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Improved nasal bioavailability of elcatonin by insoluble powder formulation

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Abstract

The bioavailability of elcatonin (ECT) via the nasal route was investigated with a powder dosage form utilizing water-insoluble calcium carbonate ($CaCO_3$) in comparison with the liquid dosage form. Total radioactivity and the radioactivity of intact [^{3}H]ECT were measured to evaluate the nasal absorption in vivo and the nasal mucosal transport in vitro. The systemic bioavailability of both total radioactivity and intact [^{3}H]ECT following intranasal administration of the powder formulation in rats was significantly greater than in the case of the liquid formulation. In contrast, similar permeability of ECT across excised rabbit nasal mucosa was seen for both formulations, and was close to that of [^{14}C]inulin, suggesting that the ECT transport is predominantly paracellular in each case. However, the powder formulation significantly prolonged the residence time of [^{3}H]ECT in the rat nasal cavity, compared with the liquid formulation. We conclude that the powder formulation utilizing $CaCO_3$ improves the nasal bioavailability by increasing the residence time of ECT in the nasal cavity and is likely to be effective in increasing systemic drug delivery.

Keywords: Nasal deliverly; Powder; Formulation; Elcatonin; Bioavailability; Calcium carbonate

1. Introduction

Nasal systemic drug delivery is a potentially valuable alternative to parenteral administration of polar molecules that are poorly absorbed from the digestive tract, such as polypeptides and water-soluble drugs, since the nasal route is non-invasive and makes self-medication practical, thus improving patient compliance (Pontiroli et al., 1989). However, low bioavailability following intranasal administration of polypeptides such as insulin and calcitonin as simple liquid formulations has been observed (Moses et al., 1983; Lee et al., 1994), due to their restricted permeability in the nasal mucosal membrane and to rapid efflux from the nasal cavity by the efficient physiological elimination mechanism, nasal mucociliary clearance, limiting the duration available for absorption. Consequently, several potential additives, both absorption promoters and mucoadhesive agents, have been evaluated in order to overcome these problems with nasal formulations (Dondeti

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et al., 1996; Behl et al., 1998). On the other hand, many reports have appeared, describing powder dosage forms, eg, microspheres or colyophilized powder using bile salts, cyclodextrins, starch, cellulose and their derivatives, and so on, for nasal delivery (Nagai et al., 1984; Illum et al., 1988; Lee et al., 1991; Edman et al., 1992; Schipper et al., 1993; Suzuki and Makino, 1999). Most of these excipients, however, can be characterized as permeation enhancers and/or mucoadhesives. In contrast, although the dry powder formulation using water-soluble lactose as a drug carrier is available for the pulmonary dry powder inhalation (Malcolmson and Embleton, 1998), this apparently does not increase the absorption of polar molecules via the nasal route (Nagai et al., 1984; Ugwoke et al., 2000). We wondered whether a powder formulation utilizing a water-insoluble compound might be effective as a drug carrier to improve nasal bioavailability.

In the present study, we chose elcatonin (ECT) and calcium carbonate ($CaCO_3$) as models of a polar polypeptide and a water-insoluble carrier, respectively, and examined whether $CaCO_3$ could increase the systemic bioavailability of ECT via the nasal route. $CaCO_3$ is extremely insoluble and has been used as an ingredient of foods, cosmetics and pharmaceuticals. ECT [(Asu^{1,7})-eel calcitonin], a synthetic derivative that is more stable biologically than native eel calcitonin and induces strong hypocalcemia, has been administered by intravenous or intramuscular injection for the treatment of Paget's disease of bone, hypercalcemia and osteoporosis found particularly among postmenopausal women. The present experiments were designed to see whether intranasal administration of ECT could be an efficient route for systemic delivery.

2. Materials and methods

2.1. Materials

[^{3}H]Elcatonin, dissolved in 50% ethanol, and unlabelled elcatonin were obtained from Sib Tech (Tenafly, NJ) and Bio Chiba (Tokyo, Japan), respectively. [^{14}C]Inulin (specific activity, 4.92 mCi/mg) was purchased from New England Nuclear (Boston, MA). Precipitated calcium carbonate ($CaCO_3$) was of JP-grade and from Sumida Shokai (Tokyo, Japan). ECT/$CaCO_3$ dry powder capsules (NDE-1003, 4 IU/(mg powder)) had been manufactured by Fuji Yakuhin (Omiya, Japan) to evaluate the hypocalcemic effect in rabbits. All other chemicals were commercial products of reagent grade and were used without further purification.

2.2. Animals

Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan) weighing 250–350 g, and male Japanese albino rabbits (Aizu Jikken Dobutsu, Fukushima, Japan), 13–16 weeks of age, were used in this study. They were housed in stainless-steel cages under environmentally controlled conditions (21–23 °C, 47–68% relative humidity, 12-h light/dark cycle) with free access to food and water prior to use in experiments.

2.3. Formulations

[^{3}H]ECT was purified in the following manner to prepare intravenous and nasal formulations. [^{3}H]ECT in 50% ethanol solution was evaporated and the resulting residue was dissolved in the mobile phase for high-performance liquid chromatography (HPLC). The reconstituted [^{3}H]ECT was applied to an HPLC column to separate the intact [^{3}H]ECT from degradation products, purified by fractionation, and lyophilized. Finally, purified [^{3}H]ECT having a specific radioactivity of 4.51 mCi/mg was obtained by dissolving lyophilized [^{3}H]ECT in 50% ethanol. The HPLC system used for separation was as follows: analytical column, Nucleosil 100-5C_8, 4.6×150 mm (Macherey-Nagel GmbH, Dueren, Germany); mobile phase consisting of water, acetonitrile and trifluoroacetic acid (650/350/1.5); flow rate, 1.0 ml/min (LC-6A pump, Shimadzu, Kyoto, Japan); and monitoring at 210 nm (SPD-6A, Shimadzu, Kyoto, Japan).

For studies of rat absorption in vivo, the liquid formulation, [^{3}H]ECT solution, was prepared by diluting purified [^{3}H]ECT to the concentration of

0.168 mg/ml with normal saline, and the powder formulation was made as follows. An appropriate amount of purified [^{3}H]ECT was added to $CaCO_3$ powder to obtain 1.68 μg of [^{3}H]ECT per 2.5 mg of powder as a final content, and the mixture was dried in vacuo for 2 h followed by milling of the resulting dry powder. For studies of transport across excised rabbit nasal mucosa in vitro, liquid and powder formulations were prepared similarly, but containing 8.09 μg of unlabelled ECT, 0.108 μg of [^{3}H]ECT and 9.76 ng of [^{14}C]inulin per 12 μl of liquid and 12 mg of powder. All formulations were freshly prepared, and undegraded ECT content and radioactivity in formulations were checked by means of HPLC and a liquid scintillation counter (LSC-1000, Aloka, Tokyo, Japan), respectively, prior to experiments.

2.4. Nasal absorption of ECT in vivo

All the animal experiments were performed according to the Guidelines for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University.

Non-fasted rats were anesthetized with 50 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL) injected intraperitoneally. They were placed on their backs, and a polyethylene catheter (SP-31, Natsume, Tokyo, Japan) was inserted into the femoral artery for serial blood sampling. Ten microliters of the liquid formulation, corresponding to a dose of 1.68 μg of [^{3}H]ECT, was instilled into the nasal cavity via the left nostril using a 50-μl microsyringe (Hamilton, Reno, NV) with polyethylene tubing (SP-45, Natsume, Tokyo, Japan) attached to the needle. Two and a half milligrams of powder dosage form in a 100-μl micropipette tip (Eppendorf, Hamburg, Germany), an equal dose to the liquid form, was administered into the nose via the left nostril with compressed air using a disposable syringe (Terumo, Tokyo, Japan) joined to the micropipette tip. To evaluate the systemic bioavailability, an intravenous bolus dose was given into the femoral vein through an indwelling cannula at a dose of 0.68 μg of [^{3}H]ECT. Rats were kept under anesthesia throughout experiments. The radioactivity of [^{3}H]ECT remaining in the micropipette tip post dose of the powder formulation was measured to calculate the exact dose administered. Arterial blood samples were withdrawn at the following time points: 5, 10, 20, 30, 60, 120 and 180 min. Plasma samples were obtained by centrifugation at 12 000 rpm and then placed on ice until analysis.

Conscious rabbits were fasted for 18 h prior to dosing while having free access to water, and were intranasally given the liquid (1000 IU/ml) or powder formulation of unlabelled ECT at a dose of 100 IU by means of a metered-dose spray (Pfeiffer, Radolfzell, Germany) or an insufflator (Jetlizer, Unisia Jecs, Gumma, Japan) modified for use with rabbits, respectively. Blood samples were taken from marginal ear vein by venipuncture using a heparinized syringe at 30, 60, 120, 180, 240, 300 and 360 min post dose, placed in micro test tubes (Eppendorf, Hamburg, Germany), and centrifuged at 3000 rpm to obtain plasma. Plasma was frozen at −80 °C until determination of total plasma calcium concentration by the *o*-cresolphthalein complexone method (Morin, 1974) using a Calcium-C Test Kit (Wako Pure Chemical Ind., Osaka, Japan).

2.5. Nasal transport of ECT in vitro

Rabbits were sacrificed by intravenous administration of a lethal dose of pentobarbital, and the nasal septum was isolated by cutting a bone block and placed in ice-cold buffer solution. Subsequently, two pieces of nasal mucosa were stripped carefully from the septum, and one of them was mounted on a horizontal diffusion chamber (Costar, Bedford, MA) with the mucosal and serosal sides facing the donor and receiver phases, respectively. Both sides of the nasal mucosa were filled with the transport medium, Ringer's solution containing 11 mM glucose, and bubbled with 95% O_2/5% CO_2 for 60 min to maintain tissue viability. The bathing medium on the mucosal surface was then removed completely by suction, and the chamber was kept under controlled conditions, at 37 °C and over 95% relative humidity, for 30 min before initiating transport.

The liquid (12 μl) or powder (12 mg) formulation tested was loaded onto the donor side to

initiate transport at 37 °C. At the scheduled time, a 50-μl aliquot of solution was taken from the receiver side and immediately replaced with an equal volume of fresh transport medium. The obtained samples were placed on ice until assay.

2.6. Intranasal deposition assessment

To evaluate residual [^{3}H]ECT in the nasal cavity, the radioactivity on the nasal mucosa was measured following administration of the liquid or powder formulation. Rats were killed by excess ether-inhalation at 60 min post dosing. The nasal mucosa was immediately picked off the septum and the concha, weighed and solubilized with SOLUENE-350 (Packard Instrument, Meriden, CT) for quantitation of the residual radioactivity in the nose.

2.7. Assay

Total and intact [^{3}H]ECT absorbed in vivo or permeated in vitro were analyzed separately and quantitatively. Rat plasma was deproteinized with an equal volume of ethanol followed by centrifugation prior to analysis. Aliquots of the resulting supernatants or the transport-medium samples were dissolved in liquid-scintillation cocktail (Clear-sol I, Nacalai Tesque, Kyoto, Japan) to measure total radioactivity. HPLC-fractionated eluates, as described above, were similarly used to determine intact [^{3}H]ECT concentration.

2.8. Data analysis and statistics

The area under the plasma concentration–time curve (AUC) was calculated by the trapezoidal method using the WinNonlin software package (Pharsight, Mountain View, CA). The maximum concentration (C_{max}) and the time at which it occurred (t_{max}) were determined from the individual plasma concentration–time data. The systemic bioavailability (BA) for the nasal formulation in rats was corrected for the actual dose (D) and the body weight (BW) based on the following equation.

$$BA\ (\%) = (\mathrm{AUC}_{in}/D_{in} \times BW_{in})/(\mathrm{AUC}_{iv}/D_{iv} \times BW_{iv}) \times 100$$

where the affixes, in and iv, denote intranasal and intravenous administration, respectively. Statistical analysis was carried out by using a Student's t-test with a significance level of $P < 0.05$.

3. Results

3.1. Nasal bioavailability of ECT in rats

Bioavailability of ECT following intranasal administration of the powder formulation was compared with that of the liquid formulation in rats. With the powder formulation, about 5–10% of the nominal dose remained in the device, so that the actual dose of [^{3}H]ECT was 1.54 μg on average. Plasma concentration–time profiles of total and intact [^{3}H]ECT after intravenous and intranasal administration are illustrated in Fig. 1. A comparison of pharmacokinetic parameters for the dosage forms is presented in Table 1. Intranasal administration of the powder formulation resulted in increased plasma levels of both total and intact [^{3}H]ECT as compared to those for the liquid formulation. Further, absorption was more rapid via the nasal mucosa with the powder form than with the liquid form, resulting in markedly increased C_{max}. The systemic bioavailability of total [^{3}H]ECT for the powder formulation was 73.6%, which was about two times higher than for the liquid formulation. Similarly, the powder formulation provided higher bioavailability, 38.1%, for the intact [^{3}H]ECT compared with the liquid formulation. The AUC ratio of intact to total [^{3}H]ECT for the intravenous dose was high, 0.82, compared with those for both nasal formulations, indicating the degradation and/or metabolism of [^{3}H]ECT in the nasal cavity.

3.2. Hypocalcemic effect of ECT in rabbits

To examine whether the powder formulations afford improved nasal absorption of ECT in different animal species, the hypocalcemic effects of

the liquid and powder formulations after administration were compared using a rabbit model. Fig. 2 shows the change in plasma total calcium concentration, which reflects the pharmacodynamics of ECT, as a function of time after administration of both formulations. With the liquid form, plasma calcium concentration decreased rapidly, reaching the minimal level at 60 min post dose, and thereafter reverted gradually to the initial level. When ECT was administered in the powder form, marked hypocalcemia was observed throughout the experimental period from 0 to 360 min. Furthermore, an extended profile, in which the maximal effect occurred at 120–180 min post dose, was seen with the powder form. This result indicates greater bioavailability of ECT with the powder formulation than with the liquid formulation in both rats and rabbits.

3.3. Permeation of ECT across excised rabbit nasal mucosa

To clarify whether or not $CaCO_3$ influences the transport process of ECT in the nasal mucosa, the permeability of ECT in the powder formulation in vitro was compared with that for the liquid formulation using excised rabbit nasal septal mucosa. Fig. 3 shows the time course of total ECT permeated across the nasal septal mucosa. The permeation of total ECT, which was corrected for the surface area and the amount loaded on the mucosal side, was linear over 120 min with an initial lag time. To estimate the permeation rate, the slope of the linear portion after the lag time on the plots of the permeation against time was calculated by linear least-square regression. There was no significant difference in the permeation rate between the two formulations: 0.218 and 0.235% of dose/cm^2/min for the liquid form and the powder form, respectively. In addition, the permeation rate of [^{14}C]inulin, 0.240% of dose/cm^2/min, which is a measure of paracellular permeability in the biological membrane, was comparable to that of ECT. Therefore, it appears that the nasal transport of ECT can be ascribed to paracellular permeation, and $CaCO_3$ used as a drug-carrier for the powder formulation did not facilitate the nasal mucosal permeation of ECT.

3.4. The deposition of ECT in the nasal cavity

The deposition of ECT in the nasal cavity following administration was compared between the liquid and powder formulations. Table 2 shows the residual radioactivity of [^{3}H]ECT in the nasal tissue 60 min after administration of the two formulations. The residual rate, given as the mean of fractional amount remaining per unit tissue weight relative to the actual dose, was calculated according to the following equation.

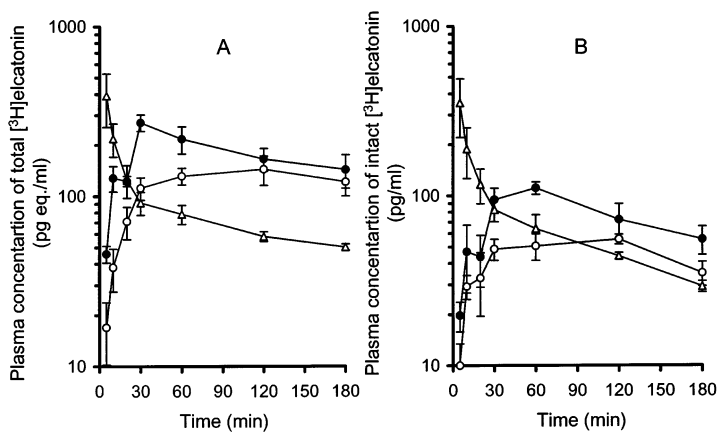

Fig. 1. Mean plasma concentration–time curves of total (A) and intact (B) [^{3}H]elcatonin after intravenous administration (△), and intranasal administration of the liquid (○) or powder (●) formulation, at actual doses of 0.68, 1.68 and 1.54 μg of [^{3}H]elcatonin, respectively, in rats. Each point and vertical bar represent the mean ± SEM of results from three rats.

Table 1
Bioavailability of [^{3}H]elcatonin following intranasal administration in rats

Route	Dosage form	Dose (μg)	C_{max} (pg eq./ml)	t_{max} (min)	AUC_{0-180} (ng eq. min/ml)	BA (%)
Total elcatonin						
Intravenous		0.68	–	–	16.7 ± 2.3	100
Intranasal	Liquid	1.68	160 ± 13	100 ± 20	21.5 ± 2.3	44.6 ± 4.4
Intranasal	Powder	1.54 ± 0.03	272 ± 30*	30 ± 0*	31.9 ± 1.6	73.6 ± 8.9*
Intact elcatonin						
Intravenous		0.68	–	–	13.7 ± 2.6 (0.82)[a]	100
Intranasal	Liquid	1.68	59 ± 6	100 ± 20	8.2 ± 2.3 (0.38)[a]	20.7 ± 2.3
Intranasal	Powder	1.54 ± 0.03	122 ± 4*	50 ± 10	13.8 ± 2.6 (0.43)[a]	38.1 ± 3.3*

[a] Values in parentheses are the ratio of AUC_{0-180} for intact [^{3}H]elcatonin to AUC_{0-180} for total [^{3}H]elcatonin. Data are presented as the mean ± SEM of three rats. *$P<0.05$, compared with each value for the liquid formulation.

Residual rate [% of dose/(g tissue)]

$$= A/W/D \times 100$$

where A, W, and D are the total [^{3}H]ECT amount contained in the isolated nasal tissue, the weight of the tissue, and the actual dose of [^{3}H]ECT, respectively, in each rat. The actual doses were from 0.96 to 1.44 μg of [^{3}H]ECT in the administration of powder formulation.

With the powder formulation, high radioactivity was retained in the nasal tissue, and the residual rate was significantly larger compared with the liquid formulation ($P < 0.05$). This result indicates that $CaCO_3$ prolongs the residence time of [^{3}H]ECT within the nasal cavity.

4. Discussion

The nasal route has received a great deal of attention as an attractive alternative to oral administration of drugs subject to first-pass metabolism and/or to invasive parenteral administration of peptide and protein drugs. Drug administration by the nasal route is convenient and makes self-medication at home possible, resulting in improved quality of life for patients and lower cost. Accordingly, a number of studies on nasal spray, gel and powder formulations have been conducted. Generally, the nasal powder formulations enhance systemic bioavailability, and are superior to liquid formulations in the following respects: increased chemical stability of the drug, no requirement for preservatives in formulations, and feasibility of administering larger amounts of drug (Lee et al., 1991; Schipper et al., 1993). Most studies on powder formulations, however, have concerned the use of a permeation enhancer or a mucoadhesive to achieve increased bioavailability. Although several reports have appeared on nasal powder formulations utilizing water-insoluble excipients such as $CaCO_3$ and microcrystalline cellulose (Nagai et al., 1984; Azria et al., 1988; Yanagawa, 1996; Kohno et al., 1997, 1998), our study is the first in which nasal bioavailability of

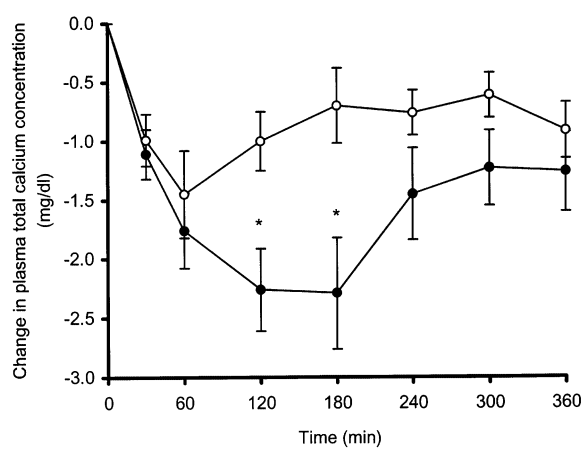

Fig. 2. Plasma total calcium concentration after intranasal administration of the liquid (○) or powder (●) formulation at a dose of 100 IU of elcatonin in rabbits. Data are expressed as the decrements in calcium concentration from the initial level as a function of time, and represent the means ± SEM of results from six rabbits. *$P < 0.05$, compared with the liquid formulation at each time.

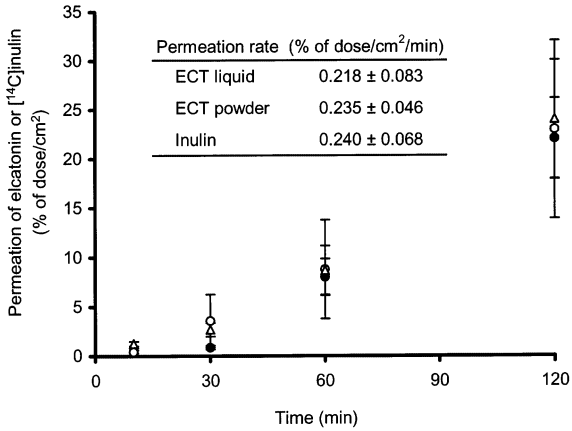

Fig. 3. Time-courses of permeation of elcatonin (○,●) and [^{14}C]inulin (△) across excised rabbit nasal mucosa following application of the liquid (open symbol) and powder (closed symbol) formulations. Each formulation contained both tritiated and unlabelled elcatonin. Data are expressed as the fractional amount permeated per unit surface area relative to the amount loaded on the mucosal side. The table inset in the figure shows each permeation rate estimated from the slope of the linear portion of the plot after an initial lag time, calculated by linear regression analysis. Each point and value represent the mean ± SEM of results from four to six determinations.

an insoluble powder formulation has been directly compared with that of a liquid formulation in terms of 'insoluble'.

We found that a powder formulation utilizing $CaCO_3$ as a carrier significantly increased the nasal systemic bioavailability of intact ECT as compared to a liquid formulation, from 21 to 38%, in rats. This is in accordance with the finding that a nasal formulation containing 0.1% lauroylcarnitine chloride, a potent absorption promoter, provided pharmacological bioavailability of 40% for nasal salmon calcitonin in rats (Kagatani et al., 1996). The permeation enhancer sodium decanoate (770 mM) and the protease inhibitor nafamostat mesilate (40 mM) each enhanced the hypocalcemic effect of ECT following nasal administration (Watanabe et al., 1998). However, $CaCO_3$ is unlikely to have a permeation-enhancing effect due to its remarkable insolubility; the solubility product is 8.7×10^{-9} M^2 at 25 °C. On the other hand, it is well-known that many enzymes, including cytochrome P-450 enzymes, conjugative enzymes, non-oxidative enzymes and proteolytic enzymes, exist in nasal secretions (Sarkar, 1992), while the nasal route permits us to avoid hepatic and intestinal first-pass metabolism. Lang et al. (1996) demonstrated that human calcitonin was metabolized by both chymotryptic- and tryptic-like endopeptidases and salmon calcitonin was degraded by the latter in excised bovine nasal mucosa. Moreover, Morimoto et al. (1995) reported an enhanced hypocalcemic effect in rats upon nasal administration of salmon calcitonin with a trypsin inhibitor such as camostat mesilate or aprotinin, and Morita et al. (1994) found improved pulmonary absorption of ECT in the presence of protease inhibitor such as bacitracin or nafamostat mesilate. Therefore, the degradation of calcitonin by proteolytic enzymes in the nasal mucosa is likely to play a role in the barrier function regarding ECT absorption from the nasal cavity. In our study, intranasal administration of both powder and liquid formulations resulted in a lower AUC ratio of intact ECT to total radioactivity as compared to that for an intravenous bolus dose (Table 1), again indicating the cleavage of ECT in the nasal mucosa. With the powder formulation, we anticipated increased absorption of intact ECT owing to saturation of

Table 2
[^{3}H]Elcatonin remaining in the nasal cavity following intranasal administration in rats

Formulation	Dose (μg)	Remaining amount of [^{3}H]elcatonin [μg eq./(g tissue)]	Residual rate [% of dose/(g tissue)]
Liquid	1.68	0.537 ± 0.081	33.8 ± 2.9
Powder	1.16 ± 0.14	0.615 ± 0.024	71.8 ± 12.8*

Remaining amounts of [^{3}H]elcatonin in the nasal cavity were measured 60 min post dose of both formulations. Residual rates are given as the fractional amount remaining per unit tissue weight relative to the actual dose. Data are presented as the mean ± SEM of three rats. *$P<0.05$, compared with the residual rate for the liquid formulation.

the enzymatic degradation processes, since this formulation increases the ECT concentration in the secreted mucus because it is in direct contact with the nasal mucosal surface. Unfortunately, however, the AUC ratio for the powder formulation was only slightly higher than for the liquid formulation (no significant difference).

Although the hypocalcemic effect of ECT in rabbit was significantly enhanced by the powder formulation (Fig. 2), in rabbit nasal mucosal transport of ECT in vitro, we demonstrated that $CaCO_3$ did not facilitate the permeation of ECT, and in addition the permeability of ECT in both liquid and powder formulations was similar to that of inulin (Fig. 3), providing evidence for paracellular transport of the drug (Tsuji et al., 1983). Recently, Cremaschi et al. (1996) claimed that cytochalasin B, which, by disassembling actin microfilaments, prevents the apical formation of vesicles in epithelial cells, and monensin, which prevents splitting of the ligand-receptor complex in endosomes, abolished the active transport, ie, endocytosis, of ECT in rabbit nasal upper concha in vitro. In vivo, however, co-administration with cytochalasin B or monensin had no effect on the hypocalcemic action of ECT dosed intranasally (Watanabe et al., 1998). These results suggest that endocytosis plays a minor role in nasal mucosal permeation of ECT, and that paracellular transport is the major mechanism. On the other hand, the values of permeated radioactivity due to undegraded [^{3}H]ECT up to 60 min were $18.2 \pm 3.7\%$ of total radioactivity (mean $\pm$ SEM) for the powder formulation and $8.6 \pm 0.5\%$ for the liquid formulation (data not shown). This significant difference in vitro implies that $CaCO_3$ may inhibit enzyme activity or that the saturation of the enzymatic degradation process may occur due to the higher drug concentration on the mucosal surface with the powder formulation. However, the reason of discrepancy between in vivo and in vitro findings regarding nasal metabolism of ECT in the present study is uncertain. Possible reasons include species difference or specific localization of enzyme activity within the nasal cavity (Gizurarson, 1990; Sarkar, 1992), or the presence of sufficient proteolytic activity to degrade ECT even in the powder formulation in vivo, but partial washout of enzymes from the mucosal preparation in vitro, resulting in a difference of proteolytic ability. Further studies are needed to clarify this phenomenon.

The overall process of nasal absorption can apparently be regarded as a balance between entry into the systemic circulation and removal from the absorption site by mucociliary clearance in the nose (McMartin et al., 1987; Harris et al., 1988). We observed that the powder formulation using water-insoluble $CaCO_3$ significantly prolonged the residence time of [^{3}H]ECT in the nasal cavity as compared to the liquid formulation (Table 2). Nagai et al. (1984) found that a nasal powder dosage form of insulin with water-insoluble microcrystalline cellulose, as well as mucoadhesives such as hydroxypropyl cellulose or carboxyvinyl polymer, provided more effective hypoglycemia than a water-soluble lactose formulation. They suggested that the insoluble or adhesive powder form did not readily flow out of the nasal cavity and thereby kept the drug concentration on the mucosal surface high, whereas the soluble powder form could be washed out by mucus in a relatively short time, as could the liquid form. Our results with $CaCO_3$ are consistent with their ideas. Accordingly, it is probable that insoluble powder improves the overall nasal absorption of ECT mainly by prolonging the contact time of the drug on the mucosa and possibly also partly by suppressing proteolytic activity in the nasal cavity.

Existence of higher drug concentration on mucosal surface, achieved by powder form administration, should be a situation favorable for its rapid diffusion through the mucosa, possibly resulting in fast onset of pharmacological action. In fact, earlier t_{max} and higher C_{max} compared with the liquid formulation were observed (Table 1). However, the observation of earlier t_{max} can be in disagreement with that of prolonged duration within the nasal cavity (Table 2). Possible reason of the conflicting findings in rats, which is uncertain, seems as follows. In the present study, since rats had held in 'supine' position throughout experiments, a part of the liquid formulation administered could be retained in the olfactory region where the epithelium is less-ciliated and -vascularized than in the respiratory region (Chien et al.,

1989), whereas another part could be transported toward the nasopharynx. Therefore, the slow absorption from the olfactory region should give the extended plasma profile in the liquid formulation. On the contrary, the profile of hypocalcemic action in the ECT powder formulation was extended in conscious rabbits who had been allowed to be in 'normal' position (Fig. 2). This finding should be in accordance with our idea that insoluble powder increases nasal bioavailability by prolonging duration of drug, while the question of body or head position in animal experiments should need to be further investigated.

The absence of a requirement for preservatives in the powder form, which may cause morphological changes in adenoid tissue in chronic use (Marttin et al., 1998), appears to be a major advantage over the liquid form. Moreover, although an insoluble powder formulation containing additives such as permeation enhancers or protease inhibitors is likely to give an additional increase of nasal absorption, these additives may cause unexpected and undesirable side effects. The particle size of $CaCO_3$ used in this investigation was 75 μm, so this powder is unlikely to reach the lung. However, it is known that intranasal deposition of particles depends on aerodynamic particle size and varying particle deposition affects the nasal absorption characteristics (Chien et al., 1989). Therefore, further studies with respect to particle size and kind of powder will be required, in particular to elucidate the relative contributions of 'absorption' and 'elimination' from the nasal cavity and to optimize nasal systemic drug delivery with powder formulations.

In conclusion, we have demonstrated that an insoluble $CaCO_3$ powder dosage form improved the nasal bioavailability of ECT in rats and rabbits. Furthermore, the permeation of ECT in the nasal mucosa could be ascribed to paracellular transport and the increase of the overall absorption was predominantly due to retardation of the outflow of ECT from the nasal cavity by the $CaCO_3$ powder. These results suggest that insoluble powder formulations are likely to be effective for nasal systemic drug delivery.

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