Studies of Drug Delivery Systems for a Therapeutic Agent Used in Osteoporosis. I.1) Pharmacodynamics (Hypocalcemic Effect) of Eclatomin in Rabbits Following Rectal Administration of Hollow-Type Suppositories Containing Eclatomin2)

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In this study, we developed a new hollow-type suppository containing eclatomin ([Asu₁²⁷]-eel calcitonin, ECT), a synthetic derivative of eel calcitonin, which produces hypocalcemia, as a pharmaceutical preparation for self-administration, to be used instead of parenteral injections for patients with osteoporosis. The absorption of ECT from the rectal mucous membrane was evaluated by observation of the decrease in serum calcium (Ca) concentrations following rectal administration in rabbits. ECT was efficiently absorbed from the rectum and effectively decreased serum Ca concentrations. The data of the area under the percent decrease in serum Ca concentration (ΔCa%)–time curve (ΔCa%–AUC), assumed to be an index of the pharmacodynamics (pharmacological effect) of ECT, indicated that similar hypocalcemic effects were obtained following rectal and intravenous administrations of ECT. In regard to the effect of coadministration of other compounds on rectal absorption of ECT, no significant difference in the ΔCa%–AUC between rectal ECT administration with or without nafamostat mesilate (a protease inhibitor) was observed. However, the coadministration of ECT with cytochalasin B or monensin (endocytosis inhibitors) significantly decreased the ΔCa%–AUC, indicating that rectal ECT absorption is probably inhibited by endocytosis inhibitors. On the other hand, it was found that sodium decanoate, a medium-chain fatty acid (sodium salt), significantly enhanced the rectal absorption of ECT. We conclude that this ECT hollow-type suppository offers promise as a new method for the administration of ECT.

Key words eclatomin; hollow-type suppository; hypocalcemic activity; protease inhibitor; endocytosis inhibitor; decanoic acid sodium salt

Calcitonin, which is a potent regulator of serum calcium (Ca) concentration, reduces bone turnover and lowers the serum Ca concentration primarily by a direct inhibition of bone resorption.5,6) Calcitonin and its analogues are indicated in the treatment of moderate to severe Paget’s disease, osteoporosis and hypercalcemia.4,1) Unfortunately, calcitonins require frequent injection in the long-term therapy of these diseases, due to degradation by proteolytic enzymes and to the gastrointestinal tract mucosa impermeability of this drug. To improve the quality of life (QOL) of these patients, new methods for calcitonin administration via nonoral and parenteral routes are desirable for the systemic administration of calcitonin. In this study, we therefore attempted to develop a new rectal suppository as a pharmaceutical preparation for self-administration.

Eclatomin ([Asu₁²⁷]-eel calcitonin, ECT), a calcitonin analogue of a synthetic derivative of eel calcitonin, has been shown to induce hypocalcemia and to be more stable than eel calcitonin under physiological conditions.5,6) We chose a new ECT dosage form of a hollow-type suppository1) containing various doses of ECT in aqueous preparation, then evaluated the rectal absorption of ECT from the rectal mucous membrane by observation of the decrease in serum Ca concentrations, which is a pharmacodynamic response index of ECT, following the rectal administration in rabbits. Only a few experiments have been reported concerning the absorption of ECT from the rectum.5) However, to our knowledge, no studies on hollow-type suppositories for ECT delivery have been reported so far. In this study, the effects of the coadministration of various compounds, such as protease inhibitors, endocytosis inhibitors and absorption-enhancing agents, on the absorption of ECT was examined, and the usefulness of the ECT hollow-type suppository was evaluated.

MATERIALS AND METHODS

Materials ECT (5200–5300 IU/mg net peptide) was obtained from Shiono Chemical Co., Tokyo, Japan. Nafamostat mesilate (Fusan™), a protease inhibitor, was obtained from Torii Pharmaceutical Co., Tokyo, Japan. Cytochalasin B and monensin (sodium salt), both endocytosis inhibitors,10) were purchased from Sigma, St. Louis, MO, U.S.A. The sodium salt of n-decanoic acid (sodium decanoate), an absorption-enhancing agent, was obtained from Tokyo Kasei Kogyo, Tokyo, Japan. The suppository base, Witepsol H-15 (H-15, Hüls Troisdorf, Troisdorf, Germany) was kindly supplied by Mitsuba Trading Co., Tokyo, Japan. All other reagents used were of analytical grade.

Preparation of Suppositories Formulations comprising the two types of hollow suppositories (approximately 2 g), those containing ECT solution without additives, and those containing a solution of ECT and each additive in various doses were prepared using Witepsol H-15 by the fusion process method reported by Watanabe et al.11) For the control experiments, a hollow-type suppository containing water for injection without drugs (blank suppository) was prepared using Witepsol H-15. The ECT and additives are listed in Table 1. Appropriate concentrations of freshly prepared solutions containing ECT and each additive except for sodium decanoate in various doses were dissolved in water for injec-

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tion. In the use of sodium decanoate, the suppository was prepared using Witepsol H-15 mixed with sodium decanoate in the body of the suppository. Two-hundred microliter of each prepared solution was filled into each suppository. The opening at the hind part of the suppository was sealed by melting the base material. All suppositories were refrigerated overnight after preparation, then examined.

Animal Experiments Male albino rabbits weighing 3.0 to 3.5 kg were used. They received food with water ad libitum and were housed individually in cages in a forced-air facility that was maintained at 23 ± 1 °C and 55% relative humidity, under a 12-h light/dark cycle. Animals with free access to water were fasted for one night prior to each experiment. For intravenous bolus administration, the ECT solution was injected into the marginal vein of one ear. The suppositories were administered into the rectum according to the method described in our previous papers.2,11) Following the intravenous or rectal administration of ECT, 2 ml blood samples were taken from the auricular vein by a syringe at pre-determined 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 an assay could be performed for calcium. The serum calcium (Ca) concentration was determined by the o-cresolphthalein complexone method12) employing a Calcium-C Test Kit (Wako Pure Chemical Co., Tokyo, Japan).

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

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

(1)

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

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

(2)

The areas under the individual ΔCa%-time curves from 0 to 6 h following administration (ΔCa%-AUC), as an index of the hypocalcemic effect of ECT, were calculated using the trapezoidal rule.13) The ECT dose-response (ΔCa%-AUC) curves were simulated according to a sigmoid model14) by use of the WinNonlin program (Scientific Consulting, Inc., Apex, NC, U.S.A.) as follows:

\[
E = E_0 + (E_{\text{max}} - Dose) \cdot (\text{ED}_{50} + Dose)
\]  

(3)

where E is the pharmacological effect intensity (ΔCa%-AUC), E₀ is the pharmacological effect intensity when drug dose is zero, E_{\text{max}} is the maximum effect the drug can produce, \text{ED}_{50} is the dose that produces 50% of the maximum effect, and n (Hill factor) is the slope factor of the sigmoidal dose-response curve.

Statistical analysis of the results was conducted by one-way analysis of variance and Dunnett’s tests. A significant difference was estimated using \( p = 0.05 \) as the minimal level of significance.

RESULTS AND DISCUSSION

Serum Ca Concentrations Following the Intravenous and Rectal Administration of ECT Solution Generally, the efficiency of the absorption of drugs is evaluated using pharmacokinetic parameter obtained following the measurements of blood (serum or plasma) concentrations of the objective drug; pharmacodynamic parameters based on the pharmacological actions of the objective drug are also accepted as indices of drug absorption.15)–17) For the determination of calcitonin concentration in blood, some problems in sensitivity and specificity have been noted.18) Therefore, more clinically viable diagnostic parameters such as Ca and phosphorus (P) concentrations in blood and serum alkaline phosphatase activity are often used as indices of calcitonin effect. Research on the evaluation of absorption using the decrease in serum Ca concentration as an index of the pharmacodynamics of calcitonin and its analogues have been reported previously.19)–21) We therefore examined the rectal absorption of ECT by determining the decrease in serum Ca concentration as an index of the pharmacodynamic response. In preliminary experiments, the relationship between the dose of ECT and the change in pharmacodynamic activity of ECT was evaluated. The change in serum Ca level (% of initial Ca concentration) observed in rabbits following the intravenous administration of ECT (30 IU) is shown in Fig. 1 (A). When the dose of ECT used for intravenous administration was gradually increased, the ΔCa%-AUC value, which was used an index of the decrease of serum Ca concentration (hypocalcemic effect) induced by ECT, increased in a dose dependent manner (shown by unfilled squares in Fig. 2). Therefore, if the ΔCa%-AUC value is obtained, by which the ECT amount in the body can be indirectly evaluated, it is possible to evaluate the efficiency of ECT absorption after rectal administration.

Hollow-type suppositories containing ECT solutions were prepared as described in Materials and Methods and administered into the rectum of rabbits. It has been reported that faster drug release is obtained with hollow-type suppositories, compared to conventional suppositories.12,22) The % of the initial Ca concentration-time curve following the rectal administration of an ECT (30 IU) suppository is shown in Fig. 1 (B). The % of initial Ca concentration (indicated by filled circles) decreased significantly (\( p < 0.01 \)) after the administration of the ECT suppository compared to that (indicated as (X)) following the administration of water-filled suppositories (the control group). The ΔCa%-AUC increased when the dose of ECT was increased, both for rectal administration as well intravenous administration. These results suggest that ECT is efficiently absorbed from the rectal mucous membrane. Figure 2 illustrates the relationship between the dose of ECT and the ΔCa%-AUC obtained after administration of ECT suppositories containing various doses of ECT.

Table 1. Doses of ECT and Additives in Solution (200μl) Added in Hollow-Type Suppositories

<table>
<thead>
<tr>
<th>Additive</th>
<th>ECT dose (IU)</th>
</tr>
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<tbody>
<tr>
<td>Without</td>
<td>0.3—1000</td>
</tr>
<tr>
<td>Nafamostat mesilate (40 mmol)</td>
<td>30</td>
</tr>
<tr>
<td>Cytochalasin B (0.02 mmol)</td>
<td>100</td>
</tr>
<tr>
<td>Monensin (0.02 mmol)</td>
<td>100</td>
</tr>
<tr>
<td>Sodium decanoate (30 mg)</td>
<td>30</td>
</tr>
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Cytochalasin B and monensin were dissolved in ethanol and added to Krebs-Henseleit saline (2.5 μl ethanol/ml) to achieve a final concentration (0.02 mmol).
As shown in Fig. 2, we were able to obtain a good approximation of the ECT dose-response (\( \Delta \text{Ca}_\text{concentration} \)) value following both the rectal and intravenous administration of ECT. In a previous study, Morimoto and coworkers\(^9\) reported that the plasma Ca concentration in rats was not decreased following the rectal administration of the conventional ECT (5 IU/kg) suppository in which a triglyceride base was used. However, we found a significant decrease in serum Ca concentration following the rectal administration of hollow-type suppositories containing at least 10–30 IU (3–10 IU/kg) ECT without additives. It may be concluded that the rectal absorption of ECT leads to satisfactory pharmacological effects. The \( ED_{50} \) values (Table 2) obtained after rectal and intravenous administration of ECT suggested the usefulness of a pharmaceutical preparation of ECT for rectal use.

**Effect of Various Materials on Rectal Absorption of ECT** In general, two types of barriers to the absorption of a drug have been defined: the enzymatic barrier (degradation by enzymes such as protease) and the physical barrier (barrier on drug permeation in the mucus membrane).\(^{23,24}\) To elucidate the effect of these barriers on the rectal absorption of ECT, we examined the effects of the addition of a protease inhibitor, endocytosis inhibitor and absorption-enhancing agent to ECT in the rectal suppository.

ECT is composed of 32 amino acids (MW, approximately 3.3 kDa) which can be decomposed into three fragments by trypsin and/or chymotrypsin.\(^{25} \) However, carboxypeptidase and aminopeptidase are less effective for the degradation of ECT.\(^6\) Therefore, nafamostat mesilate, which strongly inhibits trypsin activity,\(^{26} \) was simultaneously administered with ECT. As shown in Table 3, no significant difference in the \( \Delta \text{Ca}_\text{concentration} \) values was observed following the coadministration of ECT with nafamostat mesilate (89.1±21.2) compared to that following ECT administration without a protease inhibitor (63.2±5.4). Therefore, it is presumed that the role of the enzymatic barrier (influence of trypsins) in rectal ECT absorption is negligible.

In regard to rectal ECT absorption, endocytosis probably contributes to the process of absorption. To elucidate the effect of the addition of an endocytosis inhibitor on the rectal absorption of ECT, two hollow-type suppositories containing ECT (100 IU) and an endocytosis inhibitor (cytochalasin B or monensin)\(^{10} \) were administered into the rectum of rabbits. Cytochalasin B prevents the apical formation of vesicles in epithelial cells (disassembly actin microfilaments, and monensin prevents the split of the ligand-receptor complex in the endosome).\(^{10} \) When ECT and the endocytosis inhibitor (cytochalasin B or monensin) were simultaneously adminis-
tered, no decrease in serum Ca concentration was observed. Consequently, almost the same serum Ca concentrations were observed among the administration of suppressors containing ECT with endocytosis inhibitors, or those containing only water for injection (control group). The ΔCa%–AUC value significantly decreased following the coadministration of ECT and an endocytosis inhibitor (Table 3). The decrease in serum Ca concentration effected by ECT was clearly eliminated. These results indicate that endocytosis inhibitors significantly decrease ECT absorption from rectal mucosa. Therefore, it may be concluded that endocytosis plays an essential role in the rectal absorption mechanism of ECT.

We have reported several data concerning the rectal absorption of bioactive polypeptide drugs. Previously, it was found that the rectal absorption of insulin (MW, approximately 6 kDa) and recombinant human granulocyte colony-stimulating factor (rhG-CSF: MW, approximately 18 kDa) were promoted by the coadministration of these polypeptides and pharmaceutical additives such as cyclodextrins and medium-chain monoglycerides. In this paper, the effect of the addition of sodium deconate, the sodium salt of a medium-chain fatty acid, on rectal ECT absorption was examined. Sodium deconate has been successfully used in commercially available ampicillin suppositories. Hollow-type suppositories containing 30 mg of sodium deconate in the body (Witepsol B2, 2 g) were used, and the suppositories were filled with an ECT (30 IU) solution. The ΔCa%–AUC value obtained after the rectal administration of this suppository is summarized in Table 3. When sodium deconate was used in the suppository, the ΔCa%–AUC significantly (p<0.05) increased to 101.7±5.1%·h compared with that (63.2±5.4%·h) following the administration of ECT alone. These results indicate that the rectal absorption of ECT can be increased by the administration of ECT in combination with sodium deconate. We previously reported the enhancement of the rectal absorption of poorly-absorbed gentamicin, an aminoglycoside antibiotic, following its coadministration with medium-chain fatty acids. These results suggest the possibility of a reduction in the dose of ECT used when it is coadministered with a medium-chain fatty acid such as sodium deconate.

CONCLUSION

ECT was efficiently absorbed from the rectum and it effectively decreased the serum Ca concentration. From the data of the ΔCa%–AUC values, which is assumed to be an index of the pharmacodynamics (pharmacological effect) of this drug, a similar hypocalcemic effect was obtained following rectal and intravenous administration. When ECT was coadministered with nafamostat mesilate (a protease inhibitor), no difference in the ΔCa%–AUC was obtained between rectal ECT administration with or without nafamostat mesilate. However, the coadministration of ECT and cytochalasin B or monensin (endocytosis inhibitors) significantly decreased the ΔCa%–AUC value, indicating that rectal ECT absorption is probably inhibited by endocytosis inhibitors. On the other hand, sodium deconate, the sodium salt of a medium-chain fatty acid, significantly enhanced the rectal absorption of ECT. We conclude that the hollow-type suppository offers promise as a new method for the administration of ECT.

REFERENCES AND NOTES

1. This study was presented, in part, at the 23rd International Symposium on the Controlled Release of Bioactive Materials held in Kyoto, Japan, July 1996.