following which 2.5 μL of the loading solution was poured into each dimple and allowed to dry in a desiccator for a couple of hours, and each dimple was covered with 2.5 μL of the sealing material. The final amount of insulin and FD4 was 0.5 IU and 100 μg in a suppository, respectively. Each dimple has 1 mg of Laborasol. The obtained suppositories were stored at −20ºC until use.

2.4. *In vitro release test*

In vitro release test was performed using the apparatus illustrated in Figure 2. Namely, a suppository was placed into 9.6 mM phosphate buffered saline (pH7.2; 20 mL) with the dimple side up at 37°C and covered by #100 metal mesh. The medium was stirred by a propeller mixer (ϕ 20 mm) at 100 rpm and kept at 37°C. Five-hundred microliters of the medium was collected at 30, 45, 60, 90, 120, 180, and 300 sec and fresh phosphate buffered saline (500 μL) was added. After all the samples were collected, the residual drug was extracted. Briefly, rest of the medium and the residual suppository were collected in a test tube and methylene chloride (5 mL) was added. The mixture was shaken at 100 rpm for 30 min and centrifuged at 2,000 rpm to collect the aqueous layer. The concentration of FD4 in the samples and the aqueous layer was determined using fluorescent spectrometry using the hybrid multi-mode microplate reader (Synergy H4, BioTek Instruments, Winooski, USA). The test was performed in triplicate. The time to 50% drug release (D_{50}) was calculated from the fitting curve of quadratic approximation on each dissolution profile.

2.5. *Animal experiments*

All animal experiments were performed in line with the Guidelines for Animal Experiment at Osaka Otani University. Male Wistar rats weighing 185-230 g were allowed to fast with free access to water for 16 h and anesthetized by an intraperitoneal administration of 50 mg/kg sodium pentobarbital. The jugular vein was exteriorized by surgery, and 50 μL blood was collected as blank. A suppository was administered into the rectum and the anus was immediately tied tightly to prevent leakage of the meltage. Fifty microliters of blood sample was collected from the jugular vein at the predetermined times. The blood samples were immediately heparinized to separate the plasma by centrifugation (12,000 rpm for 5 min). The glucose concentration in plasma was measured by the glucose test kit.

2.6. *Statistical analysis*

Experiments were replicated at least thrice. The differences between means for two groups were statistically analyzed using Student's *t*-test. *P* values < 0.05 indicated significant difference.

3. Results

3.1. *In vitro drug release from the dimple suppository*

The influence of sealing materials on the drug release from a dimple suppository was examined using FD4 as a maker. As shown in Figure 3a, the suppositories containing the mixture of Suppocire AM and soybean oil (10:14) (B type) showed the smallest D_{50} of 44.8 ± 8.8 sec (mean ± S.E.), while those containing soybean oil alone (A type) and Suppocire AM alone (C type) had D_{50} values of 103.5 ± 7.6 sec and 61.6 ± 20.0 sec, respectively. The drug release was proven to be independent of the number of dimples (Figure 3b).

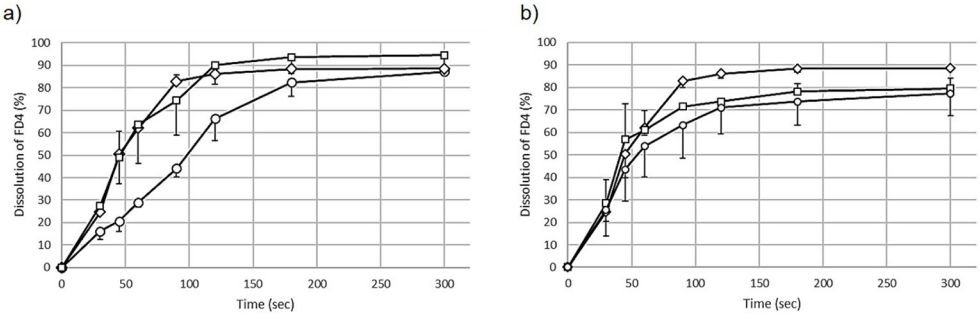

Figure 3. *In vitro* release from suppositories. a) Influence of the sealing material on drug release; ○: soybean oil (A type), ◊: the mixture of Suppocire® AM and soybean oil (= 10:14) (B type), □: Suppocire® AM (C type). **b)** Influence of the number of dimples on drug release, ◊: one-dimple, □: three-dimple, ○: five-dimple. FD4 1 mg/suppository. Labrasol 1 mg/dimple. Data represent mean ± S.E. (*n* = 3 batches).

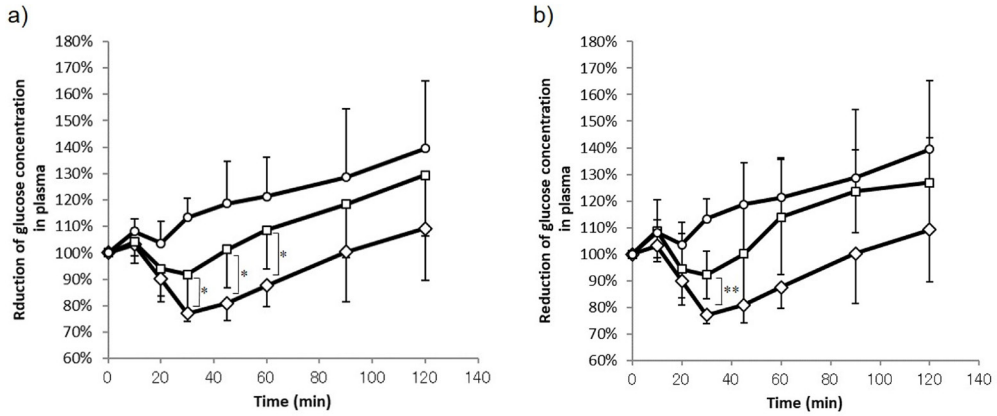

Figure 4. Plasma glucose concentration after the rectal administration of suppository. a) Effect of Labrasol on the reduction of glucose (◊: 0.5 U insulin and 1 mg LabrasolTM, □: 0.5 U insulin, ○: placebo). **b)** Influence of the number of dimples on the reduction of glucose (◊: one-dimple, □: three-dimples) or placebo (○). Insulin: 0.5 U/suppository. Labrasol^{TM}: 1 mg/dimple. Data represent mean ± S.D. (n = 6). The symbols * and ** represent $p < 0.05$ and $p < 0.01$, respectively.

The release from one-, three-, and five-dimple B-type suppositories was approximately 80-90% after 90 sec with D_{50} values of 44.8 ± 8.8 sec, 48.3 ± 9.3 sec, and 64.0 ± 27.4 sec (the mean ± S.E.), respectively. Consequently, no significant differences in drug release were detected among the suppositories with different number of dimples.

3.2. *Effect of Labrasol on the rectal absorption of insulin from the dimple suppository*

The plasma glucose level in rats after the administration of insulin-loaded one-dimple B-type suppository is shown in Figure 4a. The maximum reduction of plasma glucose level was observed at 30 min, with 22.9 ± 3.2% and 8.2 ± 5.8% (mean ± S.D. as the percentage to the initial level) reduction with and without Labrasol, respectively. The difference was statistically significant from 30 min to 60 min ($p < 0.05$).

3.3. *Effect of the number of dimples on the rectal absorption of insulin*

One- or three-dimple B-type suppositories loaded with insulin and Labrasol were administered to rats. The time profiles of plasma glucose level are shown in Figure 4b. Insulin (0.5 IU) and Labrasol (1 mg)-loaded one-dimple suppository reduced plasma glucose concentration up to 22.9 ± 3.2% (mean ± S.D.), while the three-dimple suppository in which insulin (0.5 IU) and Labrasol (3 mg) were divided into three dimples reduced plasma glucose level up to 7.8 ± 8.9% (mean ± S.D.). There was a statically significant difference in the reduction of plasma glucose level at 30 min between one- and three-dimple suppositories ($p < 0.01$).

4. Discussion

The absorption rate for a drug *via* passive transport depends on the drug concentration in the intestinal lumen. Therefore, we assumed that a rapid drug release, *i.e.* a rapid melting of the sealing material of the dimples, is required for improving the rectal absorption of insulin from the dimple suppository. First, we designed the fast release-type suppository. The drug release from the dimple-type suppository we used in this study was studied using the *in vitro* release test; the suppository with Suppocire AM alone and with the mixture of Suppocire AM-soybean oil as the sealing material, showed a more rapid release than that with soybean oil alone. FD 4 was observed to be mixed easily with soybean oil, a sealing material in a dimple, but not with the other sealing materials (data not shown). The suppository with soybean oil alone in a dimple provided a slower release of FD4 owing to the entrapment of FD4 into soybean oil, which retarded its release. With respect to variability among batches, the suppositories in which dimples were sealed with soybean oil alone and with the mixture of Suppocire AM-soybean oil showed less release variability than that with Suppocire AM alone. The melting points of Suppocire AM and soybean are around 37°C and −9°C, respectively. The 10:14 mixture of Suppocire AM and soybean oil was designed to be in solid state at 25°C and to melt at 32°C within 5 sec. It is speculated that the two sealing materials with lower melting points melt quickly with the least variability at 37°C, which leads to their sealing off within less time regardless of batches. Thus, in terms of rapid release and less variability, we selected the mixture of Suppocire AM-soybean oil as a sealing material for insulin-loaded suppository.

In this study, we used Labrasol as an absorption enhancer and showed that Labrasol is effective for the improvement of rectal insulin delivery. With respect to the mechanism of the enhancement by Labrasol, suppression of drug efflux due to P-gp (*27*), interaction with the lipid bilayer of the epithelial cell (*28*), and opening of the tight junction (*29*) are reported. However,

the suppression of drug efflux appears to contribute the least to the absorption enhancement of insulin because most of the substrates for P-gp are hydrophobic compounds.

Next, we evaluated the influence of number of dimples on rectal insulin delivery in the presence of Labrasol (Figure 4b). The results suggested that the localization of insulin in a suppository can improve the rectal absorption of insulin, although there seems to be little difference in the drug release rate between one- and three-dimple suppositories (Figure 3b). The insulin concentration in a dimple of one-dimple suppository is three times higher than that of three-dimple suppository. The loaded insulin in a dimple is covered by the hard fat except for at the sealing site. Therefore, the release of insulin from the dimple suppository is limited only towards the rectal mucosa without dilution by the luminal bulk fluid because insulin covered by the suppository base is restricted to diffuse to the lumen. The prevention of dilution of insulin by the luminal fluid or contents provides a high concentration of drug between the epithelium and a suppository. The concentration gradient between the epithelial surface and blood is the driving force for the drug to permeate across the mucosa. It is reported that dosing with a highly concentrated drug at the limited site leads to the improvement of the intestinal absorption of caffeine (*22*) *via* concentration gradient as a driving force. This could explain the improvement of the rectal delivery of insulin by one-dimple suppository.

The higher concentration may also induce the saturation of enzymatic degradation. It is reported that dosing with a highly concentrated drug at the limited site leads to saturation of enzymatic degradation to improve the intestinal absorption of erythropoietin (*23*). Among the enzymes that decompose insulin, insulin-degrading enzymes have been well investigated and it was revealed that decomposition of insulin by the enzymes played an important role in the intestinal absorption of insulin (*30-32*). It is also reported that the enzyme is located in the cytosol of rat small intestine mucosal cells, human colon mucosal cells, and Caco2 cells. The Km of the cytosolic insulin degrading activity (78 nM) is comparable to that of the enzyme (*31*). The insulin degrading activity in mucus appears to be more important for the enzymatic barrier of the rectum than that of the small intestine because of less contribution of secretory digestive enzymes in the rectum. In terms of accessibility of enzymes to insulin, the amount of the enzymes that can access insulin in the one-dimple suppository is three times lower than that for the three-dimple suppository because only one-third of the membrane area is in contact with the drug layer. Therefore, the loading of insulin per unit of enzyme for one-dimple suppository is 9 times larger than that of three-dimple suppository. If permeability through the mucosal membrane facing each dimple, which contains the same amount of Labrasol, is equally facilitated, one-dimple suppository would show. a greater reduction in plasma glucose level due to saturation of the enzymatic degradation of insulin in the intestine. For avoiding the enzymatic barrier, however, the effect of Labrasol on enzymatic activity must be considered. It is reported that Labrasol inhibits intestinal UDP-glucuronyl transferase (*33*). Although the effect of Labrasol on the activities of insulin degrading enzymes in the intestine is not clear, the condensed Labrasol in a dimple may inhibit them. If this is the case, we should estimate to what extent the enzyme inhibition by Labrasol and the saturation of enzymatic degradation by a high concentration of insulin contribute to the promotion of rectal insulin delivery.

For the absorption of insulin, the association of insulin is an important factor. Insulin exists as a monomer, dimer, trimer, or hexamer (*34*). In terms of the molecule association, addition of surfactants is one of the determinants. Shao and his co-workers report that sodium dodecyl sulfate, hexadecyl trimethylammonium bromide, and sodium glycolate dissociate porcine-zinc insulin hexamers into monomer (*35*). They also report that these surfactants enhance the bioavailability of intestinal absorption (*36*). Therefore, it is important to determine the status of insulin in the dimple of suppository in the presence of Labrasol because it appears to be an important factor for the rectal absorption of insulin at a high concentration.

We demonstrated that condensing insulin in a limited area of a suppository is a promising strategy for the enhancement of its rectal absorption. With respect to absorption enhancers, their effects generally depend more on the concentration than on the total amount of dose. When the absorption area is limited, the total dose of an absorption enhancer required and consequently, a risk of possible adverse effect can be remarkably reduced. On the other hand, this strategy not only applies to the rectal absorption of polypeptides but also can be expanded to other biomedicines such as oligonucleotides, which are poorly permeable across the mucosa and unstable against enzymes in the intestine. We demonstrated that our rectal siRNA delivery technique using lipid nanoparticles provided therapeutic gene silencing in the liver through its delivery *via* the lymphatic route (*37,38*). Feasibility of our dimple-type suppository to rectal oligonucleotide delivery is now under investigation.

5. Conclusion

The present study demonstrates that a dimple-type suppository can promote the rectal delivery of insulin by condensing it in a dimple with an absorption enhancer. This technique may lead to a new enteral delivery system for biomedicines such as peptides and oligonucleotides that are poorly permeable and unstable in the intestine.

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