STRONG DIURETIC EFFECT OF PEPSTATIN, AN ACID PROTEASE INHIBITOR

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Pepstatin, an acid protease inhibitor purified from the culture medium of *Actinomycetes*, had a strong diuretic effect in C3H/He mice. A single subcutaneous injection of pepstatin at 80 mg/kg body weight caused 3- to 4-fold increase in the urine volume over that of control mice during the first 24 hours after the injection and 6- to 7-fold increase during the second and third 24-hour periods. The effect was maximal after 72~96 hours, and gradually disappeared within 168 hours after the injection. The serum potassium concentration increased to 7.6 mEq/liter at 24 hours after the injection but the serum sodium concentration did not change significantly. This change in electrolytes disappeared within 168 hours after the injection. The soluble derivative of pepstatin, lactyl-pepstatin, which has a higher ID_{50} than pepstatin for renin, had no significant diuretic effect at the same molar dose. From these findings the strong diuretic effect of pepstatin is concluded to be due to inhibition of renin-mediated conversion of angiotensinogen to angiotensin I, which in turn is normally converted to angiotensin II, resulting in release of aldosterone.

Pepstatin is known to inhibit many acid proteases such as pepsin, cathepsin D and renin.[1,2] These inhibitory effects of pepstatin are considered to be useful in treatment of peptic ulcers and hypertension.[3]

GREENBAUM *et al.* reported that pepstatin effectively inhibited ascites accumulation in mice bearing L1210 and mastocytoma.[4] Recently, we confirmed this inhibition of ascites accumulation in mice bearing various other types of ascites tumors (to be published). This inhibitory effect was suggested to be due to inhibition of a cathepsin D-like enzyme that increases vascular permeability through formation of leukokinin, a vasoactive peptide,[5] from leukokininogen. During studies of the mechanism of inhibition of ascites accumulation we found that pepstatin has a strong diuretic effect. This paper reports studies on this diuretic effect of pepstatin and a consideration of the mechanism of this effect.

Materials and Methods

Pepstatin and lactyl-pepstatin

Pepstatin was obtained under the resources program of Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture. It was suspended in water at a final concentration of 3 mg/ml (4.5 mM) and neutralized with NaOH. Lactyl-pepstatin (lactyl-valyl-statinyl-alanyl-statin) was a generous gift from Dr. H. UMEZAWA, Institute of Microbial Chemistry, Tokyo, Japan. It was dissolved at a concentration of 2.6 mg/ml (4.5 mM) in distilled water and neutralized with NaOH. These compounds were injected subcutaneously into mice at the indicated doses. Control mice were injected subcutaneously with 27 ml/kg body weight of 4.5 mM NaCl.

Animals

Male C3H/He mice weighing about 30 g were obtained from Japanese Charles River Co., Ltd., Kanagawa, Japan.

Measurement of urine volume

Each mouse was kept in a separate metabolic cage (Natsume Seisakusho, Tokyo, Japan) with free access to pellet diet (CLEA CE-2) and water. Urine was collected for 24-hour periods for determination of its volume.

Determination of serum sodium (Na) and potassium (K)

Blood was collected by puncture of subaxillary vessels under ether anesthesia and the serum was separated by centrifugation. Serum Na and K were determined by flame photometry using a Hitachi flame photometer 205D (Hitachi Seisakusho, Co., Ltd., Tokyo, Japan) by courtesy of Dr. E. ARAKI, National Cancer Center Hospital, Tokyo, Japan.

Results

The strong diuretic effect of pepstatin is shown in Fig. 1. In this experiment, a single subcutaneous injection of 80 mg/kg of pepstatin caused 3- to 4-fold increase in urine volume during the first 24 hours, and 6- to 7-fold increase during the second and the third 24-hour periods and then its effect gradually decreased. The maximal diuretic effect was observed during the third 24-hour period. A dose of 40 mg/kg of pepstatin was as effective during the first 24 hours, but its diuretic effect decreased during the second 24-hour period and disappeared within 72 hours. These data show that the effect of pepstatin is dose-dependent and reversible.

The diuretic effect of the very water-soluble derivative of pepstain, lactyl-pepstatin was also studied. The ID_{50} of this derivative for renin (1.7×10^{-4} M) is two orders of magnitude higher than that of pepstatin. This derivative had no significant diuretic effect when injected subcutaneously at a dose of 69 mg/kg, which is equivalent to 80 mg/kg of pepstatin (data not shown).

The effect of 80 mg/kg of pepstatin on the serum Na and K levels was studied and the results are shown in Fig. 2. The serum K level showed a reversible increase with a maximum of 7.6 mEq/liter at 24 hours after subcutaneous pepstatin injection. The serum Na level also increased slightly, but since the control group showed the same increase, this increase did not seem to be a specific effect of pepstatin.

Fig. 1. Effect of pepstatin on urine volume.
Points and bars are means ± S.E. for 3 to 5 mice.

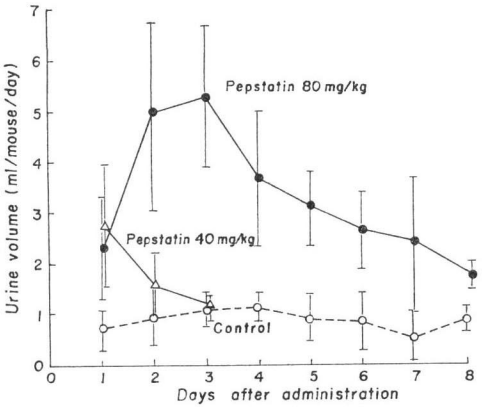
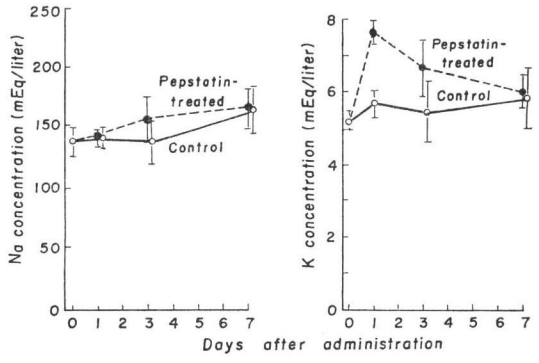


Fig. 2. Effect of pepstatin on serum Na and K levels.
Points and bars are means ± S.E. for 3 mice. The experimental group was injected subcutaneously with 80 mg/kg pepstatin on day 0.



Discussion

A strong diuretic effect of pepstatin was clearly demonstrated in the present work. Recently, pepstatin was reported to be useful for inhibiting ascites accumulation associated with cancer in mouse ascites tumor L1210 and mastocytoma P815-Y[4] and we confirmed this effect in other systems (to be published). Originally this inhibitory effect of pepstatin was supposed to be due to inhibition of a cathepsin D-like enzyme that forms leukokinin from leukokininogen.[4] However, since pepstatin has a strong diuretic effect, as shown in this paper, the reduction of ascites associated with cancer might be at least partly due to the diuretic effect.

The diuretic effect of pepstatin is probably due to an indirect antagonistic effect on aldosterone, because pepstatin increased the serum K level and this effect was reversible. Moreover, pepstatin is known to be a strong inhibitor of renin.[2] Renin is known to produce angiotensin I from angiotensinogen and a high level of angiotensin II, which is produced from angiotensin I by the converting enzyme, causes a high level of aldosterone. This possibility is supported by the finding that at the same molar dose as pepstatin, lactyl-pepstatin had no significant diuretic effect. Lactyl-pepstatin has been found to have an ID_{50} for renin two orders higher than that of pepstatin (Dr. T. AOYAGI, Institute of Microbial Chemistry, Tokyo, personal communication).

The possible clinical use of pepstatin and its derivatives requires investigation.

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References

1) UMEZAWA, H.; T. AOYAGI, H. MORISHIMA, M. TATSUZAKI, M. HAMADA & T. TAKEUCHI: Pepstatin, a new pepsin inhibitor produced by actinomycetes. J. Antibiotics 23: 259～262, 1970
2) AOYAGI, T.; H. MORISHIMA, R. NISHIZAWA, S. KUNIMOTO, T. TAKEUCHI & H. UMEZAWA: Biological activity of pepstatin, pepstanone A and partial peptides on pepsin, cathepsin D and renin. J. Antibiotics 25: 289～294, 1972
3) AOYAGI, T. & H. UMEZAWA: Structures and activities of protease inhibitors of microbial origin. pp. 429～454 *in* E. REICH, D. B. RIFKIN & E. SHAW, *eds.* Proteases and biological control. Cold Spring Harbor Laboratory, 1975
4) GREENBAUM, L. M.; P. GREBOW, M. JOHNSTON, A. PRAKASH & G. SEMENTE: Pepstatin, an inhibitor of leukokinin formation and ascitic fluid accumulation. Cancer Res. 35: 706～710, 1975
5) GREENBAUM, L. M.: Vasoactive peptides as mediators of increased vascular permeability. Trends. Biochem. Sci. 2: 41～43, 1977