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INHIBITION OF HUMAN NEUTROPHIL ELASTASE BY DIFFERENT CLASSES OF ANTIBIOTICS

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= Abstract =

사람의 혈액에 있는 호중구 엘라스테이즈에 관한 연구의 진척은, 약물로 사용가능한 생체내 혹은 인공합성의 저해제 개발을 동반해 오고 있다. 정제된 호중구 엘라스테이즈에 12종의 항생제를 처리하였더니 20mM 미만의 IC_{50}을 보인 것은 oxytetracycline, cefamandole, gentamicin, amikacin, cloxacillin, methicillin, chloramphenicol 등이었고, 10mM 농도에서 50% 이상의 저해율을 나타낸 것은 methicillin, cefamandole, oxytetracycline 등이었다. 그 결과는 세포벽 합성이나 단백질 합성을 저해하는 일반적 항생제의 약리학적 기전과는 다른 차원에서 항생제가 숙주의 조직을 보호할 수 있는 가능성을 보여준 모델이 존재한다는 것을 in vitro 실험에서 확인할 수 있었다.

INTRODUCTION

Human leukocyte proteases; collagenase, elastase, cathepsin G and specific gelatinase, present in the azurophil granules of neutrophils. These neutral proteinases, especially elastase, have been implicated as a pathogenic agent in pulmonary emphysema, rheumatoid arthritis, arteriosclerosis, acute inflammation and tumor invasion.[1,3,4,7,8,10] Endogenous and exogenous inhibitors of human leukocyte proteases for the treatment of these diseases have been developed in several laboratories.[11]

Recently, interesting investigations were reported that the activity of human leukocyte proteases were inhibited by some antibiotics. Golub, L. M., et. al.[5,6] proposed that: (i) tetracyclines can inhibit the activity of collagenase; (ii) this newly discovered property of the drugs could provide a novel approach to the treatment of diseases, including certain medical disorders, which involve excessive collagen destruction. In 1986, Doherty, J. B. and his colleagues[2] reported that neutral cephalosporins could be a potent

time-dependent inhibitor of human leukocyte elastases. Gradually, it has been evoked which antibiotic inhibits the activity of human neutrophil elastase and what is the mechanism of action of the antibiotics on the antiprotease property.

This report describes the properties of antibiotics on human neutrophil elastase activity.

MATERIALS AND METHODS

Materials

Reagents were purchased as followings : Hypaque (sodium diatrizoate), Ficoll (type 400, MW 400,000), N-Suc-$(Ala)_3$ -PNA (SANA) from Sigma; CM-sephadex C-25 from Pharmacia; Ultrogel AcA 54 from LKB. Other chemicals were used G. R. grade.

The antibiotics were purchased from following companies as listed : penicillin G (Kunhwa), methicillin (Daehan), cloxacillin (Yungjin), ampicllin and chloramphenicol (Jongkundang), cefazolin and kanamycin (Donga), cefamandole (Daewoong-Lilly), cefoperazone and oxytetracycline (Pfizer), gentamicin(Kukje), amikacin(Bristol).

Separation of Pure Neutrophils

Human neutrophils were isolated from peripheral blood of healthy volunteer donors as described previously.[9] Whole blood was mixed with EDTA to prevent clottings and carefully layered on the 3-step gradient of Hypaque-Ficoll solution (density; 1.080, 1.122, 1.133g/ml). Highly pure neutrophils were obtained through centrifugation at 300g for 25 min at room temperature.

Purification of Human Neutrophil Elastase

Separated neutrophils were homogenized and centrifuged. The supernatant was then chromatographed by two steps with Ultrogel AcA 54 and CM-sephadex. Experimental details of human neutrophil elastase isolation and electrophoresis studies are preparing for publication.

Antibiotics Test on Human Neutrophil Elastase Activity

Twelve antibiotics were selected from penicillins, cephalosporins, aminoglycosides, and others. After each antibiotics were preincubated with human neutrophil elastase in the reaction medium for 30 minutes, the reaction was started by adding SANA. Human neutrophil elastase activity was determined spectrophotometrically at 410nm by monitoring p-nitroaniline released from the synthetic substrate, SANA.

Usually the reaction mixture was incubated for 30~90 minutes at 37℃. % inhibition was determined by $100\times[1-(r_{\text{inhibitor present}}/r_{\text{inhibitor absent}})]$. IC_{50} indicates concentration of antibiotic giving 50% inhibition.

RESULTS

Separation of Pure Neutrophils

After centrifugation at 300g for 25 minutes at room temperature, 3 different cell fractions were observed at the interface of each solution. The third band was predominantly neutrophils and the cell purity was 96.2%. Cell recovery was always>80% for neutrophils and cell viability that was determined by trypan blue exclusion test was>99%.

Purification of Human Neutrophil Elastase

Highly pure human neutrophil elastase was

obtained by two steps of purification with Ultrogel AcA54 and CM-sephadex. Details of purification is out of scope of this report, therefore we will report the details about purification and characterization of human neutrophil elastase separately.

Antibiotics Test on Human Neutrophil Elastase Activity

Inhibition of human neutrophil elastase by antibiotics reveals different characteristics than that were shown against microorganisms. Penicillins; penicillin G, cloxacillin, and ampicillin showed only less than 20 percent inhibition at 10mM concentration but methicillin which is belong to similar class to above mentioned drugs, showed more than 50 percent inhibition at same concentration. When we tested the drug effect, for example like ampicillin at higher concentration, the inhibition of human neutrophil elastase was not significantly changed (Fig. 1-A). But in methicillin, the inhibition of human neutrophil elastase was significantly increased by increment of the concentration up to 8mg/100㎕ (Fig. 1-B). 80.23% of human neutrophil elastase was inhibited by 49.6mM of methicillin.

In cephalosporins series of antibiotics, cefazolin and cefoperazone showed a little inhibition of human neutrophil elastase activity but cefamandole inhibits this enzyme significantly. 10mM of cefazolin and cefoperazone inhibited less than 30% of control activity but same concentration of cefamandole inhibited up to 70% of the control activity (Table 1). 95% of human neutrophil elastase was inhibited by 48mM of cefamandole (Fig. 2), i.e., IC_{50} of cefamandole was 5.85㎍/ml (Table 2).

In aminoglycosides, most strong inhibitor

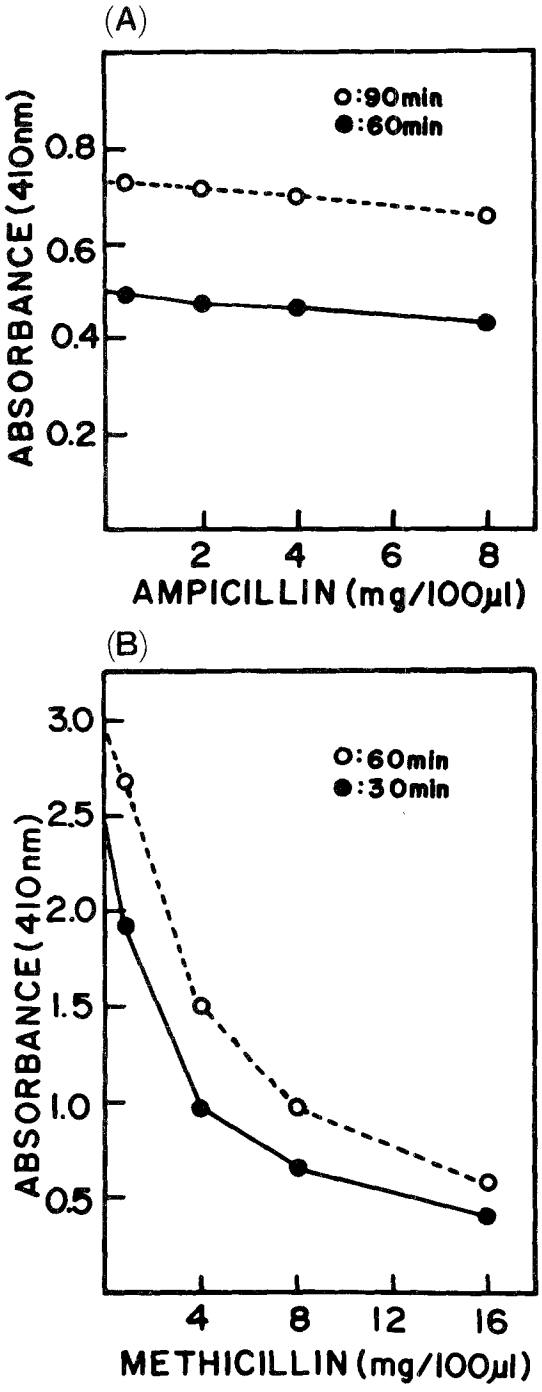

Fig. 1. Effects of Penicillins on Activity of Human Neutrophil Elastase; (A) ampicillin (B) methicillin.

Table 1. Percent Inhibition of 10mM Antibiotics against Human Neutrophil Elastase

Antibiotics	% Inhibition
Penicillins	
penicillin G	18.75
methicillin	50.98
cloxacillin	9.42
ampicillin	7.80
Cephalosporins	
cefazolin	11.99
cefamandole	66.91
cefoperazone	27.82
Aminoglycosides	
gentamicin	40.96
amikacin	26.80
kanamycin	10.71
Others	
oxytetracycline	98.55
chloramphenicol	41.24

Table 2. Activity of Antibiotics against Human Neutrophil Elastase

Antibiotics	IC_{50} (ug/ml)
Penicillins	
penicillins G	59.92
methicillin	10.00
cloxacillin	19.42
ampicillin	NA*
Cephalosporins	
cefazolin	NA*
cefamandole	5.85
cefoperazone	NA*
Aminoglycosides	
gentamicin	12.20
amikacin	16.60
kanamycin	257.40
Others	
oxytetracycline	0.25
chloramphenicol	14.56

NA* : Not Availble.

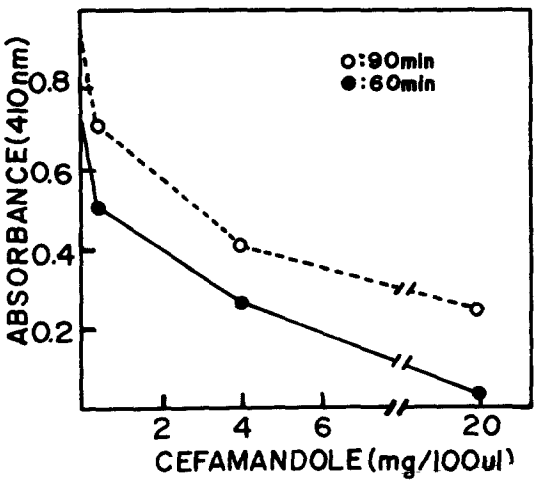

Fig. 2. Effects of Cefamandole on Activity of Human Neutrophil Elastase.

was gentamicin. 10mM of gentamicin inhibited human neutrophil elastase up to 40% of control activity, however 10mM amikacin and kanamycin inhibited 27% and 11% respectively (Table 1). Inhibition properties of kanamycin was almost negligible. It's IC_{50} was 257.40 μg/ml.

Oxytetracycline and chloramphenicol which are inhibitors of protein synthesis in microorganisms inhibited 98% and 41% respectively of human neutrophil elastase activity (Table 1). IC_{50} of oxytetracycline was 0.25 μg/ml.

DISCUSSION

Penicillins and cephalosporins are known as a inhibitors of cell wall synthesis against microorganisms to reveal their antibiotic

effects. Recently, Doherty, J. B. and his colleagues[2] reported that the activity of human leukocyte elastase can be modified by cephalosporins. Furthermore they demonstrated the ability to modify intradermal microvascular haemorrhage which was induced by injecting human PMN granules to rabbit with compound XIII which is one of the derivatives of cephalosporins. They also demonstrated the modification of the haemorrhage by inhibiting human leukocyte elastase activity with compound XIII in vitro. We could demonstrate the modification of human neutrophil elastase activity up to 50% with 10mM of methicillin and cefamandole which belong to penicillins and cephalosporins respectively. However, penicillin G, cloxacillin, ampicillin and cefazolin inhibit the human neutrophil elastase less than 20% of control activity. Similar concentration of same class of antibiotics, for example, cefazolin and cefamandole, or cloxacillin and methicillin, reveal almost same effect against microorganisms, but they showed different degree of inhibition effect on human neutrophil elastase in vitro. Therefore we can suggest that the mechanisms of action of these antibiotics to microorganisms and to the inhibition of elastase would be different.

We could demonstrate similar experimental result in other antibiotics, i.e., chloramphenicol and oxytetracycline which are known as inhibitors of protein synthesis in microorganisms. Oxytetracycline inhibited 99% of human neutrophil elastase at 10mM concentration, however, chloramphenicol inhibited only 40% of human neutrophil elastase at the same concentration. IC_{50} of oxytetracycline was 0.25 μ g/ml. It was very impressive result in controlling the activity of human neutrophil elastase. In aminoglycosides, amikacin and gentamicin showed moderate effect on the activity of human neutrophil elastase (IC_{50} : 16.6 and 12.2 respectively).

It is interesting point of view seeing different inhibition effect of some class of antibiotics on human neutrophil elstase. Overall, we can suggest that oxytetracycline, cefamandole and methicillin are strong inhibitors of human neutrophil elastase, and they could be a drug of choice for the diseases which are known the pathogenesis related to elastase. We also suggest that the mechanism of action of these antibiotics are not same as antimicrobial effects, i. e., inhibition of cell wall synthesis and protein synthesis. They may be related to the inhibition of destruction of extracellular matrix in various organs.

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