Enhanced Rectal Absorption of [Asu^{1,7}]-eel Calcitonin in Rats Using Polyacrylic Acid Aqueous Gel Base

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Abstract □ The effects of polyacrylic acid aqueous gel on the absorption of rectally administered [Asu^{1,7}]-eel calcitonin, a calcitonin analogue, were investigated in rats. The [Asu^{1,7}]-eel calcitonin (1 U/kg) was given into the rectal loop in gel bases at various pH (5.5–8.5) and polyacrylic acid concentrations (0.01–1.0% w/v). The maximum hypocalcemic effect was obtained in ~30 min after administration of the analogue in a 0.1% w/v polyacrylic acid gel base at pH 5.5. The plasma calcium level decreased by ~18% from the initial level. Rectal administration in vehicles such as polyethylene glycol 1000, triglyceride fatty acid mixture base, or saline solution had little or no hypocalcemic effect at a dose of 5 U/kg. The results indicated that a polyacrylic acid aqueous gel base significantly improved the absorption of this analogue. Furthermore, rectal administration in a polyacrylic acid gel base (0.1% w/v; pH 5.5) required a dose 35 times greater than an intravenously administered dose to achieve an equivalent hypocalcemic effect.

Keyphrases □ Calcitonin—enhanced rectal absorption using polyacrylic acid aqueous gel base, rats, bioavailability □ Rectal absorption—Calcitonin, polyacrylic acid aqueous gel base, bioavailability, rats

Calcitonin, a hypocalcemic peptide hormone, has been isolated from the thyroid of mammals and ultimobranchials and has been used for the treatment of Paget's disease as well as certain types of osteoporosis (1). Peptides, such as calcitonin, can be given effectively only by injection because, when taken orally, they are digested by the proteolytic enzymes in the GI tract and metabolized by the liver. This is a problem for patients who are unable to have injections or are in need of long-term therapy.

Rectal administration would be favorable as a dosage route for several classes of drugs because the "first-pass elimination" of high clearance drugs may be partly avoided (2, 3). However, when comparing rectal and oral administration of absorbable drugs, the former dosage requires two or three times the drug concentration of the latter dosage because the absorption surface area of the rectum is smaller than that of the duodenum (1/10,000 the surface arera of small intestine). The bioavailability of poorly absorbable drugs such as calcitonin, a relatively high molecular weight compound, needs to be enhanced by pharmaceutical modification for rectal administration. The aqueous gel bases of polyacrylic acid, which are a group of carboxyvinyl polymers cross-linked with allyl sucrose, have been shown previously to significantly improve the absorption of insulin from the rectum (4, 5) and vagina (6). The present study was designed to evaluate the feasibility of rectally administering a calcitonin analogue using a polyacrylic acid aqueous gel base as an absorption promoter.

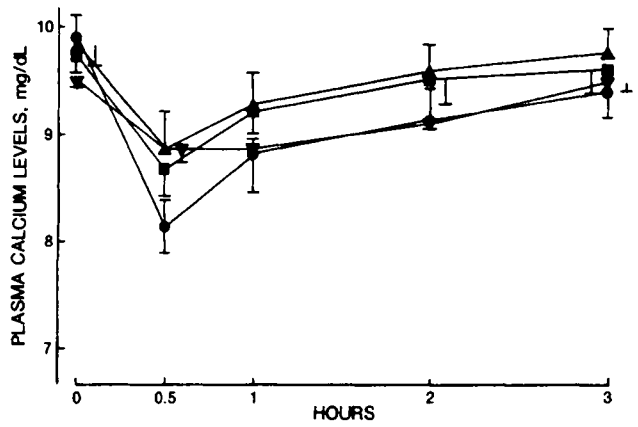

Figure 1—*Effects of the pH of polyacrylic acid gel base (0.01% w/v) on changes in plasma calcium levels following rectal administration of [Asu^{1,7}]-eel calcitonin in rats. The dose of [Asu^{1,7}]-eel calcitonin is 1 U/kg. Each point represents a mean ± SEM of five animals. Key: (●) pH 5.5; (■) pH 6.5; (▲) pH 7.5; (▼) pH 8.5 polyacrylic acid gel.*

EXPERIMENTAL SECTION

Materials—[Asu^{1,7}]-eel calcitonin[1] a synthetic analogue of eel calcitonin in which the disulfide bridge between the first and seventh amino acids in the eel-calcitonin molecule is replaced by a $-CH_2CH_2-$ bridge (7) was used in this study. A polyacrylic acid aqueous gel base[2] of molecular weight 1,250,000 was used as obtained from the manufacturers. All reagents were of analytical or reagent grade.

Preparations—Polyacrylic acid aqueous gel base was prepared by presoaking in distilled water for 15 h at room temperature and adding 10% NaOH solution to adjust each pH and each concentration, as previously described (4). The concentrations of polyacrylic acid in the gel bases were 0.01, 0.05, 0.1, and 1.0% w/v. The pH values selected for the study were 5.5, 6.5, 7.5, and 8.5. [Asu^{1,7}]-eel calcitonin was dissolved in each gel base. If the mixing process trapped air in the gel preparation, the air was removed by centrifugation for 10 min at 3000 rpm. The viscosity of the gel preparation was measured with

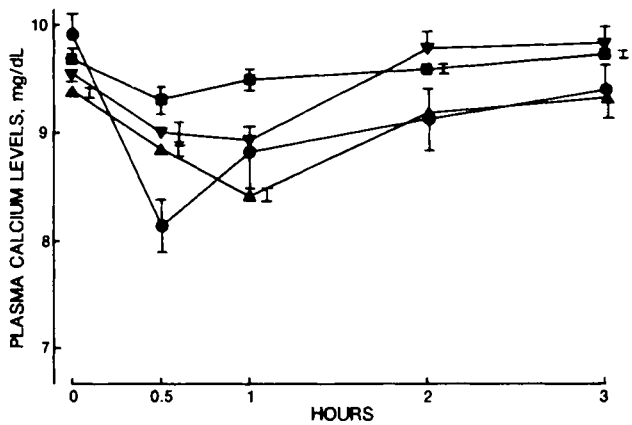

Figure 2—*Effects of the concentration of polyacrylic acid gel base (pH 5.5) on changes in plasma calcium levels following rectal administration of [Asu^{1,7}]-eel calcitonin in rats. The dose of [Asu^{1,7}]-eel calcitonin is 1 U/kg. Each point represents a mean ± SEM of five animals. Key: (▼) 0.01% w/v; (▲) 0.05% w/v; (●) 0.1% w/v; (■) 1.0% w/v polyacrylic acid gel.*

[1] Toyo Jozo Co., Ltd., Shizuoka, Japan.
[2] Carbpol 941; B.F. Goodrich Chemical Co, Cleveland, Ohio.

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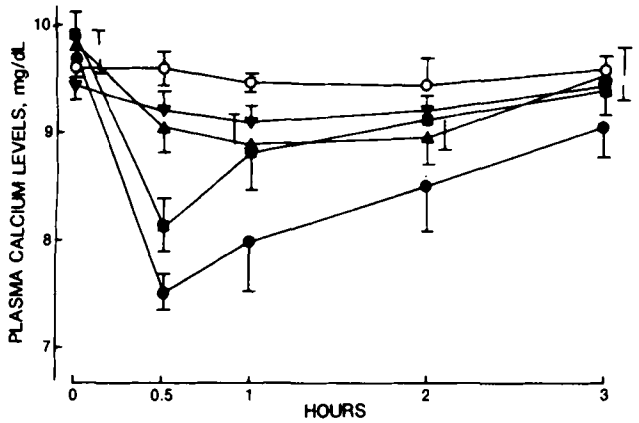

Figure 3—*Dose dependency of plasma calcium levels following rectal administration of [Asu^{1,7}]-eel calcitonin in polyacrylic acid gel base (0.1% w/v; pH 5.5) in rats. Each point represents a mean ± SEM of five animals. Key: (○) 0 U/kg; (▼) 0.1 U/kg; (▲) 0.5 U/kg; (■) 1 U/kg; (●) 5 U/kg.*

a cone and plate viscometer[3] at 37°C, and its osmolarity determined by a freezing-point osmometer[4]. The gel preparations were stored in the dark at 6°C.

Rectal Absorption—Young Wistar strain male rats (4 weeks old) weighing 80–100 g were selected as the experimental animals. The animals were fasted for 20 h prior to the experiments, but water was given freely. During the experiment, the rats were anesthetized with pentobarbital (50 mg/kg). The gel preparations were warmed to 30°C and quantities of 0.25 mL/100 g body weight were injected into the rectal loop (2.5 cm above the anus), which was isolated by ligation with thread. In comparative studies, the analogue was also formulated and administered rectally to separate groups of rats as a saline solution, in a triglyceride fatty acid mixture base[5], and in polyethylene glycol 1000. A fourth group of rats was given the material intramuscularly. Blood samples (0.3 mL) were obtained with a heparinized syringe from the femoral vein 10 min before and at 30 min and 1, 2, and 3 h postdose. The plasma was separated by centrifugation at 3000 rpm. The plasma calcium levels were determined by the *o*-cresolphthalein complexone method as described by Morin (8). The plasma concentration of calcitonin was measured by an enzyme immunoassay described earlier (9).

Protein and Calcium Release from the Rectal Mucosa—Release of protein and calcium from the rectal mucosa caused by the polyacrylic acid gel was determined by the *in situ* recirculation technique. Wistar strain male rats, weighing 260–300 g, were fasted for 20 h prior to the experiments. During the experiments, the animals were anesthetized with pentobarbital (60 mg/kg). The perfusate (20 mL) was recirculated at the rate of 2 mL/min at 37°C for 1 h in the colon and rectum (5 cm above the anus). The amount of protein was determined by Lowry's method (10) and that of calcium by atomic absorption spectroscopy.

RESULTS

Rheological Characteristics of Polyacrylic Acid Aqueous Gel—Polyacrylic acid aqueous gel behaves like a non-Newtonian liquid, and the viscosity is unchanged over a wide range of pH 4.5–12. The apparent viscosities of gel bases at concentrations of 0.01, 0.05, 0.1, and 1.0% w/v were observed to be 24, 177, 285, and 1515 cps, respectively. The values were obtained with a cone and plate viscometer at 37°C and a shear rate of 38.4 s^{-1}. As may be readily seen, the viscosity of the gel base increased with the increase in the concentration of polyacrylic acid. The viscosity of the gel preparation did not change when the calcitonin analogue was dissolved in the gel base.

Rectal Administration of [Asu^{1,7}]-eel Calcitonin—The effects of the pH of gel bases (0.1% w/v) on absorption following the rectal administration at the dose of 1 U/kg in young rats are shown in Fig. 1. The absorption increased with the lowering of the pH of the gel preparation. The hypocalcemic effects were in the following order of strength: pH 5.5 > pH 6.5 > pH 7.5 > pH 8.5 of the gel bases. The peaks of hypocalcemic effects were obtained at 30 min after administration, except with the pH 8.5 gel base. The plasma calcium values, which decreased by ~18% from their original levels, recovered after 1 h.

[3] Type E; Tokyo Keiki Co., Ltd., Tokyo, Japan.
[4] Fiske OS Osmometer; Uxbridge, Mass.
[5] Witepsol H-15; Dynamit Nobel Chemicals, Witten, West Germany.

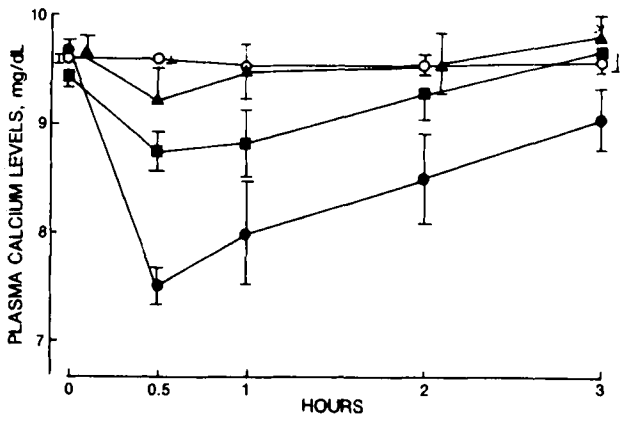

Figure 4—*Changes in plasma calcium levels following rectal administration of [Asu^{1,7}]-eel calcitonin in various bases in rats. The dose of [Asu^{1,7}]-eel calcitonin is 5 U/kg. Each point represents a mean ± SEM of five animals. Key: (○) saline solution; (▲) triglyceride fatty acid mixture base; (■) polyethylene glycol 1000; (●) 0.1% w/v polyacrylic acid gel (pH 5.5).*

The effects of the various concentrations of polyacrylic acid in the gel base (pH 5.5) on calcium levels after the rectal administration in the gel base to young rats are shown in Fig. 2. The time course of the hypocalcemic effects showed varying patterns with different concentrations of the gel after rectal administration. With the 0.1% w/v gel base, the plasma calcium level decreased rapidly for 30 min, at which time the maximum of the hypocalcemic effect was obtained. With the 0.05% w/v gel base, the plasma calcium level decreased slowly and the maximum hypocalcemic effect was found at 1 h. The 0.01% w/v and 1.0% w/v gel bases produced little hypocalcemic action; thus, the maximum hypocalcemic effect was obtained with the gel base at pH 5.5 and 0.1% w/v.

The dose response curves of plasma calcium levels following the rectal administration with the gel base (0.1% w/v; pH 5.5) are shown in Fig. 3. The rapid decrease of the plasma calcium level was observed at doses of 1 and 5 U/kg but, at doses of 0.1 and 0.5 U/kg slight hypocalcemic effects were seen from 30 min to 2 h.

In the experiment shown in Fig. 4, the hypocalcemic effect following rectal administration in polyacrylic acid gel base (0.1% w/v; pH 5.5) was compared with that obtained following administration in a triglyceride fatty acid mixture base, polyethylene glycol 1000, or saline solution at doses of 5 U/kg. The saline solution produced no hypocalcemic effect whereas the triglyceride fatty acid mixture preparation produced little hypocalcemic effect. The polyethylene glycol 1000 preparation produced a slight hypocalcemic effect; the minimum calcium level observed at 30 min after administration of [Asu^{1,7}]-eel calcitonin was ~93% of the initial level. Figure 5 compares the hypocalcemic effect produced by rectally administered material in the polyacrylic acid gel base (0.1% w/v; pH 5.5) with that administered intravenously. The plasma [Asu^{1,7}]-eel calcitonin levels obtained with rectal administration were compared with those with intramuscular administration. An enzyme immunoassay method was used to determine the [Asu^{1,7}]-eel calcitonin levels (Table I).

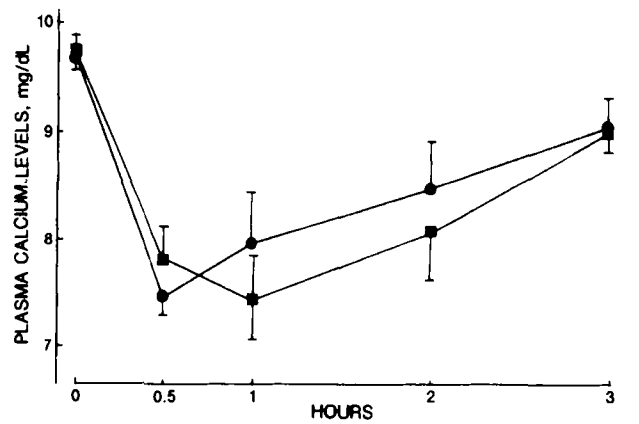

Figure 5—*Changes in plasma calcium levels following rectal administration in 0.1% w/v polyacrylic gel base (pH 5.5) and intravenous administration of [Asu^{1,7}]-eel calcitonin. Each point represents a mean ± SEM of five animals. Key (●) rectal administration (5 U/kg); (■) intravenous administration (0.15 U/kg).*

Table I—Bioavailability of [Asu[1,7]]-eel Calcitonin with Different Routes of Administration [a]

	Dose, U/kg	n	Peak Plasma Conc., mU/mL	AUC_0^{90}, mU·h/mL[b]	Apparent Bioavailability, %
Intramuscular	40	5	57.5 ± 6.1	57.3 ± 13.3	100
Rectal[c]	400	3	8.6 ± 2.1	4.3 ± 0.5	0.8 ± 0.2

[a] Each value is a mean ± *SEM*. [b] Area under plasma concentration *versus* time curve. [c] Gel base; 0.1% w/v, pH 5.5

Table II—Effects of Polyacrylic Acid Aqueous Gel Bases on Protein and Calcium Release from the Rectal Mucosa of Rats [a]

	n	Released Protein, mg	Released Ca^{+2} (mEq × 10^{-4})
Saline Solution	5	0.331 ± 0.018	6.01 ± 0.23
0.1% NaCl Solution	2	0.776 ± 0.155[b]	12.56 ± 0.94[b]
0.01% Gel Base (pH 5.5)	4	0.761 ± 0.041[b]	12.88 ± 0.50[b]
0.1% Gel Base (pH 5.5)	4	0.765 ± 0.070[b]	15.94 ± 1.01[b]
0.1% Gel Base (pH 5.5) containing 0.9% NaCl	4	0.312 ± 0.026	5.84 ± 0.66
0.1% Gel Base (pH 6.5) containing 0.9% NaCl	3	0.388 ± 0.043	6.01 ± 0.21
0.1% Gel Base (pH 7.5) containing 0.9% NaCl	3	0.347 ± 0.022	6.85 ± 0.60

[a] Each value is a mean ± *SEM*. [b] $p < 0.05$ when compared to saline using student's t test.

Rectal administration in a polyacrylic acid gel base (0.1% w/v; pH 5.5) required a dose 35 times greater than an intravenously administered dose to achieve an equivalent hypocalcemic effect (Fig. 5). When the calcitonin analogue was given rectally in the polyacrylic acid gel base, the apparent bioavailability was found to be ~0.80% (Table I) of a comparable intramuscular administration.

Protein and Calcium Releases from the Rectal Mucosa—In order to examine the effect of polyacrylic acid aqueous gel base on rectal mucosa, protein and calcium releasing effects were investigated. Table II shows the amounts of protein calcium released from the rectal mucosa. The protein and calcium releasing effects of 0.01 and 0.1% w/v polyacrylic acid gel base were increased by ~2 times that of saline. However, isotonic gel bases (at pH 5.5, pH 6.5, and pH 7.5 containing 0.9% NaCl) did not show the calcium and protein releasing effect.

DISCUSSION

It has been generally considered difficult for a peptide hormone such as [Asu[1,7]]-eel calcitonin to be absorbed through the intestinal mucosa. In this study, the rectal administration of [Asu[1,7]]-eel calcitonin in a saline solution at a dose of 5 U/kg did not lower the plasma calcium levels (Fig. 4). However, absorption from the rectum was significantly facilitated by a polyacrylic acid gel base. The pH of the gel and the concentration of polyacrylic acid in the gel base affected the rectal absorption. The rectal absorption increased with decreasing pH of the gel base in the range of pH 5.5–8.5 (Fig. 1). In the experiment where the concentrations of the polyacrylic acid in the gel base were varied, the increased absorption was in the following order of strength: 0.1% w/v > 0.05% w/v > 0.01% w/v > 1.0% w/v. The enhanced absorption (as noted by lowering of plasma calcium levels) was concentration dependent in the range of 0.01–0.1% w/v polyacrylic acid. The insignificant plasma calcium lowering effect noted with 1% w/v polyacrylic acid gel might have been due to the high viscosity of the gel and/or the interaction of the calcitonin analogue with the polyacrylic acid in the gel base.

When 1 U/kg was rectally administered in polyacrylic acid gel bases that were either isotonic (293 mOsm/kg) or hypertonic (865 mOsm/kg)—0.1% w/v; polyacrylic acid gel pH 5.5 containing 0.9% w/v and 3% w/v NaCl, respectively—the hypocalcemic effects were little or none (data not shown). Perhaps, the permeability of the calcitonin analogue through the rectal mucosal membrane in isotonic and hypertonic conditions was supressed by the exudation of fluids.

In order to clarify the mechanism of the promoting effect of the polyacrylic acid gel base, the authors studied the release of protein and calcium from the rectal mucosa. The 0.01% w/v and 0.1% w/v gel bases containing no additional sodium chloride (*viz* hypotonic) increased the release of protein and calcium from the rectum. However, the 0.1% w/v gel base containing 0.9% w/v NaCl did not show any such releasing effect. Further, microscopic examination of histological samples revealed that the polyacrylic acid gel base caused no damage to the surface of the rectal mucosa, as shown in a preliminary experiment (4). The amount of protein released by this gel base from the rectal mucosa was negligible compared with that by sodium deoxycholate and sodium lauryl sulfate (11).

The sequestering of calcium ions to polyacrylic acid was previously reported by Murata and Arai (12) and Robb and Sharples (13). These authors found that the polyacrylic acid gel base had chelating properties towards calcium. In the present study, the amount of calcium released by a hypotonic polyacrylic acid gel base from the rectal mucosa was also higher than that with a saline solution. Thus, the polyacrylic acid gel base may have altered the rectal epithelial barrier by depleting calcium ions at the mucosal membrane. Calcium ions are important for preserving a tight intercellular structure.

The comparative bioavailability of the calcitonin analogue following rectal and intramuscular administration showed that ~35 times as much drug was needed in a gel base (0.1% w/v; pH 5.5) given rectally to produce an equivalent hypocalcemic effect. The mechanism of enhancement of rectal absorption by the polyacrylic acid gel is unclear. Its major absorption pathway, perhaps, is *via* a paracellular route through the intercellular channels rather than *via* a transcellular route through the epithelial cells.

This study showed that polyacrylic acid aqueous gel base might be used to promote the rectal absorption of polypeptide drugs, such as [Asu[1,7]]-eel calcitonin.

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