Studies of Drug Delivery Systems for a Therapeutic Agent Used in Osteoporosis. II. Enhanced Absorption of Elcatonin from Nasal Mucosa in Rabbits

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In this study, the effects of a protease inhibitor, endocytosis inhibitors and an absorption-enhancing agent on the absorption of (Asu\(^{15}\))-eel calcitonin, ECT from the nasal mucous membrane in rabbits were examined, and the results were compared with those obtained following the rectal absorption of ECT reported in our previous paper. ECT was efficiently absorbed from the nasal mucous membrane and effectively decreased serum calcium (Ca) concentrations. The increase in the area under the percent decrease in serum Ca concentration (ΔCa%–AUC) value, assumed to be an index of the pharmacodynamics (hypocalcemic effect) of ECT, depended on the dose of ECT administered intranasally. When nafamostat mesilate, a protease inhibitor, was coadministered with ECT, the ΔCa%–AUC markedly increased. It is presumed that the influence (enzymatic barrier function) of protease on the nasal absorption of ECT is significant. However, no significant difference in the ΔCa%–AUC value was observed when an endocytosis inhibitor (cytochalasin B or monensin) was coadministered with ECT. ECT administration in combination with sodium decanoate, the sodium salt of a medium-chain fatty acid, effectively increased the ΔCa%–AUC value due to the enhancing effect of sodium decanoate on the nasal absorption of ECT. We conclude that the nasal application offers a promising approach for the administration of pharmaceutical preparations containing ECT with additives such as nafamostat mesilate and sodium decanoate.

Key words elcatonin; hypocalcemic activity; nasal absorption enhancement; protease inhibitor; endocytosis inhibitor; decanoic acid sodium salt

In a previous paper, we demonstrated the effectiveness of a new hollow-type suppository containing elcatonin (Asu\(^{15}\))-eel calcitonin, ECT, a synthetic derivative of eel calcitonin. The effects of the coadministration of other compounds such as protease inhibitors, endocytosis inhibitors and absorption-enhancing agents, on the rectal absorption of ECT were examined. Nafamostat mesilate (trypsin inhibitor), a protease inhibitor, did not influence the rectal absorption of ECT. However, cytochalasin B and monensin, which are endocytosis inhibitors, significantly decreased ECT absorption when coadministered with ECT. Furthermore, sodium decanoate, the sodium salt of a medium-chain fatty acid with absorption-enhancing ability, markedly increased ECT absorption from the rectum. These findings led us to investigate whether ECT absorption from another delivery route, the nasal cavity, might be useful for the coadministration of ECT with other compound. We examined the influence of these additives on the absorption of ECT from other mucous membranes such as the nasal mucous membrane.

Recently, the nasal route has been used as an alternative to parenteral injection because of its rich vascularization and ease of administration. We have reported on the effect of cycloedrinaps on the absorption of insulin from the nasal mucous membrane in rabbits, which is also the nasal delivery system of recombinant human granulocyte colony-stimulating factor (rhG-CSF). In regard to the nasal administration of ECT, Morimoto and his group and Manzoni and coworkers reported that a decrease of plasma calcium (Ca) concentration was observed after administration of ECT into the nasal cavity of rats and dogs. In the present investigation, the effects of a protease inhibitor, endocytosis inhibitors and an absorption-enhancing agent on the absorption of ECT from the nasal mucous membrane in rabbits were examined, and the results were compared with those obtained following the rectal absorption of ECT.

MATERIALS AND METHODS

Materials ECT (5200–5300 IU/mg net peptide) and nafamostat mesilate (Fusan\(^{3}\)) were obtained from Shiono Chemical, Tokyo and Torii Pharmaceutical Co., Japan, respectively. Cytochalasin B and monensin (sodium salt), both endocytosis inhibitors, were purchased from Sigma, St. Louis, MO, U.S.A. The sodium salt of \(n\)-decanoic acid (sodium decanoate) was obtained from Tokyo Kasei Kogyo, Tokyo, Japan. All other reagents used were of analytical grade.

Preparation for Nasal Administration Aqueous preparations (freshly prepared) for nasal administration were made by dissolving the appropriate amounts of ECT and additives in water for injection. The placebo formulation contained the same amount of water used for injection without any ECT or additives. The ECT and additives are listed in Table 1.

Experimental Animals and Determination of Serum Ca Concentration Male albino rabbits weighing 3.0 to 3.5 kg were used in this investigation. They had free access to water and food and were housed individually in cages under environmentally controlled conditions (23±1°C, 55% relative humidity, 12-h on/off light/dark cycle). The method for the nasal administration of drugs to the rabbits, as described in our previous reports, was applied. Briefly, rabbits were fasted overnight with freely available tap water, and they were held with their heads in a vertical position.

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hundred microliters of each prepared solution was administered into one nostril with a micropipette (Eppendorf). Immediately after the nasal administration of drugs, rabbits were placed in a supine position for 2 min, then secured in a crouching position during the experimental period. Two-ml blood samples were collected from the auricular vein using a syringe at predetermined time intervals. These samples were centrifuged at 3000 rpm for 15 min to separate the serum. Each serum sample was stored at −30°C until assays could be performed for Ca.

The serum Ca concentration was determined by the o-cresolphthalein complexone method using a Calcium-C Test Kit (Wako Pure Chemical Co., Tokyo, Japan) as described in the previous paper.

**Pharmacodynamic Analysis** Serum Ca levels after administration were calculated by Eq. (1):

\[
\text{% of initial Ca concentration} = \left( \frac{\text{Ca concentration at each time point during administration}}{\text{Ca concentration before administration}} \right) \times 100.
\]

The percent decrease in serum Ca concentration (∆Ca%) was calculated using Eq. (2):

\[
\Delta \text{Ca} \% = 100 - (\% \text{ of initial Ca concentration}).
\]

The areas under the individual ∆Ca%–time curves from 0 to 6 h following nasal administration (∆Ca%–AUC), as an index of the hypocalcemic effect of ECT, was calculated using the trapezoidal rule. The experimental data were fitted to the following equation (3) (the sigmoid E\text{max} model):

\[
E = E_0 + \left( E_{\text{max}} \times \text{Dose}^n \right) / (ED_50 + \text{Dose}^n).
\]

**RESULTS AND DISCUSSION**

**Relationship between Dosage of ECT and Pharmacodynamic Activity of ECT** The absorption and effectiveness of ECT after nasal administration was evaluated by the decrease in serum Ca concentration (pharmacological effect) as the pharmacodynamic parameter. To investigate the relationship between the dose of ECT and the pharmacodynamic response following the nasal administration of ECT, an ECT solution, prepared as described in Materials and Methods, was instilled into the nasal cavity of rabbits. The ECT dose–response (∆Ca%–AUC) curve obtained following nasal administration was compared with that observed following rectal administration.

Figure 1 illustrates the % of initial Ca concentration–time curves following nasal administration of the aqueous preparation with or without ECT. The serum Ca level in the ECT (100 IU)-administered group (shown by filled triangles) significantly decreased compared with that in the control group (shown by (X)). The relationship between the dose of ECT and the ∆Ca%–AUC is shown in Fig. 2. The ∆Ca%–AUC value increased with an increasing dose of ECT, which was consistent with the results obtained following rectal administration, reported previously. These results clearly suggest that ECT is also effectively absorbed from the nasal mucous membrane in rabbits. However, it was observed that the profile of the ECT dose–response (∆Ca%–AUC) curve following nasal administration shifts to a higher dose side compared with that obtained following the rectal administration of ECT. Consequently, the ED\text{50} value obtained following the
The rectal absorption of ECT was influenced by coadministration with endocytosis inhibitors. We therefore investigated the nasal administration of ECT (30 IU) solution in combination with cytochalasin B or monensin. Cytochalasin B prevents the apical formation of vesicles in epithelial cells by disassembling actin microfilaments, whereas monensin prevents the split of the ligand–receptor complex in the endosomes. Unfortunately, no significant difference was found between the ΔCa%–AUC values following the administration of ECT with each of the inhibitors or ECT administration without inhibitors (Table 3), indicating that the two endocytosis inhibitors do not influence the nasal absorption of ECT. These results indicate that the contribution of endocytosis to the mechanism of nasal absorption of ECT is negligible.

In a subsequent study, the effect of the sodium salt of a medium-chain fatty acid on the nasal absorption of ECT was examined. The ΔCa%–AUC values obtained are summarized in Table 3. When sodium deconate was coadministered, the ΔCa%–AUC significantly increased (78.0±5.5% h) compared with that (41.7±8.2% h) following the administration of ECT without additives. Concerning the effect of medium-chain fatty acids on the nasal absorption of polypeptide drugs, Mishima and coworkers reported the enhancement of the nasal absorption of insulin by using sodium salts of fatty acids. The increase in the nasal absorption of ECT by the use of sodium deconate indicates that medium-chain fatty acids can be added to ECT preparations for nasal administration.

### CONCLUSION

ECT was efficiently absorbed from the nasal mucous membrane and effectively decreased serum Ca concentrations. The increase in ΔCa%–AUC value, assumed to be an...
index of pharmacodynamic activity (hypocalcemic effect) of ECT, depended on the dose of ECT administered intranasally. When nafamostat mesilate, a protease inhibitor, was coadministered with ECT, the $\Delta Ca^{2+}$-AUC value markedly increased. It is presumed that the influence (enzymatic barrier function) of protease on the nasal absorption of ECT is significant. However, no significant difference in the $\Delta Ca^{2+}$-AUC value was observed when an endocytosis inhibitor (cytochalasin B or monensin) was coadministered with ECT. ECT administration in combination with sodium decanoate effectively increased the $\Delta Ca^{2+}$-AUC value due to the enhancing effect of sodium decanoate on the nasal absorption of ECT. We conclude that the nasal application offers a promising approach for the administration of pharmaceutical preparations containing ECT with additives such as nafamostat mesilate and sodium decanoate.

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REFERENCES AND NOTES


2) This study was presented, in part, at the 4th International Conference on Drug Absorption, Edinburgh, United Kingdom, June, 1997.


