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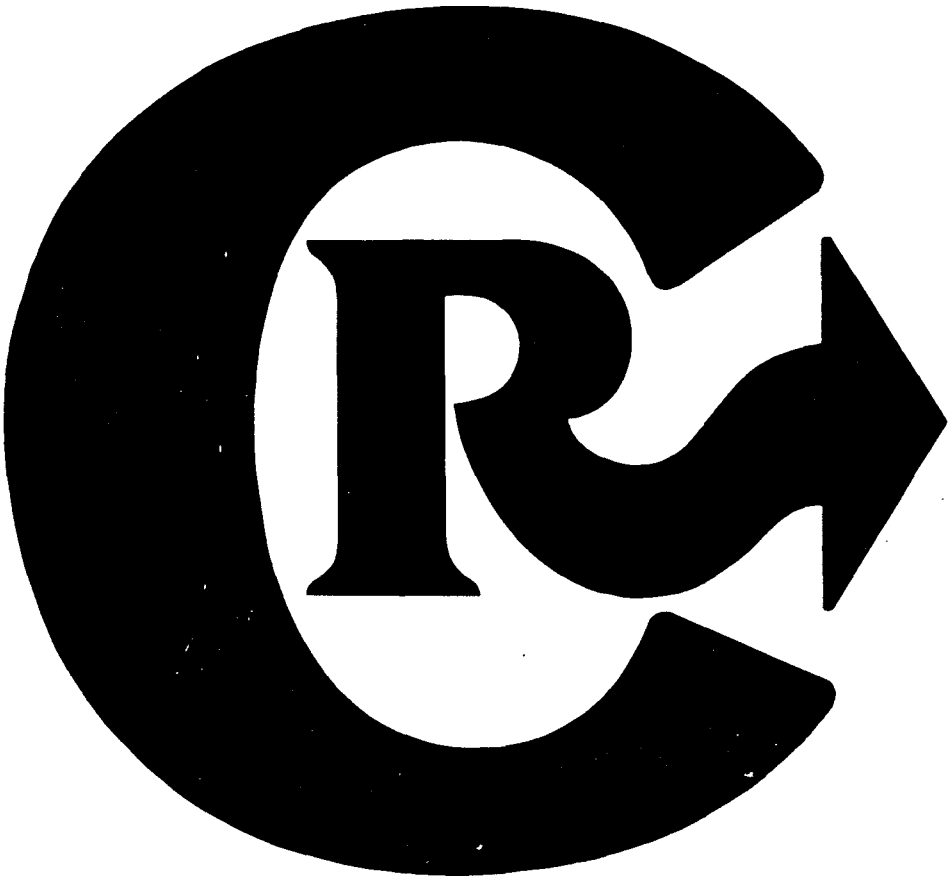

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the salting-out method, thereby avoiding the use of toxic chlorinated solvents. The use of PEO decreased the nanosphere size and might influence the degradation and drug delivery characteristics as well.

The size of the PDLLA nanospheres in PBS remained constant over 50 days, whereas the size of the PLG nanospheres decreased rapidly in time, indicating fast degradation. This suggests that by copolymerization the degradation time of the nanospheres and therefore the drug release profile could be controlled. Currently, the effect of the degradation time on the polymer molecular weight as well as the effect of PEO on the polymer degradation is under investigation.

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IV. Oral and Parenteral Delivery

EVALUATION OF ORAL DELIVERY OF SALMON CALCITONIN USING W/O/W MULTIPLE EMULSION FORMULATIONS

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Introduction

Salmon Calcitonin (sCT) is a cyclic ammo acid peptide that is synthesized and stored by the parafoUicular cells of thyroid, parathyroid and thymus glands. It is a macromolecular peptide drug having a molecular weight of -3600 Daltons and is more potent than the other calcitonin species. sCT is therapeutically effective for the management of bone diseases thus primarily used for the treatment hypercalcemia, postmenopausal osteoporosis and Paget's disease [1,2].

Generally peptides and proteins, such as insulin and calcitonin are administered parenterally due to their proteolytic degradation and limited absorption in the gastrointestmal (GI) tract. Since injections are poorly accepted by the patients, alternative routes are under investigation in formulation development studies Various approaches such as absorption enhancers, protease inhibitors and chemical modification have been examined to overcome the delivery problems of the peptides and proteins via the GI tract. Oral delivery systems for the peptide protein drugs are extensively investigated in recent literature [3-5].

The bioavailability ofsCT after oral administration is limited by enzymatic degradation in the GI tract. W/O/W (Water-in-Oil-in-Water) multiple emulsion systems seem to be promising as they enable to use two different hydrophilic active materials in different aqueous phases. They can be considered controlled release delivery systems and are used to protect encapsulated substances. The advantages of multiple emulsions as carriers of drugs for oral administration include protection against enzymatic environment and enhanced absorption through the intestinal membranes. The use of protease inhibitors has been shown to improve both the small and large intestinal absorption of peptides [2,6]. Previously in our laboratories the effect ofaprotinin on the intestinal absorption of insulin using a W/O/W multiple emulsion type of formulation was investigated and the hypoglycemic effect was observed after oral administration [6].

Table 1
W'O/W multiple emulsion formulation of sCT

W/O Primary emulsion	
Liquid paraffine	32.0 g
Lipophylic **surfactant** (Arlacel 1689)	4.4 g
Lipophylic **cosurfactant** (Atlas **SCS** 2054)	4.4 g
Cholesterol	0.8 g
Sodium Chloride	0.4 g
Salmon Calcitonin	50000 IU
Distilled water q.s.	100 g
W/O/W Multiple Emulsion	
Primary emulsion	80 g
Hydrophylic surfactant (8% Synperonic PE/F 127)	**20 g**
Aprotinin	**3300 KIU**

The objective of this present study was to evaluate the effectiveness of W/O/W multiple emulsion fomiulations of sCT when administered orally to Sprague Dawley rats by measuring the difference in serum calcium levels.

An in vivo rat model was used to evaluate the hypocalcemic effects of the formulations for comparison with each other. Formulations were grouped as I (W/O/W emulsion base), II (W/O/W emulsion containing sCT in the inner phase). III (W/O/W emulsion containing sCT in the inner phase and aprotinin in the outer phase) and IV [i.m. sCT solutions (Miacalcic®) and W/O/W emulsion base]. Three of the sCT formulations tested have shown a better hypocalcemic profile than the emulsion base control.

Experimental Methods

Synthetic sCT was donated by Novartis, Turkey; Aprotinin (Tracylol®, 500000 KIU) was donated by Bayer AG, Germany:Ariacel 1689, Atlas SCS 2054 and Synperonic PE/F 127 were kindly donated by ICI surfactants, London. Twenty four female Sprague Dawley rats, weighing about 180-220 g, were provided by Hacettepe University, animal farm.

After the formulation development study of sCT and in vitro evaluation, the formulation displayed in Table 1, was selected for in vivo trials.

Animal Studies

Four groups of rats* were housed for a period of four weeks in proper conditions and on diet. Animals were fed under ether anesthesia every other day and formulations were applied orally by using a special intragastric drill. 0.5 ml of blood was drawn from the heart of the animals with a syringe. Blood samples were collected before starting each experiment and then every week throughout the experiment. The samples were centrifaged and levels of calcium were determined each week by UV spectrophotometry at 520 nm using the method of the Ependorf Photometer [7-8].

Results and discussion

Serum calcium levels of the rats versus time are presented in Fig.1. Differences between the formulations were evaluated and statistically evaluated (Table 2).

Serum calcium levels were compared in general and on a weekly basis with a two way analysis of variance for repeated measures on one factor using the SPSS 9.0 statistical program. In case the ANOVA indicated the interaction term to be significant, the difference between consecutive time groups (1st week- initial, 2nd week-1st week and 3rd week-2nd week) was calculated and compared with one way analysis of variance for four groups. In case the difference between groups for any of the time group was significant, the Tukey HSD test was used for pairwise comparison.

According to the two way analysis of variance for repeated measures:

• The difference between four groups (independent from time) was significant (F:3.46, p: 0.036),

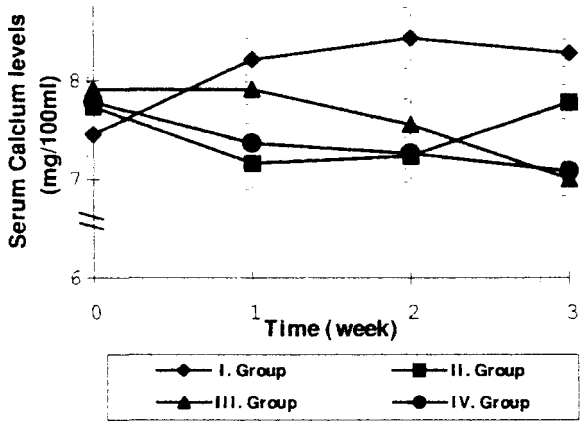

Fig. 1. Serum calcium levels measured in four different rat groups which were administered four different sCT formulations.

*In vivo experiments were performed according to the 99/27 numbered Ethic Committee decision of Hacettepe University.

Table 2
Descriptive statistics related to serum calcium levels up to groups and time

Group	Week (serum Ca levels)				
		Initial	1st week	2nd week	3rd week
	x	7.45	8.22	8.43	8.28
I.	sd	0.56	0.55	0.77	0.35
	se	0.22	0.22	0.31	0.14
	x	7.73	7.17	7.23	7.78
II.	sd	0.30	0.38	0.64	0.44
	se	0.12	0.15	0.26	0.17
	x	7.92	7.92	7.55	7.00
III.	sd	0.37	0.50	0.41	0.24
	se	0.15	0.20	0.16	0.09
	x	7.78	7.37	7.27	7.08
IV.	sd	0.63	0.67	0.65	0.44
	se	0.25	0.27	0.26	0.17

se: Standard error sd: standard deviation x: mean.

• The changes in time (independent from groups) were not significant (F:1.24, p:0.302),
• The interaction of time and groups was found significant, in other words, changes in time were different in groups (F:9.64, p: 0.000).

One way analysis of variance results for three consecutive time results were F:11.9, p: 0.000; F:4.042, p:0.021; F:3.43, p:0.037 for 1st week-initial, 2nd week-1th week and 3rd week-2nd week sequentially. In other words, three consecutive time results have shown significant difference according to the formulation groups. Up to Tukey USD test results Group I was found to be different from the other three groups and a significant difference was calculated between the group II (including aprotmin *in* sCT formulation) and group IU (not including aprotinin in sCT formulation).Also when the emulsion formulation III which contained aprotinin in the outer, and sCT in the inner phase of the multiple emulsion was compared with the commercial product of sCT, a decrease in serum calcium levels of animals with formulation III was detected for three weeks throughout the experiment.

As a result, following the administration of three sCT containing multiple emulsion formulations it was observed that they all displayed a significant hypocalcemic effect (profile) when compared to the base emulsion (Fig. 1).

Conclusions

It is observed that incorporation of the peptidc sCT in the inner aqueous phase and protease inhibitor in the outer aqueous phase of W/O/W emulsion, protects the peptide from the environmental proteolytic conditions. These results are supported by in vivo data of this study, giving hypocalcemic results similar to commercial formulations. Therefore W/O/W type emulsion formulations are suggested as promising carrier systems for oral administration of peptide-protein drugs.

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