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Absorption enhancement of calcitonin in the rat intestine by carbopol-containing submicron emulsions

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Abstract

The absorption of salmon calcitonin (sCT) in the jejunum and the colon of the rat from submicron emulsions with (MA–SME) and without (SME) the adhesive polymer Carbopol®940 was studied in side-by-side diffusion cells and closed intestinal loops of the anesthetized rat. sCT permeated the jejunal and colonic mucosa of the rat at similar rates. However, when the sCT was introduced in MA–SME a profound enhancement (3.3-fold increase) in the diffusion rate was observed in the colon, but not in the jejunal epithelium. It was found that after intra-colonic administration, the SME formulations yielded a significant reduction in plasma Ca^{2+} levels as compared to administration of sCT in normal saline. In the case of MA–SME, the reduction was more profound (80% of Ca^{2+} of basal level at 120 min, as compared to 90% of Ca^{2+} basal level at 15 min for MA–SME and SME, respectively) and was prolonged (1.5-fold) in the case of the MA–SME. Similarly, MA–SME caused a profound increase (14.7%) in the absolute bioavailability of sCT following intra-colonic administration in the rat. Indomethacin-containing MA–SME adhered rapidly, and at similar rates, to the mucosa of everted sacks taken from the jejunum or colon of the rat. It is suggested that enhancement of sCT absorption is attributable to both adherence of the emulsion droplets to the epithelium mucosa and the protease inhibition and absorption enhancement properties of Carbopol®940.

Keywords: Absorption enhancement; Salmon calcitonin; Carbopol; Colon; Colonic delivery; Rat; Submicron emulsions

Abbreviations: AAC, Area above the pharmacological response (reduction in plasma Ca^{2+} levels) versus time; PA, Relative pharmacological activity; IC, intracolonic (administration); I, indomethacin; PCP, polycarbophil; sCT, salmon calcitonin; SME, submicron emulsion; MA–SME, Carbopol®940 containing submicron emulsion; sCT–SME, sCT containing submicron emulsion; sCT–MA–SME, sCT containing MA–SME (SME with Carbopol®940).

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1. Introduction

The therapeutic uses of synthetic salmon calcitonin (sCT), a 32-amino acid hormone available commercially for over 25 years, are the long-term treatment of Paget's disease and the short-term relief of some hypercalcemia conditions (e.g. malignant hypercalcemia) (Azria, 1989). At first, injection was the only route of sCT administration. Alternative modes of administration have recently been developed. Intranasal administration of sCT is already available. Other routes such as colonic (Antonin et al., 1992, 1996; Beglinger et al., 1992), pulmonary (Kobayashi et al., 1994) or intrauterine (Golomb et al., 1993) administrations have been tested in man and in rats.

In recent studies Antonin et al. (1992, 1996) and Hastewell et al. (1995) reported that human calcitonin is rapidly absorbed from the human colon, preferably in the transverse colon. However, this rapid absorption had negligible effect on the absolute bioavailability of the drug (a typical value of 0.22% when compared to intravenous infusion). The addition of the protease inhibitor aprotinin, even decreased the colonic absorption, probably due to the formation of an insoluble complex which resulted in sedimentation of the drug (Hastewell et al., 1995). The nature of colonic absorption of calcitonin requires a formulative act to improve its bioavailability after oral ingestion. Since aprotinin was shown to be worthless, an alternative approach should be used. Lowe and Temple (1994) showed that incorporation of calcitonin into isobutylcyanoacrylate nanocapsules did not significantly improve its bioavailability after intestinal administration to rats. However, it has recently been suggested that the GI absorption of proteinaceous drugs could be improved in the presence of crosslinked acrylic acid derivatives, such as polycarbophil (PCP) and Carbomer 934P. Thus, Lehr et al. (1992), and later Luessen et al. (1994, 1995) reported that the two polymers possess absorption enhancement properties as analyzed in vitro with the peptide probe 9-desglycinamide, 8-arginine vasopressin (DGAVP). Moreover, polycarbophil and Carbomer 934P were able to protect DGAVP from mucosal homogenate degradation (Lehr et al., 1992), and inhibit trypsin activity, as analyzed by *N*-α-benzoyl-L-arginine ethylester degradation studies (Luessen et al., 1995). These findings were verified by Bai et al. (1995).

The use of emulsions for the oral delivery of lipophilic proteinaceous drugs has been suggested frequently (for example, Kararli et al., 1992). Recently, Ilan et al. have reported on a new type of submicron emulsion (SME): Carbopol®940-containing submicron emulsions (MA–SME) that were able to improve the absorption of the hydrophilic desmopressin acetate in rats (Ilan et al., 1996). The assumption was that the emulsion's nano-droplets, coated with Carbopol®940, were able to adhere for prolonged periods of time to the intestinal wall, thus increasing drug absorption.

Using salmon calcitonin (sCT), the objectives of this study were: (a) to check if SME is able to increase sCT absorption in the jejunum and colon of the rat, (b) to examine the possible absorption improvement effect of Carbopol®940 when incorporated into SME.

2. Materials and methods

2.1. Materials

Salmon Calcitonin (sCT) was obtained from Pharmachem, Lugano, Switzerland. Carbopol®940 was obtained from BF Goodrich, Cleveland, OH. All other materials were purchased from Sigma, St. Louis, MO, unless otherwise mentioned in the text. All solvents were analytical grade.

2.2. Submicron emulsion

Two types of sCT powdered SME's were prepared by Pharmos, Rehovot, Israel, as described elsewhere (Ilan et al., 1996): (a) without Carbopol®940 (denoted as sCT–SME) and (b) with Carbopol®940 (denoted as sCT–MA–SME). The concentration of Carbopol®940 in the MA–SME was 0.1% w/w and sCT activity in the SME or MA–SME formulations was 40 IU/ml (1 IU = 160 ng of sCT) unless otherwise stated. In addi-

tion, three types of indomethacin (I) containing SMEs were prepared for adhesion studies: the first did not contain Carbopol®940. The other two (MA–SMEs) contained 0.02 or 0.1% w/w Carbopol®940. In all cases I concentration was 0.4% w/w. A typical SME composition prior to lyophylization included a 5% w/w oily phases which was composed of medium chain, capric/caprylic, triglyceride oil (Miglyol®812, Huls, Germany), purified egg phosphatidylcholine (Lipoid®E-80, Lipoid, Germany) and α-tocopherol acid succinate. The aqueous phases contained Tween 80 (Emulgin®SMO-20, Henkel, Germany), disodium edetate USP, anhydrous glycerol USP (Merck, Germany) and sCT or I. The typical mean droplet size of the reconstituted SMEs was 110 ± 50 nm as analyzed by Coulter N4MD (Coulter Electronics, UK) (Fig. 1). The emulsion viscosity after reconstitution was 1.7 ± 0.2 cps (Brookfield, LVDV, Stoughton, MA) and the pH of the reconstituted preparations was 4.5.

2.3. sCT in vitro diffusion studies

The diffusion rate of sCT through the intestinal epithelium of the rat was assessed in side-by-side diffusion cells (Grass and Sweetana, 1988). Mucosal preparations from the jejunum and the colon were made by trimming the serosa and muscle layers as close as possible to the mucosa

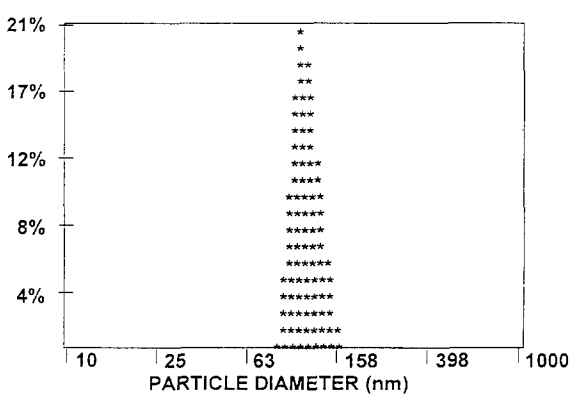

Fig. 1. Typical size distribution (% in nm) of the sCT–MA–SME droplets as analyzed by photon correlation spectroscopy analyzer.

to form 30 mm by 15 mm slices. The mucosal sheets were rinsed with Krebs buffer pH 7.4 and mounted in the cells. The study was performed at 37°C, while bubbling a mixture of O_2: CO_2 at a ratio of 95:5. In separate experiments 100 μl of sCT containing SME, MA–SME or sCT in normal saline, each containing 1 IU/μl of sCT, were placed in the mucosal compartment (volume: 8.5 ml) of the diffusion cell. The serosal compartment (same volume) was sampled (500 μl) at 5, 30, 60 and 120 min. 500 μl of fresh Krebs buffer were used to replenish the withdrawn samples that were kept at −20°C until sCT analysis.

2.4. In situ absorption study of sCT in the rat

2.4.1. Animals and anesthesia

After an overnight fast, male Sabra (Lutsky et al., 1984) rats (200–250 g) were anesthetized by an intra-peritoneal injection of Equitensine solution (equivalent to 6 mg sodium pentobarbitone/100 g rat body weight). At the end of each study the anesthetized rats were sacrificed by chest wall puncturing. All animal studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication #85–23, revised 1985). Protocols were reviewed by the mutual committee of Hadassah and the Faculty of Medicine for Animal Welfare.

2.4.2. Intravenous administration of sCT

The right vena cava of six anesthetized male Sabra rats (200–250 g) was exposed and cannulated with 0.98 mm O.D. silastic tubing. A solution of sCT in normal saline at a dose of 1 IU/kg body weight was administered intravenously followed by a 0.5 ml of normal saline rinse of the silastic catheter. At 0 (2 min before administration), 5, 15, 30, 60 and 120 min 500 μl samples of blood were withdrawn through the silastic catheter into heparinized test tubes kept at 0°C (crushed ice). Clotting was avoided by the addition of 100 μl of 50 U/ml heparin aqueous solution to each test tube. The blood samples were centrifuged and the plasma separated and kept at −20°C until sCT analysis.

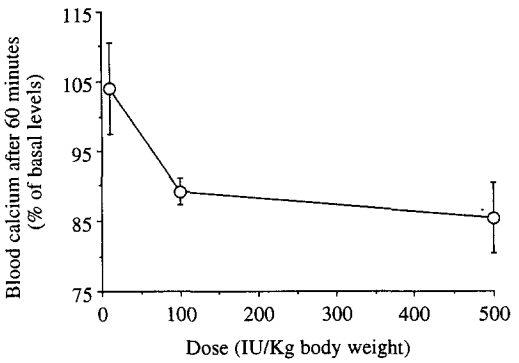

Fig. 2. Dose-response (reduction in blood Ca^{2+} levels as measured 60 min after the administration) curve of sCT after IC administration of 10, 100 and 500 IU/kg rat body weight of sCT in normal saline into six rats.

2.4.3. Intracolonic (IC) administration of sCT

The colon of anesthetized rats was exposed through a midline incision. Two mm horizontal cuts were made at two ends of the exposed colon to allow saline rinse. The portal vein of the rats was cannulated with silastic tubing. The rinsed colon was then ligated at two ends to form a 7 cm closed pouch without removing the organ from the anesthetized animal and without impairing the blood flow to the ligated segment. In separate experiments 50 μl of each formulation, SME (n = 13 rats) or MA–SME (n = 18 rats), containing a dose of sCT equivalent to 10 IU/kg rat body weight after reconstitution in distilled water, or aqueous solution of sCT (n = 6 rats), or sCT in saline containing 0.1% w/w Carbopol®940 (n = 6 rats) of the same dose were injected into the ligated colon using a 25 G hypodermic needle. The sCT dose was selected as the minimal dose causing a hypocalcemic response over 60 min after preliminary experiments in which 10, 100 and 500 IU/kg of sCT in saline was administered IC to six rats each (Fig. 2). After the IC administration of the three formulations portal blood samples (500 ml) were withdrawn through the polyethylene catheter at 5, 15, 30, 60, 120 and 180 min. Additional two samples, at 240 and 300 min, were taken for the Ca^{2+} analysis of the two sCT formulations of SME and MA–SME. All blood samples were collected (separately) in heparinized test tubes kept at 0°C (crushed ice). The plasma was separated and collected into heparinized test tubes which were stored at −20°C until sCT analysis.

2.5. Adhesion studies

2.5.1. Everted sack studies with I-containing SME and MA–SME

Jejunum or colon segments (5 cm length) were separated from the anesthetized rats and everted sacks were prepared and secured with a 3/0 silk suture at two ends. The jejunal everted sacks were soaked for 1, 10 or 30 min in I-containing SME or I-containing MA–SME. The latter contained 0.02% or 0.1% w/w of Carbopol®940. The colonic everted sacks were soaked for 1 and 10 min in the same formulations. After soaking the everted sacks were rinsed with normal saline and rapidly transferred into a glass tissue homogenizer containing 5 ml methanol. The contents of the test tube were homogenized manually for 2 min, centrifuged (3000 rpm for 5 min), and the supernatant liquid was separated and kept at −20°C for I analysis.

2.6. sCT assays

sCT determination was performed by radioimmunoassay (DSL 1300 Kit, Diagnostic Systems Laboratories, TX). The plasma or Krebs buffer samples were suitably diluted before analysis. All assays were performed in duplicate using a standard curve for each assay at a concentration range of 0.1–50 ng/ml of sCT.

2.7. Calcium assay

Plasma calcium levels were determined by atomic absorption spectroscopy (Varian Spectra AA 300 equipped with Zeeman Atomic Absorption Spectrometer).

2.8. Indomethacin assay

Samples (200 μl) were acidified with 200 μl of 0.066 M phosphate buffer pH 5 and the mixtures were vortexed for 1 min. Methanol (100 μl) containing 0.1 mg/ml flufenamic acid as an internal

standard were added to each mixture. The mixture was vortexed for 1 min, 5 ml of diethyl ether were added, followed by additional vortexing (2 min) and centrifugation of 5 min (3400 rpm). The organic phase was separated, evaporated and the residue was re-dissolved in the mobile phase (45:55 acetonitril and phosphate buffer, 0.01 M, pH 7.5). 20 μl of the solution were injected into the HPLC system (Hewlett Packard 1050 pumping system, Jasco 875 Intelligent UV/Vis detector, and Hewlett Packard 3365 Chemstation data analyzer). The wavelength was 280 nm, the column was 5 microns, 250 × 4.6 mm RP-18 (LichroCart 250-4, E. Merck, Germany), the mobile phase flow rate was 1.2 ml/min, the R.T. for indomethacin was 4.0 min and the R.T. for the flufenamic acid was 3.3 min.

2.9. Data analysis

The relative pharmacological activity (RPA) of the sCT formulations was calculated after measuring the area *above* the plasma levels versus time curves obtained after IC administration of sCT formulations using the following equation:

$$RPA = [AAC_{SME}/AAC_{AS}]$$

where AAC_{SME} is the area above the plasma calcium versus time curve resulting from SME formulations and AAC_{AS} is the area above the plasma calcium versus time curve resulting from sCT aqueous solution.

The pharmacokinetic parameters derived from the plasma sCT levels versus time curves, such as the area *under* the curve (AUC) were calculated by a computerized program (PC-Nonline program, Version 4.1, SCI Software, Clintrials, USA).

2.10. Statistical analysis

A Kruskal–Wallis test was performed to check whether the various groups of rats were from different populations. A difference was considered to be statistically significant when the p value was less than 0.05. When the difference between the groups was validated, a Mann–Whitney U test was used to analyze the significance of the differences between the obtained data ($p < 0.05$).

Table 1
Diffusion rate constants of sCT through mucosal epithelium of the rat intestine as measured in vitro in side-by-side diffusion cells in two different studies: (a) mucosal tissue of the rat jejunum; (b) mucosal tissue of the rat colon was mounted in the cell. Shown are the mean data of two measurements. Individual values are detailed in parenthesis.

Formulation	Jejunum (ng/ml)	Colon (ng/ml)
Normal saline	**0.04** (0.032; 0.054)	**0.05** (0.02; 0.08)
sCT in MA–SME with 0.1% w/w Carbopol®940	**0.03** (0.030; 0.032)	**0.17** (0.11; 0.24)
sCT in normal saline with 0.1% w/w Carbopol®940	—	**0.01** (0.006; 0.017)

3. Results

The diffusion rate constants of sCT in different formulations, as measured in side-by-side diffusion cells, are summarized in Table 1. Similar mean values were measured for sCT diffusion in the jejunum and the colon in normal saline. The diffusion rate of sCT in the case of MA–SME was 5.6-fold higher when tested with colonic epithelium than jejunal epithelium and 3.3-fold higher than the diffusion rate in the colon when tested in normal saline.

sCT plasma profile after intravenous administration is shown in Fig. 3 and the derived pharmacokinetic parameters are summarized in Table 2.

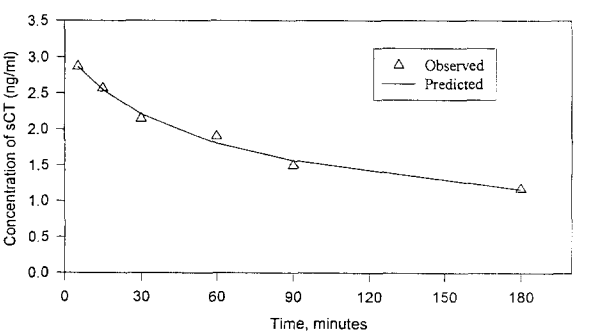

Fig. 3. Mean plasma sCT levels after intravenous administration of 1 IU/kg rat body weight of sCT in normal saline to 3 rats. (O): measured data, (-) fitted data as calculated from the PC-nonline program.

Table 2
Pharmacokinetic parameters of sCT in the rat as calculated after intravenous administration of 1 U/kg body weight of sCT (Fig. 3).

Pharmacokinetic parameter	Measured value
$T_{1/2}$ (min)	102 ± 14
CL (ml/min)	0.07 ± 0.01
MRT (min)	147 ± 20
V_{ss} (ml)	11.2 ± 0.7
AUC (ng/ml per min)	438 ± 45

These parameters, especially the AUC one, were required for the absolute bioavailability (F%) calculations as summarized in Table 4.

The reduction in plasma calcium concentrations in the anaesthetized rat after IC administration of 10 IU/kg rat body weight of sCT are shown in Fig. 4 and the RPA values that were calculated from the curves of Fig. 4 are summarized in Table 3 which shows that the administration of sCT in

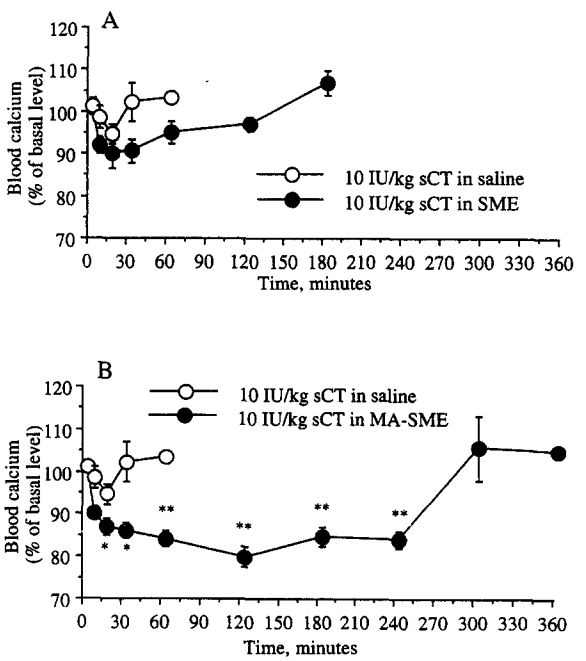

Fig. 4. Reduction in plasma Ca^{2+} levels in anesthetized rats after IC administration of 10 IU/kg rat body weight of sCT in the following formulations: A: sCT in SME versus sCT in normal saline; B: sCT in MA–SME (0.1% w/w Carbopol®940) versus sCT in normal saline.

Table 3
The relative pharmacological activity (RPA) of sCT after its IC administration in SME or MA–SME formulations. The dose was 10 IU/kg rat body weight and the PA was calculated by comparing the area above the plasma Ca^{2+} curves (AAC) over 300 min of the two formulations to the AAC obtained after the administration of the same concentration of sCT in normal saline ($p < 0.05$ compared to saline).

sCT formulation	AAC (0–300 min)	RPA
SME	972 ± 148	4.3
MA–SME	3625 ± 332	15.9

MA–SME was 3.7-fold more effective as compared to its administration in SME.

The plasma sCT levels after IC administration of 10 IU/kg rat body weight of sCT in normal saline, normal saline with 0.1% w/w of Carbopol®940 and MA–SME (0.1% w/w Carbopol®940) are shown in Fig. 5. While normal saline with and without Carbopol®940 yielded C_{max} values of 800 and 600 pg/ml (respectively) at T_{max} of 10 min, the C_{max} plasma levels following MA–SME administration was 4300 pg/ml at T_{max} of 20 min. The AUC values are summarized in Table 4 and so are the calculated absolute bioavailability (F%) and the relative bioavailability (F*, compared to IC administration of sCT) values.

The adhesion properties of three types of I-containing SME, without or with two different con-

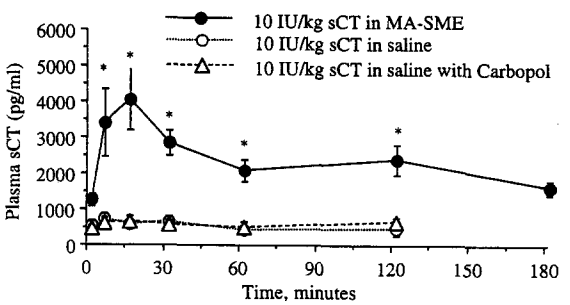

Fig. 5. Plasma sCT levels after IC administration of 10 IU/kg rat body weight of sCT in (a) normal saline, (b) normal saline with 0.1% w/w of Carbopol®940, (c) MA–SME (0.1% w/w Carbopol®940) to 6 anesthetized rats. Shown are the mean values ± SE (*$p < 0.05$ compared to the sCT level at 15 min). Shown are the mean results from 10 rats ± SE (*$p < 0.05$, **$p < 0.01$ compared to the calcium level at 15 min).

Table 4
sCT bioavailability in the rat after IC administration of three formulations, each containing 10 IU/kg rat body weight of sCT.

Formulation	AUC (ng/ml per min)	F (%)	F*
Normal saline	82.5 ± 38	1.9	1
Saline with 0.1% Carbopol®940	71.5 ± 66	1.7	0.9
MA–SME	629 ± 290	14.7**	7.7**

* Relative to the same dose of sCT administered in normal saline.
** $p < 0.01$ compared to saline.

centrations of Carbopol®940, to the epithelium of everted sacks taken from the rat jejunum or colon of the rat are shown in Fig. 6. In all cases adhesion occurred rapidly (starting at 1 min after soaking the everted sack in the sCT preparation) and adherence was larger when MA–SME was used, although no significant differences in the tissue I concentrations could be demonstrated between the two types of the MA–SME.

4. Discussion

In this study we examined the possibility of increasing the bioavailability of sCT after oral administration by incorporating the drug into a new type of powdered submicron emulsion (SME). We also checked if the incorporation of the loosely crosslinked acrylic acid product Carbopol®940 into the SME to form MA–SME could improve sCT absorption even more. The working hypothesis was that, by being located at the surface of the emulsion droplets (Ilan et al., 1996), Carbopol®940 would serve as an adhesive layer that would cause the emulsion droplets to stick to the intestinal mucosa for prolonged periods of time. Improved drug absorption would result from the intimate contact between the emulsion droplets and the tissue.

This assumption was first tested in side-by-side diffusion cells in which epithelial sheets from the jejunum or colon of the rat were mounted. It was found that sCT permeated the jejunal and colonic mucosa of the rat at similar rates (Table 1). However, when sCT was introduced in MA–SME (containing 0.1% w/w of Carbopol®940) a profound enhancement (3.3-fold increase in the diffusion rate constant) in the diffusion rate was observed in the colon, but not in the jejunal epithelium. The addition of Carbopol®940 to sCT in normal saline (not in SME) did not affect the diffusion rate of sCT. It is noteworthy that MA–SME increased the diffusion rate of sCT through the colonic mucosa of the rat, but did not affect its diffusion through the jejunal epithelium, an observation that may indicate that in the rat colon most of the sCT uptake is via diffusion and not a result of active transport of any kind. Similarly, differences in the absorption rates of the peptide drug captopril were observed in the jejunum and the colon of the rat (Sintov et al.,

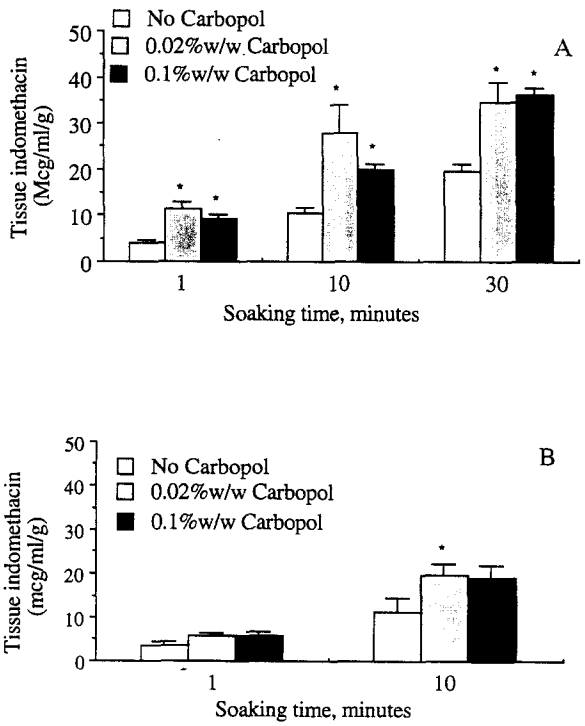

Fig. 6. Adherence of indomethacin SME and two types of indomethacin MA–SMEs (containing 0.02% w/w Carbopol®940 or 0.1% w/w Carbopol®940) to everted sacs of rat jejunum (A) and rat colon (B) as assessed from epithelial indomethacin levels after soaking for 1, 10 and 30 min (for jejunum) or 1 and 10 min (for colon). Shown are mean results of six rats ± SD (*$p < 0.05$ compared to tissue indomethacin after soaking in indomethacin SME).

1996; Hu and Amidon, 1988). These studies suggest that, in the colon, the absorption of proteinaceous drugs, such as sCT, results primarily from diffusion and may therefore be more profoundly increased by absorption promoters (Muranishi, 1990; Sintov et al., 1996).

The reduction in plasma calcium concentrations in the anesthetized rat after IC administration of 10 IU/kg rat body weight of sCT (Fig. 4) shows that in both cases the use of SME yielded a significant reduction in plasma Ca^{2+} levels as compared to administration of sCT in normal saline. However, in the case of MA–SME, the reduction was more profound (80% of Ca^{2+} of basal level at 120 min, as compared to 90% of Ca^{2+} basal level at 15 min for MA–SME and SME, respectively) and was prolonged (1.5-fold) in the case of the MA–SME. RPA values that were calculated from the curves of Fig. 4 are summarized in Table 3. The relative pharmacological activity (RPA) of sCT (as measured by the ability to lower blood Ca^{2+}) after IC administration of sCT to the anesthetized rat was improved by SME (RPA = 4.3) and improved even more by MA–SME (RPA = 15.9). The difference in the RPA values after the administration of sCT in either SME or MA–SME (3.7-fold difference) was supported by sCT pharmacokinetic profiles (Fig. 5) and the derived bioavailability values summarized in Table 4. MA–SME caused a profound increase (14.7%) in the absolute bioavailability (F) of sCT following IC administration in the rat. A similar increase (F value of 13%) in colonic calcitonin absorption was obtained by Baudys et al. (1996) who formulated human calcitonin into emulsions with absorption enhancers such as sodium dodecyl sulfate. The two investigators also concluded that the colon of the rat is the appropriate site of absorption when oral delivery of calcitonin is required. The F^* value of 7.7 for MA–SME verified the RPA value of 15.9 obtained in our study. Again, the IC administration of sCT together with Carbopol®940 without formulating it into SME yielded negligible F value (1.7% compared to 1.9% after IC administration in normal saline).

The last section of the study examined, with a different drug probe-indomethacin, the adhesion properties of the MA–SME to the epithelium of everted sacks taken from the rat jejunum or colon (Fig. 6). In all cases adhesion occurred rapidly (starting at 1 min after soaking the everted sack in the sCT preparation) and the number of emulsion droplets adhering (assessed by measuring the tissue I concentrations) was larger when MA–SME was used, although no significant differences in the tissue I concentrations could be demonstrated between the two types of the MA–SME. Fig. 6 demonstrates that emulsion adherence occurred in both colonic and jejunal epithelium of the rat. However, the absorption enhancement was observed in the colon only. Therefore, it would be an over-simplification to relate the increase in sCT absorption to a mucoadhesion phenomenon only. A more likely explanation would be absorption–enhancement coupled with protease–inhibition properties of Carbopol®940, as has been indicated already by Lehr et al. (1992) and Luessen et al. (1994, 1995). It is speculated that sCT was stabilized by the SME formulation without interacting with the phosphatidylcholine fraction (Epand et al., 1983). Therefore, the close encounter between stabilized sCT in the SME formulations and the intestinal mucosa enabled the increase in its bioavailability. Without adherence (the control studies with Carbopol®940 in normal saline) no effect was observed. Since it is expected that Carbopol®940 would have served as both absorption enhancer and protease inhibitor, even when not included in the formulation, it is reasonable to relate this lack of activity to its low concentration (less than 0.5% w/w) in the normal saline studies.

It has been shown previously by Antonin et al. (1992) that calcitonin can be absorbed from the human transverse colon. If not affected by mucus turnover (Rubinstein and Tirosh, 1994), MA–SME in a proper colonic delivery system may be a reasonable formulative solution for the increase of sCT bioavailability after oral administration.

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