METABOLISM OF PEPTIDE DRUGS BY THE MICROORGANISMS IN RAT CECAL CONTENTS

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The metabolism of insulin and calcitonin by microorganisms was examined in rat cecal contents. Both insulin and calcitonin were markedly degraded. Calcitonin was more susceptible to proteolysis in rat cecal contents than insulin. Calcitonin was also rapidly degraded in supernatant, while we found few degradation products of insulin. These findings suggest that care should be taken to metabolize the peptide drugs by microorganisms when they are administered to the large intestine for colon-specific drug delivery.

KEY WORDS insulin; calcitonin; microorganism; colon-specific drug delivery

It is well known that the activities of various proteases which are responsible for peptide degradation in the large intestine are generally lower than in the small intestine.[1-4] Therefore, many studies have investigated the delivery of peptide and protein drugs to the colon.[8-11]

On the other hand, various microorganisms are distributed throughout the gastrointestinal tract, and most of these are found in the large intestine where they mediate hydrolytic digestive functions using carbohydrate and proteins as substrates. In addition, these microorganisms have the potential to metabolize drugs and other foreign compounds including peptide drugs. However, there have been very few studies concerning the metabolism of peptides in the large intestine by these microorganisms.[7] Therefore, in this study, insulin and calcitonin were chosen as model peptides, and the metabolic characteristics of these peptides by the microorganisms were examined in rat cecal contents.

MATERIALS AND METHODS

Preparation of Drug Solutions

Bovine insulin (Sigma, Chemical Co., St. Louis, MO, USA) and human calcitonin (SUNTORY, Osaka, Japan) were dissolved in isotonic phosphate buffer at pH 7.4 to yield the final concentration of 0.1 mM.

Preparation of Cecal Contents Suspension and Its Supernatant

Fresh cecal contents from non-fasted rats were suspended in two-fold their volume of bicarbonate buffer ($NaHCO_3$, 9.240 g ; $Na_2HPO_4 \cdot 12H_2O$, 7.125 g ; NaCl, 0.470 g ; KCl, 0.450 g ; $CaCl_2 \cdot 2H_2O$, 0.073 g ; $MgCl_2 \cdot 6H_2O$, 0.087 g/l). The pH of the buffer was adjusted to 7.0 by bubbling with CO_2 gas prior to use. The suspension was filtered through four layers of gauze. Supernatant was obtained by centrifuging the cecal suspension at 3,000 rpm for 5 min.[7]

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In Vitro Stability Experiments

The degradation of insulin and human calcitonin in 33% suspension and supernatant of rat cecal contents was studied by incubating 0.1 mM insulin or calcitonin solution with 33% suspension or supernatant of rat cecal contents. Samples were withdrawn from the incubation mixture, and 50% acetic acid was added to terminate the reaction. Then the resulting mixture was centrifuged for 5 min to remove the precipitated protein and cecal contents. Twenty-five microliters of the supernatant were injected into HPLC.[7]

RESULTS AND DISCUSSION

The concentration-time profiles for the degradation of insulin and calcitonin from the rat cecal contents are shown in Fig. 1. Both insulin and calcitonin were metabolized in 33% suspension of rat cecal contents, but the degradation of calcitonin was much faster than that of insulin.

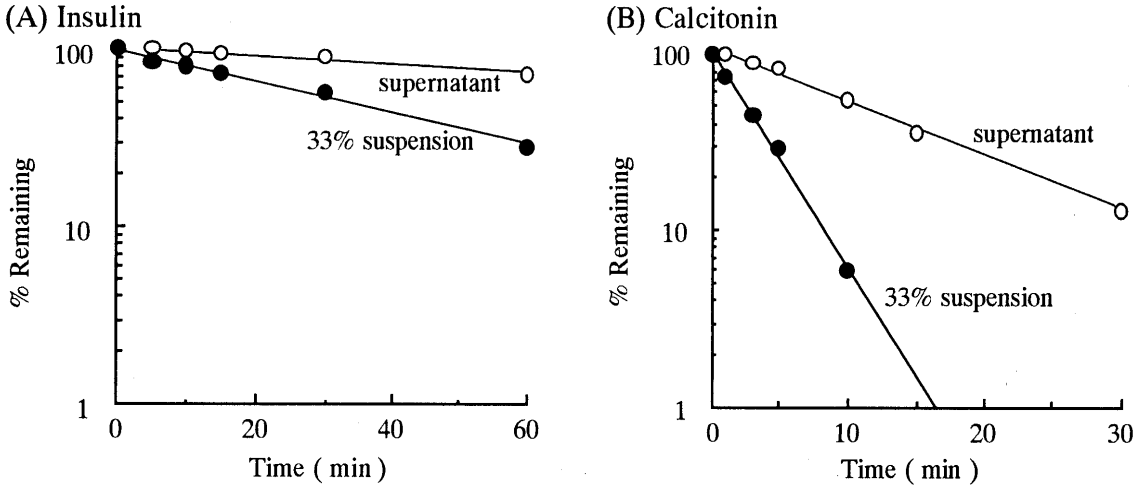

Fig. 1. Degradation Profiles of (A) Insulin and (B) Calcitonin in Rat Cecal Contents Results are expressed as the mean ± S.D. of 3 experiments. Keys: ● , 33% suspension of rat cecal contents; ○ , supernatant of 33% suspension of rat cecal contents.

Table 1 shows the degradation half-lives of insulin and calcitonin in 33% suspension of rat cecal contents. The half-life for the proteolysis of calcitonin in 33% suspension of rat cecal contents was 13-fold smaller than that of insulin. These findings suggested that insulin and calcitonin might be degraded by some peptidases of microorganisms in rat cecal contents as well as proteolytic enzymes in the intestinal homogenates and intestinal fluid of rats.[3-6] Furthermore, it was suggested that microorganisms in rat cecal contents might contain some proteolytic enzymes which are responsible for calcitonin hydrolysis rather than insulin degradation.

Table 1. Degradation Half-Lives of 0.1 mM Insulin and Calcitonin in Rat Cecal Contents

	Insulin (min)	Calcitonin (min)
33% suspension	33.6 ± 5.8	2.5 ± 0.3
Supernatant	125.9 ± 3.4	9.8 ± 0.4

Results are expressed as the mean ± S.D. of 3 experiments.

We also examined the metabolism of these peptides in supernatant of 33% suspension of rat cecal contents. As shown in Fig. 1, calcitonin was rapidly degraded in supernatant of rat cecal contents: less than 20% of calcitonin remained after 30 min. In contrast, insulin was almost stable in such supernatant of rat cecal contents. Table 1 shows the degradation half-lives of insulin and calcitonin in supernatant of 33% suspension of rat cecal contents. The half-life for the proteolysis of calcitonin in this supernatant was 13-fold smaller than that of insulin. This finding indicated that calcitonin was metabolized by both enzymes released from the microorganisms and their membrane enzymes in rat cecal contents. On the other hand, insulin was mainly metabolized by the membrane enzymes of the microorganisms.

We have not determined the proteolytic enzyme types of these microorganisms in rat cecal contents. However, in our pilot studies, it may be considered that there exist aminopeptidases, trypsin, and chymotrypsin-like peptidases in the microorganisms in rat cecal contents, since the degradation of insulin was inhibited by camostat mesilate, aprotinin, and soybean trypsin inhibitor in rat cecal contents. We are now determining the types of proteolytic enzymes and the effects of various protease inhibitors on the metabolism of these peptides in rat cecal contents, and these results will be reported in a subsequent paper.

In conclusion, the present study indicated that peptide drugs such as insulin and calcitonin were degraded by the microorganisms in rat cecal contents. The metabolism of peptide drugs by the microorganism in the large intestine should be taken into account, when colon-specific peptide drug delivery systems are designed.

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