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Absorption enhancement of orally administered salmon calcitonin by polystyrene nanoparticles having poly(N-isopropylacrylamide) branches on their surfaces

Shinji Sakuma [a,*], Norio Suzuki [a], Hiroshi Kikuchi [a], Ken-ichiro Hiwatari [b], Kiyotaka Arikawa [b], Akio Kishida [b], Mitsuru Akashi [b]

[a] *Pharmaceutical Formulation Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Kita-Kasai, Edogawa-ku, Tokyo 134, Japan*
[b] *Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Kagoshima University, Korimoto, Kagoshima 890, Japan*

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Abstract

Polystyrene nanoparticles having poly(N-isopropylacrylamide) branches on their surfaces (PNIPAAm nanoparticles) were synthesized and various attempts were made in rats to increase the absorption enhancement of orally administered salmon calcitonin (sCT) by these nanoparticles. The hypocalcemic effect after oral administration of a mixture of sCT and PNIPAAm nanoparticles depended greatly on the administration schedule. When one half of a dose of the mixture was given orally 40 min after the other half, sCT-induced hypocalcemic effect was markedly enhanced by PNIPAAm nanoparticles and the area of the reduction of the blood ionized calcium concentration was about 3 times that after administration of a single full dose of the same mixture. However, there was no further enhancement of the pharmacological activity of sCT when the half-doses were administered 120 min apart. sCT absorption was also affected by the hydrophobicity of the PNIPAAm nanoparticles. The hydrophobic PNIPAAm nanoparticles dispersed in hydrochloric acid-sodium chloride solution of pH 1.2, increased in sCT-induced hypocalcemic effect considerably. When two half-doses of the mixture containing these hydrophobic nanoparticles were given orally 40 min apart, the hypocalcemic effect remained strong, even though the dose was reduced to less than half. These changes probably depended on the bioadhesion of PNIPAAm nanoparticles to the gastric mucosa. It was demonstrated that PNIPAAm nanoparticles are good drug carriers that substantially enhance sCT absorption via the gastrointestinal tract. © 1997 Elsevier Science B.V.

Keywords: Nanoparticle; Poly(N-isopropylacrylamide); Absorption enhancement; Salmon calcitonin; Bioadhesion; Phase transition

* Corresponding author. Tel.: +81 3 56968307; fax: +81 3 56968228.

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1. Introduction

The development of a dosage form that improves the absorption of peptide and protein drugs via the gastrointestinal (GI) tract, is one of the greatest challenges in the pharmaceutical field (Lee and Yamamoto, 1990b). Many researchers have taken up the challenge, using approaches including particulate drug delivery such as nanoparticles (Kreuter, 1991; Couvreur and Puisieux, 1993), colon delivery (Ushirogawa et al., 1992), mucoadhesive drug delivery (Gruber et al., 1987), chemical modification of drugs (Fujita et al., 1996), and the use of an absorption enhancer (Muranishi, 1990) or a protease inhibitor (Lee, 1990a).

We also demonstrated previously that nanoparticles composed of new graft copolymers having a hydrophobic backbone and hydrophilic branches, are very useful as drug carriers (Akashi et al., 1985, 1989a). These nanoparticles are prepared by the free radical copolymerization of hydrophilic macromonomers—polyvinyl compounds terminating in vinylbenzyl groups—with hydrophobic styrene (Akashi et al., 1990). The surface of the nanoparticles obtained is covered by hydrophilic macromonomer chains (Akashi et al., 1989b). By designing and synthesizing different functional macromonomers, a variety of nanoparticles having functional polymeric branches on their surfaces can be obtained (Capek and Akashi, 1993).

In our previous study (Sakuma et al., 1997), it was reported that the absorption of salmon calcitonin (sCT) via the GI tract was enhanced by these nanoparticles. This absorption enhancement effect was affected by the macromonomer structure, and sCT absorption was significantly enhanced by nanoparticles having poly(N-isopropylacrylamide) branches on their surfaces (PNIPAAm nanoparticles). However, there was insufficient absorption of sCT to retain the hypocalcemic effect and it was suggested that further improvement is necessary for achieving oral peptide delivery by this technology.

In this article, we examined several approaches to increase the absorption enhancement effect of sCT by PNIPAAm nanoparticles.

2. Materials and methods

2.1. Materials

sCT was purchased from Sigma Chemical (St. Louis, MO). N-isopropylacrylamide (NIPAAm) monomer was obtained from Kohjin (Tokyo, Japan). *p*-Chloromethyl styrene (*p*-CMSt) was furnished by Nippon Oil and Fats (Tokyo, Japan). NIPAAm monomer and *p*-CMSt were used without further purification. All other chemicals were commercial products of reagent grade and were purified in the usual manner if necessary (Riza et al., 1995; Chen et al., 1996).

2.2. Preparation of PNIPAAm nanoparticles

2.2.1. Preparation

PNIPAAm nanoparticles were prepared by the procedure of Chen et al. (1996) as follows. NIPAAm monomer was oligomerized by using 2,2′-azobisisobutyronitrile (AIBN, less than 1 mol% to monomer) in the presence of 2-mercaptoethanol in 50 ml of ethanol at 60°C under nitrogen for 7 h. After oligomerization, ethanol was removed by evaporation. The resulting hydroxyl group-terminated NIPAAm oligomers were dissolved in purified water (20 w/v%), heated to 50°C so that only oligomers were deposited, and isolated by centrifugation (3000 rpm for 15 min) at more than 50°C. NIPAAm oligomers were condensed with *p*-CMSt in 50 ml of N,N-dimethylformamide containing 50 w/v% potassium hydroxide (KOH) aqueous solution at 30°C for 72 h in the presence of tetra-butylphosphonium bromide (TBPB). KOH and TBPB were used in amounts that were 5 and 0.5 times the molar quantity of the oligomers. The precipitated potassium chloride was removed by filtration and the resulting vinylbenzyl group-terminated NIPAAm macromonomers were dialyzed in purified water. PNIPAAm nanoparticles were prepared by dispersion copolymerization between the macromonomers and styrene using AIBN in 5 ml of ethanol at 60°C for 24 h in a vacuum. Finally, these nanoparticles were dialyzed in purified water and then lyophilized. The amounts of the respective reagents used are tabulated in Table 1.

Table 1
Conditions of PNIPAAm nanoparticle preparation

Run	Oligomerization		Condensation			Copolymerization		
	Monomer	2MEtOH[a]	Oligomers		*p*-CMSt[b]	Macromonomers		Styrene
	(mmol)	(mmol)	Mn[c]	(mmol)	(mmol)	Mn[c]	(mmol)	(mmol)
1	73	1.6	3.4	3.0	30	3.5	0.15	5.0
2	88	2.7	4.3	0.55	5.5	4.4	0.12	4.4

[a] 2-Mercaptoethanol
[b] *p*-Chloromethyl styrene
[c] Number-average molecular weight ($\times 10^{-3}$).

2.2.2. Characterization

The nanoparticles were characterized using the method described in an earlier article (Chen et al., 1996). Briefly, the number-average molecular weight (Mn) of macromonomers on the nanoparticle surface was determined by gel permeation chromatography. The particle size of the nanoparticles was measured by dynamic light-scattering spectrophotometry.

2.3. Animal experiments

2.3.1. Preparation of PNIPAAm nanoparticles incorporating sCT

Twenty milligram of the lyophilized PNIPAAm nanoparticles were redispersed in 1 ml of purified water or 1 ml of hydrochloric acid-sodium chloride (HCl-NaCl) solution of which pH was 1.2 and ionic strength was 0.1. sCT was dissolved in the same solution and the concentration was adjusted to 0.08 or 0.2 mg/ml. The nanoparticle dispersion was mixed with the same volume of sCT solution prior to the in vivo study. The final concentration of sCT in this mixture for use as a dosing solution was 0.04 or 0.1 mg/ml. The nanoparticle concentration in the mixture was constant at 10 mg/ml.

2.3.2. The incorporation rate of sCT in nanoparticles

One milliliter of the mixture of sCT and PNIPAAm nanoparticles was centrifuged at 10 000 rpm for 60 min at room temperature to separate the sCT not associated with the nanoparticles, from that incorporated in nanoparticles. The concentration of sCT in the supernatant was determined by the HPLC procedure reported in our previous study (Sakuma et al., 1997). After the measurement, the incorporation rate of sCT was calculated from the difference in sCT concentration between the supernatant and the original mixture.

2.3.3. In vivo study

Male Sprague-Dawley (SD) strain rats weighing 190–230 g were used. The rats (four or five per group) were fasted overnight with free access to water before the in vivo study. Each mixture of sCT and PNIPAAm nanoparticles (containing both incorporated sCT and non-incorporated sCT) was given orally (intragastrically) to rats in a single administration by using a feeding needle at a dose of 0.1 or 0.25 mg of sCT and 25 mg of nanoparticles in a 2.5 ml mixture/kg of body weight. When necessary, the mixture was heated to 50°C for 10 min just before administration to make the PNIPAAm nanoparticles hydrophobic (Chen et al., 1996). Separately, the dose was divided in half before oral administration was carried out in rats, one half being given 40 or 120 min after the other (0.05 or 0.125 mg sCT with 12.5 mg nanoparticles in a 1.25 ml mixture/kg of body weight). The total dose given in single and divided administrations was the same. Blood samples (approximately 0.1 ml) were obtained from the tail vein without anesthesia until 8 h after administration. The blood ionized calcium concentration was measured with an analyzer using

calcium electrodes (634 automated Ca^{++}/pH analyzer, Ciba Corning Diagnostics, Tokyo, Japan). As a control, sCT dissolved in purified water or the HCl-NaCl solution, was administered to rats under the same conditions.

2.3.4. Statistical analysis

The change in the blood ionized calcium concentration from before to after oral administration of the dosing solution was calculated, and the means and standard errors were determined. Each value was plotted as a function of time. The area between the curve for the change in the ionized calcium concentration versus time and a horizontal line representing zero change was calculated by the trapezoidal method until the ionized calcium concentration returned to the initial value. The value obtained (area of ionized calcium reduction) was used as an index of the biological effect of sCT, along with a minimum ionized calcium concentration level (Morita et al., 1994; Kobayashi et al., 1994). Statistical significance was assessed with Student's t-test, and p values of 0.05 or less were considered significant.

3. Results

3.1. Preparation of PNIPAAm nanoparticles

Figs. 1 and 2 and Table 2 show the chemical structure, a scanning electron micrograph and the characteristics of PNIPAAm nanoparticles, respectively. Spherical and monodispersed PNIPAAm nanoparticles were prepared by this method as described in previous studies (Chen et al., 1996; Sakuma et al., 1997). The weight-average diameter of nanoparticles was less than 1000 nm and these nanoparticles possessed good water-dispersibility. The incorporation rate of sCT in nanoparticles dispersed in HCl-NaCl solution was about 10%, and equal to that in purified water.

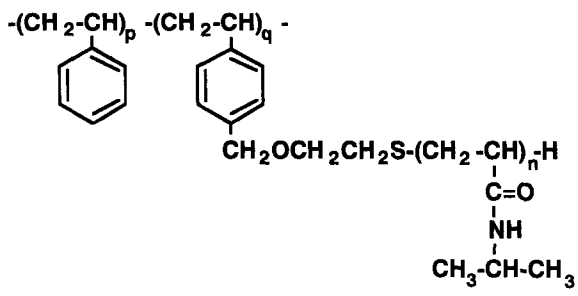

Fig. 1. Chemical structure of PNIPAAm nanoparticles.

Fig. 2. Scanning electron micrograph of PNIPAAm nanoparticles. The PNIPAAm nanoparticle size and the molecular weight of the macromonomers were 530 nm and 3500, respectively.

3.2. Absorption enhancement of orally administered sCT by PNIPAAm nanoparticles

3.2.1. Effect of administration schedule

Fig. 3 and Table 3 show the change in the blood ionized calcium concentration after a single administration of the mixture of sCT and PNIPAAm nanoparticles in aqueous solution (0.1 or 0.25 mg of sCT/kg of rat body weight). The decrease in the blood ionized calcium concentration was greater than that after oral administration of sCT aqueous solution, as was the case in the previous study at a dose of 0.25 mg sCT/kg of rat body weight (Sakuma et al., 1997). This hypocalcemic effect changed less when the dose of sCT was 0.1 mg/kg of rat body weight, irrespective of the presence of PNIPAAm nanoparticles.

The hypocalcemic effect after oral administration of the mixture (0.25 mg of sCT/kg of rat body weight) was greatly affected by the administration schedule, as shown in Fig. 4 and Table 3. When the dose was halved and the halves were

Table 2
Characteristics of PNIPAAm nanoparticles incorporating sCT

Run	Mn[a]	Diameter[b] (nm)	sCT Incorp.[c] (%)	Solvent[d]	Conc. of sCT[e] (mg/ml)	Conc. of NPs[f](mg/ml)
1	3.5	530	16	water	0.1	10
2	4.4	750	9	water	0.1	10
3	4.4	750	9	HCl-NaCl[g]	0.1	10
4	4.4	750	11	HCl-NaCl[g]	0.04	10

[a] Number-average molecular weight of macromonomers on nanoparticle surface ($\times 10^{-3}$).
[b] Weight-average diameter.
[c] Rate of sCT incorporated in nanoparticles (mean of three experiments).
[d] Solvent for use as dosing solutions.
[e] Concentration of sCT.
[f] Concentration of nanoparticles.
[g] Hydrochloric acid-sodium chloride solution of pH 1.2.

given orally 40 min apart (0.125 mg of sCT/kg of rat body weight × 2 times), the hypocalcemic effect was considerably stronger. In this case, the area of ionized calcium reduction was about 3 times that after a single administration of the same mixture ($p < 0.001$). The difference in the minimum ionized calcium level between two experiments was also significant ($p < 0.01$). However, there was no significant difference in the area of ionized calcium reduction between single (0.56 ± 0.08 mMhr) and divided administration whose interval was 120 min (0.77 ± 0.06 mMhr), nor was the minimum ionized calcium level.

3.2.2. *Effect of hydrophobicity of PNIPAAm nanoparticles*

We next examined the effect of the hydrophobicity of PNIPAAm nanoparticles on the absorption enhancement of sCT, as shown in Fig. 5 and Table 3. PNIPAAm nanoparticles in the mixture whose solvent was HCl-NaCl solution aggregated within a few minutes when heated to 50°C because the PNIPAAm branches on the nanoparticle surface become hydrophobic above 35°C (Chen et al., 1996). These micro-size aggregations of hydrophobic PNIPAAm nanoparticles were still dispersed in the solution (data not shown). When this mixture was given orally in a single administration at a dose of 0.25 mg sCT/kg of rat body weight, the hypocalcemic effect was significantly improved, compared with that after oral administration of the same dose of the unheated mixture containing the hydrophilic PNIPAAm nanoparticles in water (minimum ionized calcium level, $p < 0.05$; area of ionized calcium reduction, $p < 0.001$). On the other hand, the degree of the reduction of the calcium concentration after oral administration of the mixture containing the hydrophilic PNIPAAm nanoparticles was not much affected by the solvent (both indices: not significant).

3.2.3. *Absorption enhancement effect under optimized conditions*

Two half-doses of the mixture of sCT and hydrophobic PNIPAAm nanoparticles aggregated in HCl-NaCl solution were given orally 40 min apart (0.05 mg of sCT/kg of rat body weight × 2 times). In this experiment, the dose of sCT was decreased to 0.1 mg/kg of rat body weight. As is obvious from Fig. 6 and Table 3, there was a greater hypocalcemic effect after the divided administration of the mixture, compared with an normal single administration of the same dose of the mixture (minimum ionized calcium level, $p < 0.001$; area of ionized calcium reduction, $p < 0.05$). This effect was almost equal to that of a single administration of the mixture containing the hydrophobic nanoparticles, and to that of the divided administration of the mixture of sCT and hydrophilic PNIPAAm nanoparticles in water with a 40-min interval, although only two-fifths of sCT dose was used.

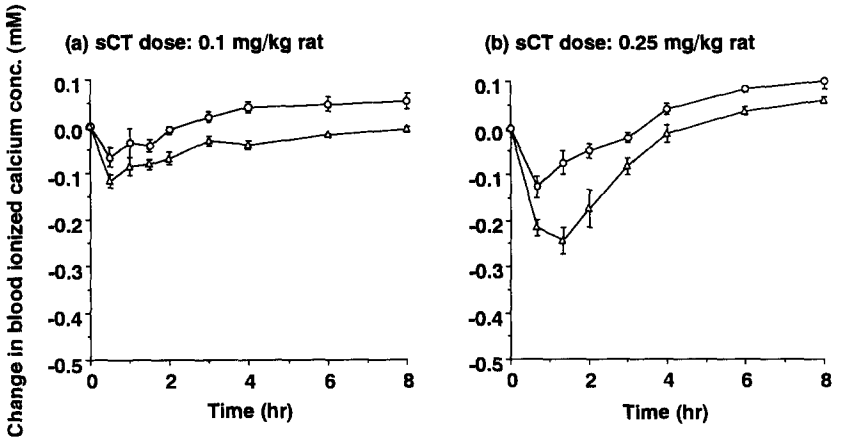

Fig. 3. Concentration-time profiles of ionized calcium in blood after oral administration of sCT solution (○) and a mixture of sCT and PNIPAAm nanoparticles (△) in rats. The sCT solution and the mixture were given orally in a single administration at a dose of 0.1 mg (a) or 0.25 mg (b) of sCT with 25 mg nanoparticles in 2.5 ml dosing solution/kg of rat body weight. The nanoparticle size and the molecular weight of the macromonomers were 530 nm and 3500, respectively. Water was used as the solvent. Each value represents the mean ± S.E.

4. Discussion

The above results suggest that PNIPAAm nanoparticles are drug carriers which substantially enhance sCT absorption via the GI tract. As Figs. 3–6 and Table 3 show, the hypocalcemic effect after oral administration of the mixture of sCT and PNIPAAm nanoparticles was markedly increased by the several approaches which we proposed. The plasma concentration of sCT after oral administration of the mixture has been also examined and it has already been confirmed that the increase in the plasma concentration of sCT enhances the pharmacological activity of sCT (data not shown). We cannot yet explain completely the mechanism of the absorption enhancement of sCT by nanoparticles composed of graft copolymers having a hydrophobic backbone and hydrophilic branches (Sakuma et al., 1997). In addition, there are insufficient data to explain the further absorption enhancement effect of sCT by PNIPAAm nanoparticles.

In the previous article (Sakuma et al., 1997), we proposed the hypothesis that the nanoparticles composed of the graft copolymers which we developed have not only the property of bioadhesion to the GI mucosa but also that of stabilizing peptide and protein drugs in the GI tract, and that the improvement of drug absorption results from these actions. We consider that the bioadhesion of PNIPAAm nanoparticles mainly affected the further absorption enhancement of sCT in this study, as follows. When the mixture of sCT and PNIPAAm nanoparticles was administered orally, a number of nanoparticles most likely adhere to the gastric mucosa. However, sCT is never absorbed from the gastric mucosa and there was no hypocalcemic effect after oral administration of the mixture of sCT and PNIPAAm nanoparticles to rats ligated the pyloric region (data not shown). These facts suggest that the adhesion of PNIPAAm nanoparticles to the gastric mucosa is unnecessary for inducing the absorption enhancement of sCT by nanoparticles, and that sCT absorption increases with increasing the amount of nanoparticles which reach the intestine with sCT. It is clear that there is an interaction between the hydrophilic mucous layer (Gu et al., 1988) and hydrophilic PNIPAAm nanoparticles just after administration. After a while, the nanoparticles become hydrophobic at body temperature because a phase transition of PNIPAAm branches on the nanoparticle surface occurs at 35°C (Chen et al., 1996). It is probable that these hydrophobic PNIPAAm nanoparticles subsequently desorb from the hydrophilic mucous layer

Table 3
Hypocalcemic effect after oral administration of sCT alone and of a mixture of sCT and PNIPAAm nanoparticles

Run	Dose (mg/kg rat)		Administration schedule	Minimum Ca^{++} level[a] (% of initial)	Area of Ca^{++} reduction[b] (mM·hr)
	sCT	Nanoparticles			
1[c]	0.1	0	Single[g]	94 ± 1.4	0.08 ± 0.03
2[c]	0.1	25 (Hydrophilic)[e]	Single[g]	91 ± 1.2	0.33 ± 0.04
3[c]	0.25	0	Single[g]	90 ± 1.3	0.24 ± 0.06
4[c]	0.25	25 (Hydrophilic)[e]	Single[g]	81 ± 1.5	0.56 ± 0.08
5[c]	0.25	0	Divided (40 min)[h]	89 ± 3.7	0.32 ± 0.17
6[c]	0.25	25 (Hydrophilic)[e]	Divided (40 min)[h]	69 ± 1.1 (0.01[j])	1.50 ± 0.16 (0.001[j])
7[c]	0.25	0	Divided (120 min)[i]	91 ± 1.1	0.23 ± 0.03
8[c]	0.25	25 (Hydrophilic)[e]	Divided (120 min)[i]	79 ± 1.6 (NS[jk])	0.77 ± 0.06 (NS[jk])
9[d]	0.25	0	Single[g]	87 ± 1.5	0.26 ± 0.07
10[d]	0.25	25 (Hydrophilic)[e]	Single[g]	76 ± 2.7 (NS[jk])	0.97 ± 0.37 (NS[jk])
11[d]	0.25	25 (Hydrophobic)[f]	Single[g]	73 ± 1.5 (0.05[j])	1.52 ± 0.10 (0.001[j])
12[d]	0.1	0	Divided (40 min)[h]	90 ± 1.6	0.32 ± 0.07
13[d]	0.1	25 (Hydrophobic)[f]	Divided (40 min)[h]	72 ± 1.4 (0.001[l])	1.26 ± 0.23 (0.05[l])

[a] Minimum blood ionized calcium concentration level (mean ± S.E.).
[b] Area between blood ionized calcium concentration versus time curve and horizontal at 0 change (mean ± S.E.).
[c] Water was used as a solvent.
[d] Hydrochloric acid-sodium chloride solution of pH 1.2 was used as a solvent.
[e] Mixture was left at room temperature.
[f] Mixture was heated to 50°C to make nanoparticles hydrophobic just before administration.
[g] Mixture was given orally in a single administration.
[h] Mixture was administered orally in two equal parts 40 min apart.
[i] Mixture was administered orally in two equal parts 120 min apart.
[j] Statistically significant difference from Run No. 4.
[k] Not significant.
[l] Statistically significant difference from Run No. 2.

by degrees. Chen et al. (1996) reported that PNIPAAm nanoparticles require about 1 h to become stably hydrophobic at 40°C and this time decrease with increasing temperature. Forty minutes after administration of the mixture of sCT and PNIPAAm nanoparticles, the hydrophilicity of the nanoparticles was still high, so that most nanoparticles which adhered to the gastric mocosa probably remained there. It seems that the PNIPAAm nanoparticles in the second dose, which were administered in this condition, did not adhere to any great extent to the gastric mucosa, because the surface of the mucosa is already covered with the nanoparticles of the first dose. It is expected that many PNIPAAm nanoparticles from the second dose could consequently reach the intestine with sCT, and that sCT absorption was effectively enhanced by them in comparison with the results of the single administration. On the other hand, 120 min after administration of the mixture, most PNIPAAm nanoparticles probably became hydrophobic, although body temperature is slightly below 40°C. It appears that there were few PNIPAAm nanoparticles left in the gastric mucosa at this time. As a result, it is considered that the PNIPAAm nanoparticles in the second dose probably adhered to the gastric mucosa in the same way as those in the first dose and that there was therefore no further enhancement of sCT absorption by them.

We next examined the absorption enhancement effect of sCT by the hydrophobic PNIPAAm nanoparticles. In this experiment, HCl-NaCl solution of pH 1.2 was used as a solvent to regenerate

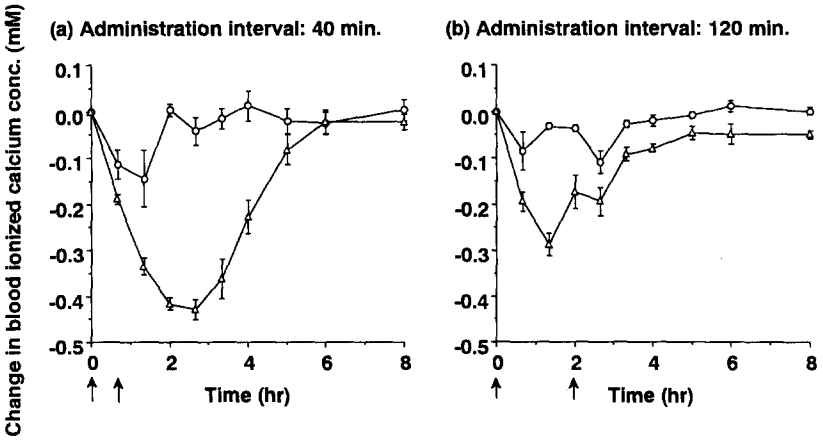

Fig. 4. Concentration-time profiles of ionized calcium in blood after oral administration of sCT solution (○) and a mixture of sCT and PNIPAAm nanoparticles (△) in rats (0.25 mg sCT with 25 mg nanoparticles in 2.5 ml dosing solution/kg of rat body weight). A dose of each dosing solution was halved and the halves were given orally 40 and 120 min apart, as denoted by the upward arrows. The PNIPAAm nanoparticle size and the molecular weight of the the macromonomers were 530 nm and 3500, respectively. Water was used as the solvent. Each value represents the mean ± S.E.

the condition just after the hydrophobic PNIPAAm nanoparticles were desorbed from the gastric mucosa. After a single administration of the mixture of sCT and hydrophobic PNIPAAm nanoparticles, the hypocalcemic effect was as strong as that after the divided administration, with a 40-min interval, of the mixture of sCT and hydrophilic PNIPAAm nanoparticles in water. In addition, the divided administration of the mixture containing hydrophobic PNIPAAm nanoparticles caused greater enhancement of sCT absorption. These results seem to indicate that many nanoparticles reached the intestine with sCT. It is known that PNIPAAm has some hydrophilicity above the temperature at which the phase transition occurs (Tokuhiro et al., 1991). In addition, it has been confirmed that PNIPAAm nanoparticles are still wettable, even when heated to 50°C (Chen et al., 1996). These hydrophobic PNIPAAm nanoparticles also seem to adhere to the GI mucosa because of a hydrophilic interaction between them and the mucous layer although it is necessary to examine the effect of the balance of hydrophilicity and hydrophobicity on the PNIPAAm nanoparticle surface, on the interaction between the mucous layer and the nanoparticles. The divided administration perhaps reduced the loss of the hydrophobic PNIPAAm nanoparticles resulting from their adhesion to the gastric mucosa. In our preliminary study, it has already been confirmed that the transit time through the GI tract of PNIPAAm nanoparticles was longer than that of nanoparticles having surface poly(ethylene glycol) branches, which do not enhance sCT absorption via the GI tract. These data suggest that the absorption enhancement of sCT is affected by the bioadhesion of nanoparticles. However, it is not clear how sCT not incorporated in PNIPAAm nanoparticles behaves in the GI tract, or where sCT incorporated in nanoparticles is released from them. We have to examine the effect of nanoparticles on the kinetics of sCT in the GI tract, taking account of the interaction between sCT and nanoparticles, because the interaction is probably affected by the temperature and by components such as ions, enzymes and mucin. In addition, it may be necessary to discuss this absorption enhancement effect from the standpoint of the stabilizing effect, which is the other mechanism of absorption enhancement. Further work will be successively discussed in future reports.

5. Conclusions

We examined several approaches for augmenting the absorption enhancement effect of sCT by PNIPAAm nanoparticles. The hypocalcemic effect after oral administration of the mixture of sCT and PNIPAAm nanoparticles was affected by the administration schedule and hydrophobicity of the nanoparticles. When a dose of the mixture was halved and the halves were given orally 40 min apart, sCT absorption was markedly enhanced by the PNIPAAm nanoparticles. In addition, the hydrophobic PNIPAAm nanoparticles dispersed in HCl-NaCl solution of pH 1.2, increased in sCT-induced hypocalcemic effect considerably. The divided administration of the mixture containing these hydrophobic nanoparticles caused greater enhancement of sCT absorption. It was considered that the additional absorption enhancement effect was mainly affected by the bioadhesion of PNIPAAm nanoparticles to the gastric mucosa.

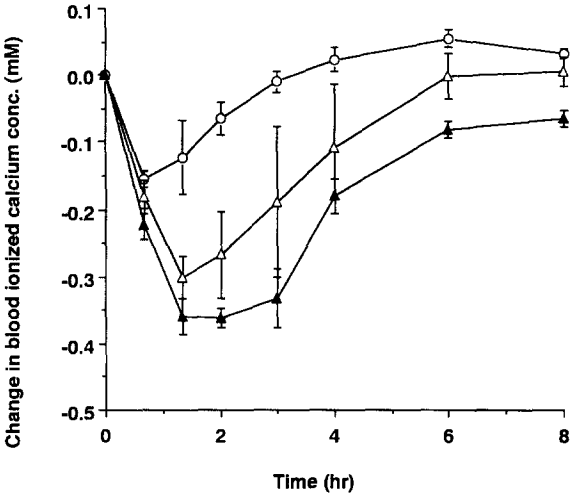

Fig. 5. Concentration-time profiles of ionized calcium in blood after oral administration of sCT solution (○), a mixture of sCT and hydrophilic PNIPAAm nanoparticles (△) and a mixture of sCT and hydrophobic PNIPAAm nanoparticles (▲) in rats. The sCT solution and the mixtures were given orally in a single administration at a dose of 0.25 mg sCT with 25 mg nanoparticles in 2.5 ml dosing solution/kg of rat body weight. The nanoparticle size and the molecular weight of the macromonomers were 750 nm and 4400, respectively. HCl-NaCl solution of pH 1.2 was used as the solvent. Each value represents the mean ± S.E.

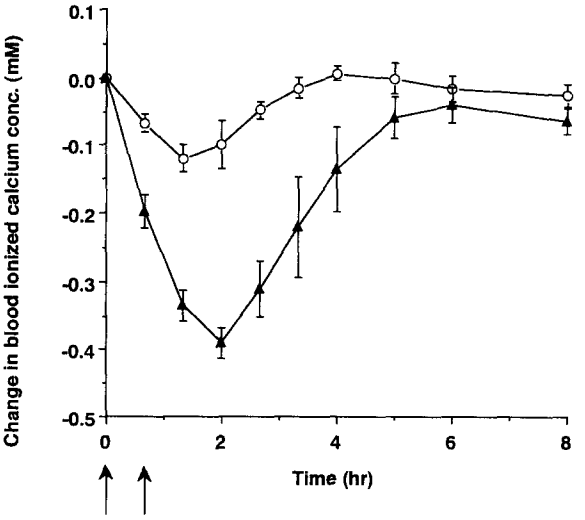

Fig. 6. Concentration-time profiles of ionized calcium in blood after oral administration of sCT solution (○) and a mixture of sCT and hydrophobic PNIPAAm nanoparticles (▲) in rats (0.1 mg sCT with 25 mg nanoparticles in 2.5 ml dosing solution/kg of rat body weight). One dose of each dosing solution was halved and the halves were given orally 40 min apart, as denoted by the upward arrows. The nanoparticle size and the molecular weight of the macromonomers were 750 nm and 4400, respectively. HCl-NaCl solution of pH 1.2 was used as the solvent. Each value represents the mean ± S.E.

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