EFFECT OF ORAL ADMINISTRATION OF A MOLD PROTEASE (ONOPROSE SA) ON THE CONCENTRATION OF ANTIBIOTICS IN RAT BRONCHIAL WASH

JUNNOSUKE KIDA and KUNIO KANO
Ono Pharmaceutical Co., Central Research Laboratory
SUSUMU YASUOKA and EIRO TSUBURA
The Department of Internal Medicine, School of Medicine, Tokushima University

(Received June 18, 1967)

INTRODUCTION

Various kinds of proteases have been demonstrated to have antiinflammatory[1,2] and mucolytic[3,4] actions. They havy have been also reported to increase the concentrations of antibotics in various tissues and inflammatory lesions when administered with the latter[5,6]. On the basis of these reports, they have been used in treatment of acute and chronic infections of the respiratory tract with antibiotics[7]. Few investigators, however, have demonstrated that proteases increase the concentration of antibiotics into normal and diseased respiratory tracts, or the alveoli. Therefore, we examined the effect of oral administration of a mold protease, Onoprose SA to rats on the concentrations of penicillin and cefazolin sodium salt in bronchial wash.

MATERIALS AND METHODS

Animals : Male Wistar strain rats, weighing 200 to 250 g, were used after they had been fasted overnight.

Enzymes : Onoprose SA (specific activity 2,150 units/mg) was purified from the culture filtrate of *Aspergillus melleus* by the method of ITO and SUGIURA[8]. *Serratia* peptidase (specific activity 3,155 units/mg) was extracted and purified from commercially available tablets (Dasen). Bromelin was purchased from Wako Pure Chemical Co. The specific activities of these proteases were measured with casein as substrate.

Antibiotics : Penicillin G potassium salt and aminobenzyl penicillin potassium salt were purchased from Meiji Seika Co., and cefazolin sodium salt from Fujisawa Pharmaceutical Co.

Drug administration and bronchial washing

Various amounts of Onoprose SA in 1 ml of distilled water were administered orally to normal and triolein-treated rats.

Various amounts of antibiotics in 0.4 ml of saline were administered intramuscularly in the left hind leg, with or after oral administration of Onoprose SA and 10 to 180 minutes later the rats were exsanguinated through the aorta abdominalis under nembutal anesthesia. In some experiments, blood was withdrawn from the external jugular vein before exsanguination, and serum was obtained by centrifuging the blood at 3,000 rpm for 15 minutes. The inferior vena cava was ligated, and the pulmonary vasculature was perfused by a syringe with physiological saline *via* the right ventricle.

The tracheal organs were removed *en bloc*. The trachea was canulated, and the bronchi were washed 5 times with 5 ml volumes of saline. The washing fluid was collected and centrifuged at 400×g for 10 minutes at 4°C. The concentration of antibiotics in the supernatant was measured.

Measurement of the concentrations of antibiotics in bronchial wash and serum

The concentration of penicillin G in the serum or bronchial wash was measured after extracting it with amylacetate as described by HUMPHREY and JOULES[9]. Cefazolin and aminobenzyl penicillin in the serum and bronchial wash could not be extracted with amylacetate. Therefore, their concentrations were measured directly in samples which had been diluted 5-fold with saline.

The concentrations of antibiotics were measured by the thin layer cup method with *S. aureus* 209 P as test organism according to the method of MIYAMURA *et al*[10].

Protein concentration was measured by the method of LOWRY *et al.* with bovine serum albumin as a

standard[11].

Intravenous administration of triolein

A dose of 0.25 ml of triolein was administered intravenously to rats through the tail vein to induce fat embolism in the lungs[12] and 24 hours later the rats were used for experiments.

RESULTS

(1) Effects of rat serum and bronchial wash on the antibacterial activity of penicillin G

Penicillin G in saline was added to the system for assay of the antibacterial activity of antibiotics at the various concentrations shown in Fig. 1.

Rat serum and bronchial wash were treated with amylacetate to extract penicillin G from them, and the extracted materials were dissolved in the original volume of saline. The bronchial wash and the material extracted from them were then added to the assay system at the concentrations indicated in Fig. 1. As shown in this figure, the bronchial wash at concentrations of 20 and 50% did not affect the activity of penicillin. Moreover, the extracted material at a concentration of 50% and serum and the material extracted from it at a concentration of 20% did not inhibit the activity. Therefore, the concentration of penicillin G in the serum and bronchial wash was measured using extracts of serum and bronchial wash at concentrations of 20 and 50%, respectively.

(2) Time course of change in concentration of penicillin G in the serum and bronchial wash after its intramuscular administration

Doses of 100,000, 250,000 and 625,000 units of penicillin G were administered intramuscularly to normal rats, and then the concentrations of penicillin in the serum and bronchial wash were measured at intervals of 180 minutes.

As shown in Fig. 2, the concentration of penicillin in the serum reached a maximum within 10 minutes, and then gradually decreased. Penicillin was scarcely detectable in the bronchial wash after administration of doses of 250,000 and 625,000 units per kg.

(3) Effects of simultaneous and previous administration of Onoprose SA on the concentrations of penicillin G in the serum and bronchial wash

Doses of 100, 200, 300 and 600 mg of Onoprose SA per kg body weight were administered with 100,000 or 250,000 units of penicillin per kg body weight. Results showed that the concentrations of the antibiotic in the serum and bronchial wash were not affected within 180 minutes by simultaneous admin istration of Onoprose SA.

Next a dose of 250,000 units of penicillin per kg body weight was administered 1, 2, 3 or 4 hours after administration of 300 to 600 mg of Onoprose SA per kg body weight. Results showed that the concentration of penicillin in the serum was not affected by administration of Onoprose SA, but that its concentration in the bronchial wash was markedly increased when it was administered 2~4 hours after Onoprose SA, although it was not significantly affected by Onoprose SA when administered 1 hour after the latter, as shown in Fig. 3.

Fig. 4 shows that the increase in the concentration of penicillin in the bronchial wash caused by Ono-

Fig. 1 Effect of bronchial wash and the material extracted from it with amylacetate on the antibacterial activity of penicillin G

For details see text.

●——● Control, ○·····○ bronchial wash, 20%, ×——× bronchial wash, 50%, △·····△ material extracted from bronchial wash with amylacetate, 50%.

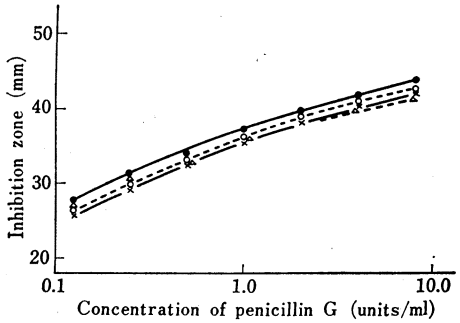
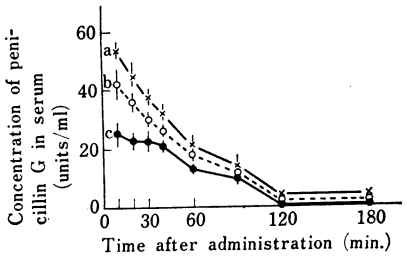


Fig. 2 Changes of concentration of penicillin G in the serum after its intramuscular administration without Onoprose SA

Points are means of values in 4 rats, and vertical lines show SD.

Dose of penicillin G : a 625,000, b 250,000, c 100,000 units/kg body weight.



prose SA was proportional to the dose of the latter.

(4) Effect of previons administration of Onoprose SA on the concentration of penicillin G in the bronchial wash of triolein-treated rats

Doses of 250,000 units of penicillin G per kg body weight were administered to normal and triolein-treated rats 4 hours after administration of 100 mg of Onoprose SA per kg body weight. Table 1 shows that Onoprose SA increased the concentration of penicillin G in the bronchial wash significantly.

Fig. 3 Effect of previous administration of Onoprose SA on the concentration of penicillin G in bronchial wash at various times after oral administration of 300 or 600 mg of 600 mg of Onoprose SA per kg body weight.

Values are means±SD of those for 4 rats. Onoprose SA was administered orally a 0 hr; b 1 hr; c 2 hr; d 3 hr; e 4 hr and f 5 hr before penicillin G.

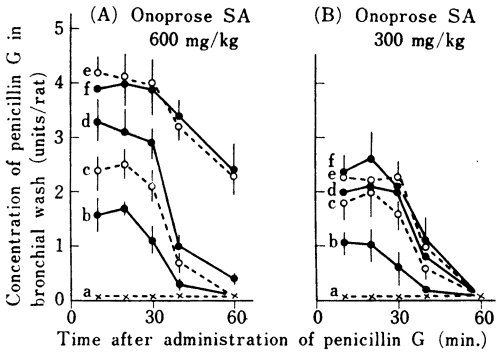
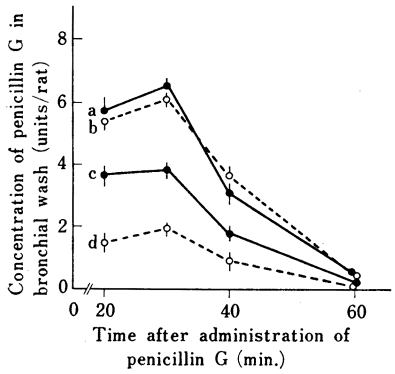


Fig. 4 Effect of dose of Onoprose SA on the concentration of penicillin G in bronchial wash.

Penicillin G (250,000 units/kg) was administered 4 hr after various amounts of Onoprose SA.

Dose of Onoprose SA: a 200, b 100, c 75, d 0 mg/kg body weight.



Also, as shown in Table 1, when penicillin was administered, the concentration of penicillin in the bronchial wash of triolein-treated rats was as low as that in normal rats. But Onoprose SA increased the concentration of penicillin in the bronchial wash more in triolein-treated rats than in normal rats. Therefore, triolein-treated rats were used in all experiments described below.

Table 1 Effect of oral administration of Onoprose SA on concentration of penicillin G in bronchial wash in normal and triolein-treated rats

Rats	Concentration of penicillin G in bronchial wash	
	Dose of Onoprose SA	
	0 mg/kg	100 mg/kg
	units/rat	
Normal	1.46±0.40	3.96±0.21*1
Triolein-treated	1.72±0.38	6.94±0.28*2

Penicillin G (250,000 units/kg body weight) was administered intramuscularly to normal and triolein-treated rats 4 hours after Onoprose SA.

Values are means ± SD of those in 4 rats. (30 minutes after penicillin G administration)

*1 Significant difference against Onoprose 0 mg/kg ($p<0.001$)

*2 Significant difference against normal ($p<0.001$)
Significant difference against Onoprose 0 mg/kg ($p<0.001$)

Table 2 Effects of oral administration of Bromelin and *Serratia* peptidase on concentration of cefazolin in rat bronchial wash

Protease administered	Concentration of cefazolin in bronchial wash Time after administration of cefazolin	
	30 minutes	60 minutes
	μg/rat	
Control	4.43±0.68	0.52±0.12
Bromelin (100 mg/kg)	7.13±1.12*1	2.93±0.72*3
Serratia peptidase (100 mg/kg)	8.12±0.93*2	2.22±0.82*4

Cefazolin (200 mg/kg body weight) was administered intramuscularly to rats 4 hours after Bromelin or *Serratia* peptidase.

Values are means±SD of those in 4 rats.

*1 Significant difference against control ($p<0.05$)

*2 Significant difference against control ($p<0.001$)

*3 Significant difference against control ($p<0.01$)

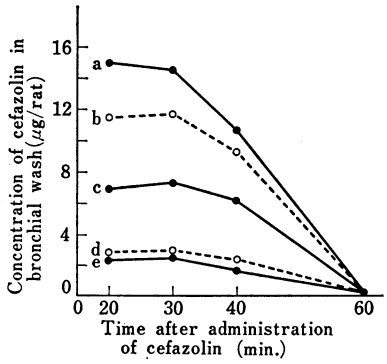
*4 Significant difference against control ($p<0.05$)

Fig. 5 Effect of dose of Onoprose SA on concentration of cefazolin in bronchial wash

Cefazolin (200 mg/kg) was administered 4 hr after various amounts of Onoprose SA

Values are means±SD of those in 4 rats.

Dose of Onoprose SA : a 200, b 100, c 50, d 25, e 0 mg/kg body weight.



(5) Effect of Onoprose SA on the concentration of cefazolin and aminobenzyl penicillin in the bronchial wash

Two other antibiotics, cefazolin (200 mg/kg) and aminobenzyl penicillin (100 mg/kg) were administered in the same way as penicillin G, 4 hours after various concentrations of Onoprose SA. As shown in Figs. 5 and 6, the concentrations of both cefazolin and aminobenzyl penicillin in the bronchial wash were significantly increased by administration of 50 to 200 mg of Onoprose SA per kg body weight.

(6) Effects of previous administration of *Serratia* peptidase and Bromelin on the concentration of cefazolin in the bronchial wash

A dose of 200 mg of cefazolin per kg body weight was administered intramuscularly to rats 4 hours after 100 mg of Bromelin or *Serratia* peptidase. Table 2 shows that both Bromelin and *Serratia* peptidase increased the concentration of cefazolin in the bronchial wash significantly.

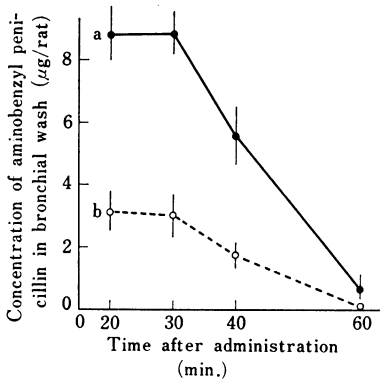
DISCUSSION

This work was on the effect of protease on the appearance of antibiotics in the respiratory tract and alveolus. We found that previous oral administration of Onoprose SA, a mold protease, increased the concentration of intramuscularly administered antibiotics, in the bronchial wash. Onoprose SA was only effective when administered orally some time before the antibiotics were injected intramuscularly. This is probably due to the difference in

Fig. 6 Effect of Onoprose SA on concentration of aminobenzyl penicillin in bronchial wash

Amino (100 mg/kg) was administered 4 hr after Onoprose SA.

Dose of Onoprose SA : a 100, b 0 mg/kg.



the times when the concentrations of Onoprose SA and antibiotics in the serum were maximal : the concentrations of antibiotics in the serum decreased rapidly from about 40 minutes after their intramuscular administration, whereas protease is probably absorbed about 30~60 minutes after its oral administration in an intact or degradative form from the intestine. Two other proteases, Bromelin and *Serratia* peptidase, also increased the concentration of antibiotics in the bronchial wash when administered orally. However, it is uncertain whether the effects of these proteases were actually due to their proteolytic activity, because they were administered much earlier than the antibiotics.

Intravenous injection of triolein into rats induces fat embolism in the lungs[12]. Onoprose SA increased the concentration of antibiotics in the bronchial wash more in triolein-treated rats than in normal rats. This may be due in part to increased permeability of the blood vessels of lungs with fat embolism.

Present results indicate that oral administration of Onoprose SA stimulates the appearance of antibiotics in normal and inflammatory respiratory tracts and alveoli. Further studies are required on the mechanism of its stimulatory effect.

SUMMARY

Oral administration of a mold protease, Onoprose SA before intramuscular administration of penicillin G, aminobenzyl penicillin or cefazolin to rats significantly increased the concentrations of these antibiotics in the bronchial wash. Other proteases, such as Bromelin and *Serratia* peptidase had similar

effects. The effect of Onoprose SA was greater in rats with fat embolism in the lungs than in normal rats.

These results indicate that Onoprose SA stimulates the appearance of antibiotics in normal and diseased respiratory tracts and alveoli.

REFERENCES

1) INNERFIELD, I.; A. SCHWARZ & A. ANGRIST : Intravenous trypsin : Its anticoagulant fibrinolytic and thrombolytic effects. J. Clin. Invest. 31 : 1049～1055, 1952
2) INNERFIELD, L.; A. ANGRIST & A. SCHWARZ : Parenteral administration of trypsin. Clinical effects in 538 patients. J. A. M. A. 152 : 597～605, 1953
3) MASAOKA, A.; M. MAEDA, T. MORI, S. OSHIMA & H. MANABE : Mechanism of liquefaction of sputum by the protease in bronchial secretion. Jap. J. Thoracic Dis. 9 : 491～492, 1971
4) ITO, H.; H. UCHIDA, J. KONISHIIKE, M. YAMAZAKI, Y. YAMAMOTO, S. KO & M. SUNAHARA: Liquefaction of sputum by a protease, Seaprose S. Nihon Rinsho 31 : 2339～2344, 1973
5) NEUBAUER, R.: A plant protease for potentiation of and possible replacement of antibiotics. Exp. Med. Surg. 19 : 143～160, 1961
6) SHIBATA, K.: Antibiotics and protease in Koso Ryoho edited by YAMAMURA U., p. 185～197, Nankodo, 1969
7) MISE, S.: Effects of various kinds of mucolytic drugs on the concentration of antibiotics in sputum. Chemotherapy 14 ; 381～391, 1966
8) ITO, M. & M. SUGIURA : Studies on *Aspergillus* protease, II, Purification, crystallization and some properties of semi-alkaline proteinase from *Aspergillus melleus*. Yakugaku Zasshi 88 : 1583～1590, 1968
9) HUMPHREY, J. H. & H. JOULES : Penicillin inhalation in pulmonary disease. Lancet 2 : 221～225, 1946
10) MIYAMURA, S. & Y. KANAZAWA : On cup assay of terramycin. J. Antibiotics 4 : 470～474, 1951
11) LOWRY, O. H.; N. J. ROSEBROUGH, A. L. FARR & R. J. RANDALL : Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193 : 265～275, 1951
12) PAREDES, S ; F. COMER, S. RUBIN, R. SIDNEY, F. ADLER & L. F. PELTIER : Fat embolism. J. Bone & Joint Surg. 47 A : 1216～1220, 1965