Morphologic Examination of Rabbit Nasal Mucosa after the Nasal Administration of Insulin Peanut Oil Suspension and a Powder Dosage Form with Soybean-Derived Sterylglucoside

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Soybean-derived sterylglucoside mixture (SG) is a potentially effective absorption enhancer for the nasal absorption of insulin. Insulin in a peanut oil suspension dosage form and in a powder dosage form with SG were administered into the rabbit nasal cavity. After 0.5 or 1 h, nasal mucosa was taken from the nasal cavity and side effects were examined by an optiphot light microscope. The insulin in the peanut oil suspension produced a histological change in the nasal epithelium mucosae, as well as mucodermal phlogistic cellular infiltration. The peanut oil suspension with SG showed side effects slightly stronger than without SG. SG alone and the insulin powder dosage form with SG produced no signs of inflammation, erosion or squamous metaplasia. The results from this study indicate that SG can be considered safe.

Key words nasal absorption; insulin; peanut oil; powder dosage form; sterylglucoside; morphologic examination

Interest in the drug delivery of peptides and proteins through nonparenteral routes has increased in recent years. Insulin is not effective when administered orally, and must be administered by intravenous or dermoclysis injection. Nonparenteral routes for insulin delivery have been investigated, including nasal, buccal, rectal, vaginal, pulmonary and transdermal routes. The results to date indicate that the nasal route is considered to be the most promising.

In an attempt to increase the bioavailability of insulin after nasal administration, a number of enhancers have been investigated. Among them, bile salts, sodium taurodihydrofusidate and didecanoyl-t-α-phosphatidylcholine are effective as enhancers for the nasal absorption of insulin, but are also reported to alter nasal morphology.

We have already reported on the effect of a soybean-derived sterylglucoside mixture (SG) as an enhancer for the nasal absorption of insulin in rabbits. The bioavailability of insulin was 6.0% in peanut oil without SG, and it increased to 11.6% with SG as an enhancer. SG possesses excellent properties as an enhancer in a peanut oil suspension and powder dosage form. It is superior to the best known absorption enhancer, sodium glycocholate.

It is necessary to examine histological changes in nasal mucosae caused by peanut oil and SG if it is to be considered for practical use. In this study, we examined the side effects on nasal mucosae caused by the administration of insulin peanut oil suspension and powder dosage forms with SG.

MATERIALS AND METHODS

Materials Bovine insulin (24.4 IU/mg) and sodium glycocholate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Peanut oil was of guaranteed reagent grade from "The Japanese Pharmacopoeia XII." SG was kindly supplied by Ryukakusan Co., Ltd. (Tokyo, Japan). All other materials used were reagent grade. SG is a mixture of the monoglucoside of β-sitosterol (49.9%), campesterol (29.1%), stigmasterol (13.8%), and brassicasterol (7.2%).

Preparation of Dosage Form and Administration Methods Preparations were administered to female Japanese white rabbits weighing between 2.5 and 3.0 kg (Saitama Experimental Animal Supply Co., Japan). A polyethylene tube with a diameter of 1.05 mm and a length of 10.0 cm was installed at the top of a syringe and inserted into the nose of a rabbit.

The liquid dosage form was prepared from 40 mg insulin suspended in 10 ml of peanut oil with or without 1% (w/w) SG, and a pH 7.31 phosphate buffer saline solution (PBS) with 1% (w/w) sodium glycocholate (97.6 IU/ml). About a 250 μl dosage was loaded into the syringe and administered through the tube into either nasal cavity of a rabbit (10 IU/kg). The powder dosage forms, 10 mg of insulin, 10 mg of SG, or 10 mg of insulin with SG (insulin:SG = 2:1, weight), were administered through the tube, again into either nasal cavity of a rabbit (10 IU/kg).

In the powder dosage form, spraying was effected by the attachment of a sprayer (rubber bulb with reservoir) as previously reported. The control group received no administration.

Evaluation of Stimulus on Nasal Mucosa Rabbits were sacrificed using an anesthetic at 0.5 or 1 h after the administration of each preparation. The ventral nasal conchae was immediately removed from the anterior nasal cavity. The nasal mucosa was fixed in 10% neutral carbonate-buffered formalin for at least 24 h before routine processing and then cut vertically against the nasal mucosa surface at the central region in 4 mm widths. Each section was dehydrated using a graded series of ethanol solutions, and was then embedded in paraffin wax. Tissues were divided into small pieces (about 3 μm in thickness) and stained with hematoxylin and eosin. All sections were examined by an optiphot light microscope (Optiphot, © 1995 Pharmaceutical Society of Japan

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Fig. 1. Microscopic Photos of Nasal Mucosa 1 h after Administration of 250 μl of the Liquid Dosage Form (H and E Stain, × 100)
(a) control (untreated, normal respiratory epithelium), (b) peanut oil, (c) insulin in peanut oil, (d) insulin with SG in peanut oil.

Nikon). The state of the nasal epithelium on the dosed side of the septum was qualitatively compared with the untreated tissue.

RESULTS AND DISCUSSION

The tissue was excised 1 h after administration. The 1 h contact time was chosen since it showed the lowest glucose level; blood glucose levels started to drop quickly after dose administration, and fell most rapidly after the first 1 h.\(^{81}\) To observe the epithelium damage, additional samples were taken 0.5 h after SG powder administration.

The effect of peanut oil and SG on nasal mucosa was examined. Fig. 1 shows some of the effects of peanut oil and SG on rabbit nasal mucosa structure 1 h after administration of 250 μl of the liquid dosage form.

Figure 1a shows the control mucosa, normal respiratory epithelium stained with hematoxylin–eosin. Figures 1b, c and d show peanut oil, insulin in peanut oil, and insulin with SG in peanut oil, respectively, in contact for 1 h after the administration of a 250 μl dose. They show the destructive effects of peanut oil on the mucosa, which included degeneration of epithelium. From Fig. 1, it could be estimated that peanut oil lightly disrupts the nasal mucosa. Added SG did not strongly disrupt the mucosa; however SG in peanut oil doubled the bioavailability.\(^{80}\)

Histopathology was investigated using sodium glycocholate, which is one of the most effective enhancers for the nasal absorption of insulin\(^{21}\) (Fig. 2). Extensive damage caused by sodium glycocholate was seen, and in some regions, erosion of the mucosa. Hirai\(^{21}\) and Hermens\(^{80}\) reported that sodium glycocholate shows ciliary toxicity.
This result corresponds with the suggestion that the increased mucosal permeability of insulin caused by sodium glycocholate may be the result of mucosal disruption. Peanut oil and sodium glycocholate may enhance insulin absorption because they affect nasal mucosa.

The powder dosage form is less effective for insulin absorption than the peanut oil suspension dosage form,\(^8\) but it is nevertheless useful to examine histological changes caused by insulin and SG.

Figure 3 shows microscopic photos of nasal mucosa 0.5 and 1 h after the administration of 10 mg of the insulin powder dosage form. Figure 3a shows control mucosa treated with insulin alone. Figure 3b, c and d show the nasal mucosa 0.5 (b) and 1 h (c) after the administration of SG, and 1 h after the administration of insulin with SG (d), respectively. They show no destructive effect of SG on the mucosa. Insulin and SG do not appear to affect the mucosae since no histological change in nasal mucosae was observed after their administration in a powder dosage form.

These results, histopathological findings and irritation scores are summarized in Table 1.

The administration of insulin in a peanut oil suspension with 1% SG showed high nasal bioavailability, but that without SG did not. The main cause of enhancement by peanut oil and SG is presumed to be a histological change in nasal epithelium mucosae, and subsequent promotion of insulin permeation and diffusion through the nasal

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**Table 1. Histopathological Findings of Nasal Mucosas of Insulin Peanut Oil Suspension and Powder Dosage Forms**

<table>
<thead>
<tr>
<th>Histopathological findings</th>
<th>Prep. Animal No.</th>
<th>Control</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degeneration of epithelium</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Inflammatory cell infiltration</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>in lamina propria mucosa</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Erosion in mucosa</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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Abbreviations: - , no change; ±, very slight; +, slight; ++, moderate; I, peanut oil; II, insulin in peanut oil; III, insulin and SG in peanut oil; IV, sodium glycocholate; V, insulin powder; VI, SG powder (0.5 h); VII, SG powder (1 h); VIII, insulin and SG. Nasal mucosae were excised at 1 h except for group VI (0.5 h). Each finding is the result of duplicate experiments.
mucoderm by peanut oil, not by SG. The result of coadministration of peanut oil and SG appears to indicate no close relationship between increasing insulin absorption and the degree of epithelial damage (Table 1). Peanut oil showed weak chelation and SG has chelation as previously reported. These suggest that SG has a possible alternative mechanism which does not involve epithelium disruption to any great extent. Therefore, the powder dosage form with SG may prove to be a more useful system for the nasal absorption of insulin.

Duration of the effect of SG in the powder dosage form has already been investigated. The result showed that pre-administration of SG 60 min before insulin administration did not cause a significant reduction in glucose levels. It suggested that the recovery of nasal mucosa occurred in less than 60 min. This result coincided with the morphologic results that mucosa showed no damage 0.5 and 1 h after the administration of SG (Table 1). SG may be considered to be safe regarding the nasal mucosa, though its safety concerning the olfactory epithelium must still be examined.

CONCLUSIONS

The bioavailability of insulin was 6.0% in peanut oil without SG, and it increased to 11.6% by the addition of SG, but no correlation between enhancing efficiency and epithelium disruption was found in peanut oil and SG. These histological effects prove that SG is safe and has great practical significance.

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REFERENCES