# EFFECT OF PEPSTATIN ON ACID PROTEASES

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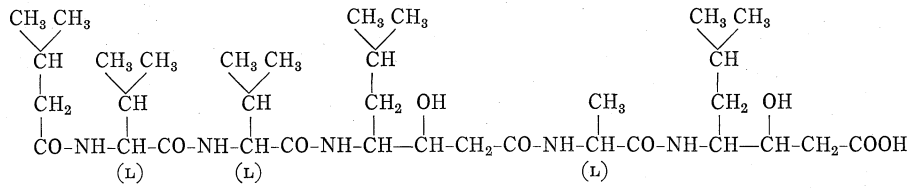
(Received for publication July 10, 1971)

Pepstatin is shown to be a specific inhibitor of acid proteases. It also inhibits human gastricsin, but the effect is weaker than against human pepsin. The content of pepsin and gastricsin in gastric juice of stomach ulcer patients is described and the pepstatin which remains in gastric juice collected at 60 minutes after its administration is also determined. These results and those obtained by the direct measurements of the peptic activity of the gastric juice indicate that a sufficient amount of pepstatin to inhibit pepsin remains in the gastric juice 60 minutes after oral administration. The peptic activity of gastric juice is 0~9.8 % of the activity of the gastric juice collected before the administration of pepstatin. A method of testing for peptic activity of gastric juice containing pepstatin is described. Free and bound sialic acid in gastric juice of pylorus-ligated rats treated with pepstatin is determined, and pepstatin was shown to inhibit release of bound sialic acid from the mucous membrane. The activity of pepstatin derivatives are described.

As reported in a previous paper[1], pepstatin obtained from cultured broth of actinomycetes was shown to be a specific inhibitor of acid proteases. It did not inhibit trypsin, chymotrypsin, plasmin, kallikrein, thrombin, thrombokinase and papain, but showed a strong inhibition against pepsin and a strong protective effect against stomach ulcer of pyrolus-ligated rats. It has a low toxicity to mice, rats, rabbits, dogs and monkeys, and the effect on stomach ulcer has been studied clinically. The structure of pepstatin reported in another previous paper[2] is as follows:



In this paper, the effects of pepstatin on acid proteases are reported in detail.

## Materials and Methods

Enzymes: Porcine pepsin (3,200 units/mg) was purchased from Sigma Chemical Co., U. S. A. Acid proteases, Type A and Type B, obtained from *Aspergillus niger* var. *macrosporus* was purchased from Seikagaku Kogyo Co., Ltd., Tokyo. Acid protease of *Trametes sanguinea* was obtained from Takeda Chemical Industries Ltd., Osaka. Acid protease of *Aspergillus saitoi* was obtained from Fujisawa Pharmaceutical Co., Osaka. Acid protease of *Xylaria* sp. was prepared and purified using a slight modification of the method described by KOAZE[3]. These enzymes were dissolved and diluted in 0.001 N HCl

immediately before use. Human pepsin and gastricsin were prepared by the method described by RICHMOND[4,5]. Human gastric juice was supplied by Dr. KASUGAI and Dr. ITO, Aichi Cancer Center, Nagoya.

Substrates: Casein was purified according to the procedure described by NORMAN[6] and a 0.6 % casein solution in 0.75 % lactic acid was employed as a substrate. Bovine hemoglobin was purchased from Nutritional Biochemical Co., U. S. A., and a 0.5 % hemoglobin solution in 0.03 N HCl was used for the experiment. N-Acetyl-L-phenylalanyl-L-diiodotyrosine (APDT) was purchased from Sigma Chemical Co., U. S. A., and acetyl-L-phenylalanyl-L-tyrosine (APT) was obtained from the Protein Research Foundation in the Institute for Protein Research, University of Osaka.

Inhibitors: Pepstatin was prepared by fermentation of *Streptomyces argenteolus* var. *toyokaensis* as described in a previous paper[1]. Pepstatin derivatives were prepared by MORISHIMA and TAKITA at the authors' institute. For the experiments, pepstatin and its derivatives were first dissolved in methanol and diluted with a buffer. Heparin for medical use containing 5,000 units/5 ml in an ampule was obtained from Takeda Chemical Industries Ltd., Osaka. Chondroitin sulfate was purchased from Sigma Chemical Co., U. S. A. Carrageenin, phenylbutazone and basic aluminum sucrose sulfate were obtained from Banyu Seiyaku Co., Tokyo.

Reaction system for hydrolysis of hemoglobin by acid proteases: One ml of 0.5 % hemoglobin solution, 0.8 ml of 0.02 M KCl-HCl buffer (pH 2.0) and 0.1 ml of the same buffer with or without an inhibitor were mixed and incubated for 3 minutes. Then, 0.1 ml of a solution of porcine pepsin (10 $\mu$g/ml) or other acid proteases was added. The concentration of the other acid proteases used was that which had the same activity as 10 $\mu$g/ml of porcine pepsin. After incubation at 37°C for 25 minutes, 2.0 ml of 1.7 M perchloric acid was added and the mixture kept for 1 hour at room temperature. It was then centrifuged and the extinction of the acid soluble fraction was read at 280 m$\mu$.

Reaction system for hydrolysis of casein by acid proteases: One ml of 0.6 % casein solution, 0.8 ml of 0.02 M KCl-HCl buffer (pH 2.0) and 0.1 ml of the same buffer with or without an inhibitor were mixed and incubated for 3 minutes. Then, 0.1 ml of a solution of porcine pepsin (40 $\mu$g/ml) or other acid proteases was added. The concentration of acid proteases in the solutions was 4 times higher than that used for the hydrolysis of hemoglobin. After incubation at 37°C for 30 minutes, 2.0 ml of 1.7 M perchloric acid was added and the extinction at 280 m$\mu$ measured as described above.

Reaction system for hydrolysis of synthetic dipeptide substrate by pepsin: In pepsin-catalyzed hydrolysis of APT the incubation mixture contained 0.25 ml of $10^{-2}$ M APT in 0.065 % $Na_2CO_3$, 0.2 ml of 0.05 M sodium acetate buffer (pH 4.0) with or without an inhibitor and 0.05 ml of pepsin solution containing 50 $\mu$g in 0.001 N HCl. After incubation at 37°C for 22 hours, 0.1 ml of the incubation mixture was mixed with 0.9 ml of 0.02 M Tris-HCl buffer (pH 8.0) to stop further hydrolysis. Released tyrosine was determined by the ninhydrin method[7]. Peptic hydrolysis of APDT was measured according to the method of JACKSON *et al.*[8] The incubation mixture contained 0.17 ml of 2 mM APDT in 4 mM NaOH, 3.03 ml of 0.01 N HCl with or without an inhibitor and 0.2 ml of pepsin 300 $\mu$g/ml solution in 0.001 N HCl. After 10 minutes at 37°C, 0.1 ml of 0.7 N NaOH was added and the extent of hydrolysis was determined by the ninhydrin method as slightly modified by JACKSON *et al.*[8]

Determination and preparation of human pepsin and gastricsin and the method of testing the effect of pepstatin on them: The chromatographic separation of pepsin and gastricsin from human gastric juice was carried out according to the procedure described by RICHMOND[4,5]. One half to one ml of gastric juice from each individual was filtered, dialyzed against 0.2 M sodium citrate buffer (pH 3.0) to remove dialyzable ninhydrin-positive materials and then subjected to ion-exchange column chromatography with Amberlite CG-50. A column of 0.4×5 cm was prepared and equilibrated with 0.2 M sodium citrate

buffer (pH 3.0). After passing the gastric juice through the column, the pH of eluting buffer was changed to 3.7, 4.16 and 4.4 successively and the eluate was assayed for proteolytic activity using hemoglobin as the substrate. The amounts of pepsin and gastricsin separated were calculated from their specific activities in the hemoglobin digestion method. Quantitative determination of gastricsin and pepsin in a mixture of the two enzymes was carried out according to the method of CHIANG *et al.*[9,10] The total proteolytic activity in human gastric juice was determined by the hemoglobin hydrolysis method and the quantity of human pepsin in the mixture was determined using APDT as a specific substrate for pepsin since it is not hydrolyzed by gastricsin. The difference between the total proteolytic activity using hemoglobin substrate and that using APDT as the substrate was taken as the activity of gastricsin. The quantities of pepsin and gastricsin in gastric juices were calculated from their specific activity values as described by CHIANG *et al.*[9,10]

Determination of proteolytic activity of gastric juice of stomach ulcer patients before and after administration of pepstatin: The gastric juice of stomach ulcer patients was collected before and after pepstatin administration and its proteolytic activity was measured by another method using hemoglobin as the substrate. The hemoglobin hydrolysis method described above was suitable to measure small quantities of acid proteases, but was unsuitable to measure the peptic activity of gastric juice. If the hemoglobin hydrolysis method described above is utilized, then the gastric juice must be diluted approximately 1,000 times. Dissociation of the enzyme-inhibitor complex in the gastric juice collected after administration of pepstatin would occur at such a high dilution, and the peptic activity shown by highly diluted juice would not be valid in estimating the activity of the undiluted juice. Therefore, the activity of the gastric juice was determined by the following method. One and one half ml of 2.0 % hemoglobin solution adjusted to pH 2.0 with HCl, 0.2 ml of 0.02 M KCl-HCl buffer (pH 2.0) and 0.3 ml of human gastric juice were mixed. After incubation at 37°C for 3 minutes, 2.0 ml of 1.7 M perchloric acid were added and the extinction at 280 m$\mu$ was measured as described above. The percent inhibition was estimated as described below.

Determination of free and bound sialic acid in gastric juice of pylorus-ligated rats (SHAY rats): Sialic acid in the gastric juice of pylorus-ligated rats with or without the administration of pepstatin was measured by the thiobarbituric method (TBA) of AMINOFF[11]. Crystalline N-acetyl neuraminic acid (NANA) was used as a reference standard with each assay. Free sialic acid was determined by the TBA method directly, and the total sialic acid was determined by the TBA method after hydrolysis. The bound sialic acid was obtained from the difference between the total and free sialic acid. Four tenth ml of 0.125 N $H_2SO_4$ was added to 0.1 ml of gastric juice and the contents were then hydrolyzed at 80°C for 1 hour. After hydrolysis, total amount of sialic acid was determined by TBA directly.

Estimation of percent inhibition of proteolytic reactions[12]: The percent inhibition of the reactions was calculated as follows:

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100$$

A = Optical density at 280 m$\mu$ without inhibitor

B = Optical density at 280 m$\mu$ with inhibitor

The concentration inducing 50 % inhibition was obtained by plotting the probit of the percent inhibition on the ordinate and the log of the inhibitor concentration on the abscissa. In all cases, a linear relation was observed between 30 % and 70 % inhibition.

## Results and Discussion

The effects of pepstatin on hydrolysis of casein and hemoglobin by porcine pepsin, protease Type A and Type B of *Aspergillus niger*, acid protease of *Trometes sanguinea*,

Table 1. Inhibitory activity of pepstatin on acid proteases

| Enzymes | Substrates | Pepstatin $ID_{50}$ (M) |
|---|---|---|
| Pepsin (porcine) | Casein | $1.5\times10^{-8}$ |
| | Hemoglobin | $4.5\times10^{-9}$ |
| | APT[1] | $1.1\times10^{-6}$ |
| | APDT[2] | $2.3\times10^{-7}$ |
| Protease A | Casein | $>3.6\times10^{-4}$ |
| | Hemoglobin | $>3.6\times10^{-4}$ |
| Protease B | Casein | $1.3\times10^{-8}$ |
| | Hemoglobin | $1.0\times10^{-8}$ |
| *Trametes sanguinea* | Casein | $5.5\times10^{-8}$ |
| | Hemoglobin | $2.6\times10^{-8}$ |
| *Aspergillus saitoi* | Casein | $1.8\times10^{-8}$ |
| | Hemoglobin | $8.7\times10^{-9}$ |
| *Xylaria* sp. | Casein | $1.0\times10^{-7}$ |
| | Hemoglobin | $4.0\times10^{-7}$ |

1) N-Acetyl-L-phenylalanyl-L-tyrosine
2) N-Acetyl-L-phenylalanyl-L-diiodotyrosine

Table 2. Effect of pepstatin, its derivatives and other agents on pepsin-casein system

| Inhibitors | $ID_{50}$ ($\mu$g/ml) | Activity |
|---|---|---|
| Pepstatin | 0.01 | 100 |
| 〃 methyl ester | 0.008 | 125 |
| 〃 ethyl ester | 0.01 | 100 |
| 〃 *p*-bromophenacyl ester | 0.01 | 100 |
| 〃 Na | 0.0096 | 104 |
| 〃 Mg | 0.009 | 111 |
| 〃 Ca | 0.01 | 100 |
| Dehydropepstatin[1] | 0.015 | 67 |
| Dehydroacetyl pepstatin[2] | 1.31 | 0.76 |
| Heparin | >250 | <0.004 |
| Chondroitin sulfate Na | >250 | <0.004 |
| Carrageenin | >250 | <0.004 |
| Phenylbutazone | 74 | 0.014 |
| Basic aluminum sucrose sulfate | >250 | <0.004 |

1) Ivaler-Val-Val-AHMHA-Ala-dehydro AHMHA
2) Ivaler-Val-Val-O-acetyl-AHMHA-Ala-dehydro AHMHA,
AHMHA : 4-amino-3-hydroxy-6-methyl heptanoic acid
dehydro-AHMHA : 4-amino-6-methyl-2-heptenoic acid

acid protease of *Aspergillus saitoi* and acid protease of *Xylaria* sp. are shown in Table 1. As shown by 50% inhibition concentration ($ID_{50}$), pepstatin shows strong inhibition against all acid proteases except protease Type A of *Aspergillus niger* which is thought to be a different type. Comparing the 50% inhibition concentrations of hydrolysis using casein and hemoglobin substrates, with the exception of the enzyme obtained from *Xylaria* sp., the inhibitory effect was greater when hemoglobin was employed as a substrate. One reason is thought to be the higher concentration of the enzyme used in the casein system than in the hemoglobin system. It should be noted that the 50% inhibition concentration against the protease of *Xylaria* sp. is higher against casein than against hemoglobin. Moreover, the activity of pepstatin against this protease is weaker than against the other proteases. As will be described later, the activity of pepstatin against this enzyme is similar to gastricsin. The results shown in Table 1 indicate that pepstatin is a specific inhibitor of acid proteases in general with the exception of some of the fungal enzymes, because $3.6\times10^{-4}$ M of pepstatin showed no inhibition against the following systems when tested by the methods described in previous pepers[12~14]: hydrolysis of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride by thrombin, fibrinogen by plasmin, casein by trypsin, $\alpha$-N-benzoyl-L-arginine ethyl ester by kallikrein, casein by $\alpha$-chymotrypsin, casein by papain, and plasma by thrombokinase.

Pepstatin derivatives were tested for their effects on the hydrolysis of casein by porcine pepsin and the results are described in Table 2. As shown in Table 2, the esters of pepstatin are equally or slightly more active than pepstatin, which indicates that the free carboxyl group is not essential for the activity. This is consistent with the effects seen in acid conditions. As reported in another paper, the binding of pepstatin with pepsin can be shown using Sephadex G-50 column chromatography of

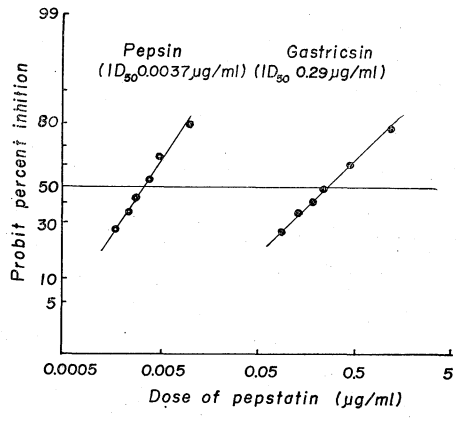
a solution containing pepsin and $^{3}$H-pepstatin. If a solution of the pepsin-pepstatin complex is neutralized, pepstatin is released from the enzyme which is simultaneously inactivated. Apparently pepstatin binds with active pepsin and the carboxyl group does not participate in the binding, since the ester which contains a relatively large group such as *p*-bromophenacyl shows a similar activity as pepstatin. Sodium, magnesium and calcium salts of pepstatin show similar activity as pepstatin. When these salts are added to the reaction mixture at pH 2.0, they must be converted to the undissociated form in the acid solution. One of two hydroxyl groups of pepstatin is thought to participate in the binding of pepstatin with pepsin. Dehydropepstatin (Ivaler-Val-Val-AHMHA-Ala-dehydro AHMHA) (AHMHA: 4-amino-3-hydroxy-6-methyl heptanoic acid; dehydro AHMHA: 4-amino-6-methyl-2-heptenoic acid) in which the terminal amino acid is dehydrated is slightly less active than pepstatin. However, dehydroacetyl pepstatin (Ivaler-Val-Val-O-acetyl-AHMHA-Ala-dehydro AHMHA) in which the hydroxyl group of dehydropepstatin was acetylated is 150 times less active. Based on these results and the structure of pepstatin it is suggested that hydrophobic binding and hydrogen bonding through one of the hydroxyl groups is necessary for pepstatin to inhibit the activity of pepsin.

The effect of sulfate esters of carbohydrates which have been reported to inhibit pepsin are shown in Table 2. A number of them are marketed as pepsin inhibitors for the treatment of peptic ulcer. However, as shown in the table, they are very weak in inhibiting enzyme activity. In the test system against casein, no inhibition even at 250 $\mu$g/ml was seen with heparin, chondroitin sulfate, carrageenin and sucrose sulfate. They are therefore at least 25,000 times less active than pepstatin. In general, polyanionic compounds exhibit inhibitory activity against various enzymes and thus, sulfate esters of carbohydrate are probably not specific inhihitors of pepsin. It is possible that pepstatin is the first specific inhibitor of acid proteases to be isolated. Phenylbutazone which is known to be an anti-peptic ulcer agent is 7,000 times less active than pepstatin.

The effects of pepstatin on acid proteases of human, porcine and rat origin were compared, and on the basis of the $ID_{50}$ values pepstatin show the same inhibitroy activity on the proteases of all three species. Human gastric juice contains an acid protease other than pepsin. Pepsin and gastricsin were prepared from human gastric juice by the method of RICHMOND[4,5], and the effects of pepstatin on these enzymes were tested using the hemoglobin hydrolysis system. As shown in Fig. 1, both the pepsin and gastricsin obtained from human gastric juice are inhibited

Fig. 1. Inhibition of pepstatin against hydrolysis of hemoglobin by human pepsin and gastricsin.

The effect of pepstatin was tested using standard hemoglobin assay system at pH 2.0. The amounts of human pepsin and gastricsin in the incubation mixture were 1 $\mu$g and 0.5 $\mu$g, respectively.
These amounts showed the same activity as 1 $\mu$g of porcine pepsin.



by pepstatin, but gartricsin appears to be about 100 times more resistant to pepstatin than was pepsin on the basis of the $ID_{50}$ value. The 50 % inhibition concentration obtained is as follows : $5.4\times10^{-9}$ M against human pepsin ; $4.2\times10^{-7}$ M against human grstricsin ; $4.5\times10^{-9}$ M against porcine pepsin.

Table 3. The contents of pepsin and gastricsin in human gastric juice

| Individuals | pH | Pepsin ($\mu$g/ml) | Gastricsin ($\mu$g/ml) | Pepsin / Gastricsin Ratio |
|---|---|---|---|---|
| 1 | 1.7 | 111 | 18 | 6.1 |
| 2 | 1.7 | 144 | 28 | 5.1 |
| 3 | 5.2 | 142 | 95 | 1.5 |
| 4 | 2.0 | 471 | 94 | 5.0 |
| 5 | 2.0 | 312 | 105 | 3.0 |
| 6 | 1.7 | 160 | 107 | 1.5 |
| 7 | 1.6 | 612 | 117 | 5.2 |
| 8 | 3.0 | 734 | 158 | 4.6 |
| 9 | 3.5 | 551 | 148 | 3.7 |

Pepstatin is now being studied clinically for its effect on stomch ulcer. In order to determine a suitable dose, it was necessary to know the contents of pepsin and gastricsin in gastric juice of stomach ulcer patients. Pepsin and gastricsin in gastric juice were determined by the two methods described in "Methods and Materials". Gastric juice from two patients with stomach ulcers were analyzed by both methods. After chromatographic separation of these enzymes, the pepsin levels were 346 $\mu$g/ml and 92 $\mu$g/ml, while gastricsin levels were 62 $\mu$g/ml and 7 $\mu$g/ml respectively. These results were compared with those obtained by another method based on the difference of substrate specificity between pepsin and gastricsin. The contents of pepsin and gastricsin determined by the second method in the two samples of gastric juice described above are as follows: pepsin 471 $\mu$g/ml and 111 $\mu$g/ml ; grstricsin 94 $\mu$g/ml and 18 $\mu$g/ml. The two methods therefore gave similar results. Using the second method, the contents of pepsin and gastricsin in gastric juice of 9 peptic ulcer patients were determined. As shown in Table 3, the pepsin concentration was found to be 111~734 $\mu$g/ml and gastricsin concentration 18~158 $\mu$g/ml. The ratio of pepsin to gastricsin was 1.5~6.1.

The residual pepstatin content in the gastric juice of the above-mentioned stomach ulcer patients at 60 minutes after the administration of one or two capsules containing 50 mg of pepstatin were determined by two methods. One is based on the fact that two acid proteases in gastric juice are irreversibly inactivated in alkaline condition (pH 9.0, for 10 minutes) while pepstatin is not inactivated. The pepstatin level in gastric juice after the inactivation of the enzymes was determined by testing the activity against hemoglobin hydrolysis by porcine

Table 4. Content and antipeptic activity of pepstatin in human gastric juice 1 hour after administration

| Individual* No. | Pepstatin administration (mg) | Time** (min.) | pH | Pepstatin ($\mu$g/ml) | | Percent*** inhibition |
|---|---|---|---|---|---|---|
| | | | | Alkali denaturation | Butanol extraction | |
| 4 | 50 | 60 | 1.8 | 20.5 | 14.3 | 93.6 |
| 5 | 50 | 60 | 1.9 | 8.6 | 6.9 | 90.2 |
| 7 | 50 | 60 | 1.5 | 26.6 | 15.6 | 92.7 |
| 8 | 50 | 60 | 2.5 | 24.4 | 17.6 | 100 |
| 9 | 50 | 60 | 1.9 | 27.8 | 32.8 | 98.4 |
| 10 | 50 | 60 | 4.7 | 5.8 | 3.9 | 100 |
| 11 | 100 | 60 | 1.5 | 12.9 | 11.9 | 100 |

* The individual No. is the same as in Table 3.
** The sampling time of gastric juice were shown by the time after administration.
*** Calculated from the peptic activities of the juices collected before and after the administration of pepstatin.

pepsin. In the second method, the pepstatin content was determined by extracting it into butanol and determining the inhibitory activity of the extract. The results are shown in Table 4. The results of the determination by two methods are fairly similar. As reported in the next paper[15], pepstatin binds with pepsin in an equimolar ratio and the dissociation constant is less than $3\times10^{-9}$ M. Therefore, an equimolar concentration of pepstatin and pepsin shows almost 100 % inhibition. The molecular weight of pestatin is 686 and that of pepsin is approximately 34,500. As shown in Tables 3 and 4, one would expect that the pepstatin concentration in gastric juice at 60 minutes after administration is sufficient to inhibit pepsin activity. Indeed, when the gastric juice before and after administration of pepstatin is assayed using hemoglobin hydrolytic activity, as shown in the last column of Table 4 pepstatin inhibits the proteolytic enzymes in gastric juices almost completely. The peptic activity of the juice collected 60 minutes after the administration of pepstatin is 0~9.8 % of that of the juice collected before the administration.

Free sialic acid and bound sialic acid in gastric juice of pylorus-ligated rats (SHAY rats) were determined by the thiobarbituric method (TBA) of AMINOFF[11]. When 0.5 mg/kg of pepstatin is orally administered to SHAY rats, the peptic activity in gastric juice is completely inhibited and a marked decrease of bound sialic acid is observed as shown in Table 5. The decrease of bound sialic acid in the gastric juice of rats treated with pepstatin suggests that the bound sialic acid (sialoglycoprotein) may be released into the gastric juice by the action of an acid protease on the mucous membrane. The marked decrease of bound sialic acid might be one of the mechanisms whereby pepstatin prevents stomach ulceration in SHAY rats. Protein concentration of human gastric juice was measured by the LOWRY[16] reaction. The value was lower in juice collected after pepstatin administration. This result suggests that pepstatin inhibits the release of protein (sialoglycoprotein) from the mucous membrane due to pepsin. These results indicate that pepstatin can be a useful tool for the study of the mechanism of peptic ulcer formation.

Table 5. Effect of pepstatin on free and bound sialic acid in the gastric secretions of pylorus-ligated rats (SHAY rats)

| Dose mg/kg (Oral) | No. of rat | Gastric secretion | | | Grade of ulcer [2] | | | | | Sialic acid (μg/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Volume (ml) | pH | Pepsin[1] (μg/ml) | − | + | ++ | +++ | ++++ | Free | Bound |
| Control | 5 | 12.3 | 2.0 | 475.1 | 0/5 | 0/5 | 0/5 | 2/5 | 3/5 | 19 | 89 |
| 0.5 | 5 | 13.8 | 2.1 | 0 | 3/5 | 1/5 | 1/5 | 0/5 | 0/5 | 24 | 16 |
| 2.0 | 6 | 12.5 | 2.2 | 0 | 4/6 | 2/6 | 0/6 | 0/6 | 0/6 | 19 | 14 |
| 32 | 6 | 13.0 | 2.2 | 0 | 6/6 | 0/6 | 0/6 | 0/6 | 0/6 | 14 | 13.5 |

Rat : male Wister. Pepstatin treatment : 15 minutes after pyloric ligation. Body weight : 200 g±15 g.
Pyloric ligation : 22 hours. Fasted : 48 hours. Administration route : oral.
1) Pepsin activity corresponding to porcine pepsin μg/ml.
2) − : No ulceration. + : A single ulceration. ++ : Some points of ulceration. +++ : Generalized ulceration.
++++ : Perforated ulceration.

As reported in a previous paper[1], pepstatin has a very low toxicity and more than 90 % of orally administered pepstatin is excreted in the feces in 24 hours after administration. It was also shown that intraperitoneal injection of pepstatin inhibited carrageenin edema of rats[17]. These results suggest that an enzyme inhibited by

pepstatin is one of the factors responsible for the formation of edema. The low toxicity, the effect against carrageenin edema and the strong inhibitory effect against acid proteases are the biological properties of pepstatin so far observed.

Acknowledgement

The authors wish to thank Misses M. NAMBO and F. KOJIMA for technical assistance throughout the course of study.

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