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Antibiotics; Methicillin, Cefamandole and Oxytetracycline, Can Modulate the Activity of Human Neutrophil Elastases

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ABSTRACT

Human neutrophil elastase (HNE, EC 3, 4 21, 11), a major causative factor in the induction of pulmonary emphysema, were purified by two steps of liquid chromatography. Purified elastases were cross-reacted with antibody to human neutrophil elastases.

Methicillin and cefamandole, which are known as inhibitors of cell wall synthesis of microorganisms, could inhibit the activity of human neutrophil elastase up to 50% with 10 mM of both agents and IC_{50} of methicillin was 9.8 mM. Gentamicin, one of the aminoglycosides, also inhibits human neutrophil elastases up to 60% of original activity with 10 mM of this agent and IC_{50} was 9.0 mM. We could demonstrate similar effects in oxytetracycline. 10 mM of oxytetracycline inhibited 95% of human neutrophil elastase and IC_{50} was 0.3 mM.

Overall, oxytetracycline, cefamandole and methicillin are strong inhibitors of human neutrophil elastase, and they could be a drug of cholice for the diseases which were known as pathogenesis related to elastase. We also suggest that the mechanism of action of these antibitics are different from the mechanism of antimicrobial effects like inhibition of both cell wall synthesis and protein synthesis.

Key Words: Neutrophil, Elastase, Antibiotics

Abbreviations: HNE, Human Neutrophil Elastase; PMN, Polymorphonuclear Leukocyte; SANA, N-Succinyl-L-Alanyl-L-Alanyl-L-Alanine p-Nitroanilide

INTRODUCTION

Human neutrophil elastases are present in the azurophil granules of human polymorphonuclear leukocytes (PMNs) (Dewald *et al.*, 1975). Normally, the granules fused with pagosomes containing engulfed foreign material, and human leukocyte elastase catabolizes the particles (Weissmann *et al.*, 1981). Under certain pathological conditions, however, PMN become attached host protein (elastin fibers, basement membrane, connective tissues, immune complexes) (Schmidt & Havemann, 1974; Starkey & Barrett, 1976), and in response to this adherence, the granules may fuse with the PMN outer membrane and release their contents directly onto the tissues, and its potential involvement of various inflammatory diseases such as emphysema and rheumatoid arthritis makes human neutrophil elastase inhibitors of considerable interest. It is known that penicillins and cephalosporins are inhibitors of bacterial cell wall synthesis, and aminoglycosides, tetracyclines and chloramphenicol are inhibitors of bacterial protein synthesis. We now report that methicillin, cefamandole, gentamicin, and oxytetracycline are potent inhibitors of HNE.

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MATERIALS AND METHODS

Materials

Hypaque, Ficoll, and N-Suc-(Ala)3-PNA (SANA) were purchased from Sigma. CM-Sephadex C-25 and Utrogel AcA_{54} were purchased from pharmacia and LKB respectively. Antibiotics were purchased from following companies: penicillin G (Kunhwa), methicillin (Daehan), cloxacillin (Yungjin) ampicillin and chlor-

amphenicol (Jongkundang), cefazolin and kanamycin (Dong-A), cefamandole (Daewoong-Lilly), cefoperazone and oxytetracycline (Pfizer), gentamycin (Kukje), amikacin (Bristol). All other chemicals were of the highest quality of obtainable.

Separation of human neutrophils

Human neutrophils were isolated from peripheral blood of healthy volunteer donors as described previously (Kang *et al.*, 1987).

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 C-25. Experimental details of purification of human neutrophil elastase and electrophoresis studies were described previously (Ghim *et al.*, 1989; Jeong *et al.*, 1987).

Elastase assay

After antibiotics were preincubated with human neutrophil elastase in the reaction medium containing 50 mM Tris-Cl, 150 mM NaCl and 5 mM $CaCl_2$, pH 7.3 for 30 minutes, the reaction was started by adding SANA. The activity was determined using a Titertek Multiskan Spectrophotometer (Flow Lab. Model MCC) at 410 nm 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

Inhibition of HNE by 10 mM of each of penicillin G, cloxacillin and ampicillin was not significant, less than 10% of control activity, but same concentration of methicillin inhibited up to 50% of original activity of HNE (Table 1), and the inhibition was dose dependent. IC_{50} of methicillin on HNE was 9.8 (Table 2).

Cephalosporins; cefazolin and cefoperazone inhibited HNE not significantly, *i.e.*, 10 mM of each of them inhibits HNE only 5% and 28% respectively, however, same concentration of cefamandole inhibited this enzyme up to 67% (Table 1) and inhibition was dose dependent. IC_{50} of cefamandole was 24.5 mM (Table 2).

Aminoglycosides; kanamycin and amikacin inhibited not significantly, i.e., 10 mM of each kanamycin and amikacin inhibited HNE only 5% or 7% respectively, but same concentration of

Table 1. Percent inhibition of 10 mM antibiotics on human neutrophil elastase

Antibiotics	% inhibition
Penicillins	
penicillin G	7.5
methicillin	51.7
cloxacillin	9.4
ampicillin	7.8
Cephalosporins	
cefazolin	5.3
cefamandole	66.9
cefoperazone	27.8
Aminoglycosides	
gentamicin	56.5
amikacin	7.2
kanamycin	5.1
Others	
oxytetracycline	95.7
chloramphenicol	4.3

Table 2. IC_{50} (mM) of antibiotics on activity of human neutrophil elastase

Antibiotics	IC_{50} (mM)
Penicillins	
penicillin G	130
methicillin	9.8
cloxacillin	19.4
ampicillin	NA*
Cephalosporins	
cefazolin	NA*
cefamandole	24.5
cefoperazone	NA*
Aminoglycosides	
gentamicin	9.0
amikacin	31.0
kanamycin	198
Others	
oxytetracycline	0.3
chloramphenicol	46

NA*: Not available

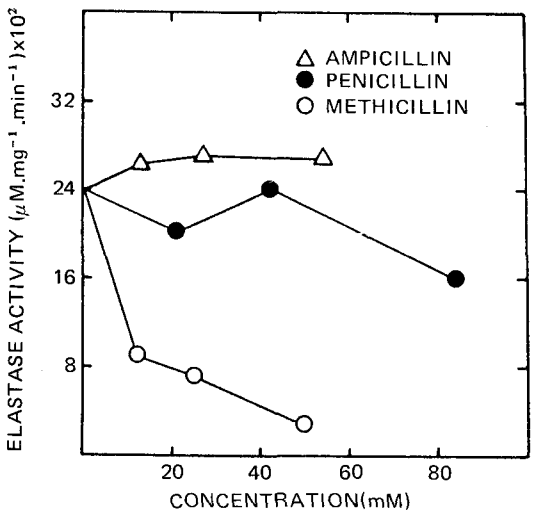

Fig. 1. Effects of penicillins on activity of human neutrophil elastase.

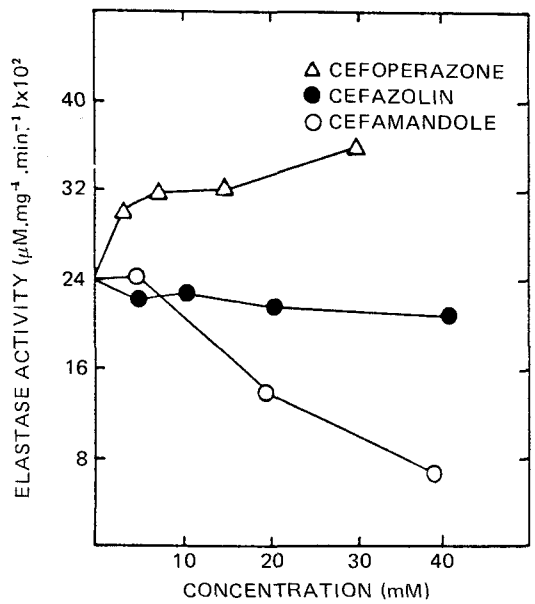

Fig. 2. Effects of cephalosporins on activity of huyman neutrophil elastase.

gentamicin inhibited HNE up to 50% of control activity. The inhibition of HNE by gentamicin was dose dependent.

Oxytetracycline and chloramphenicol, which

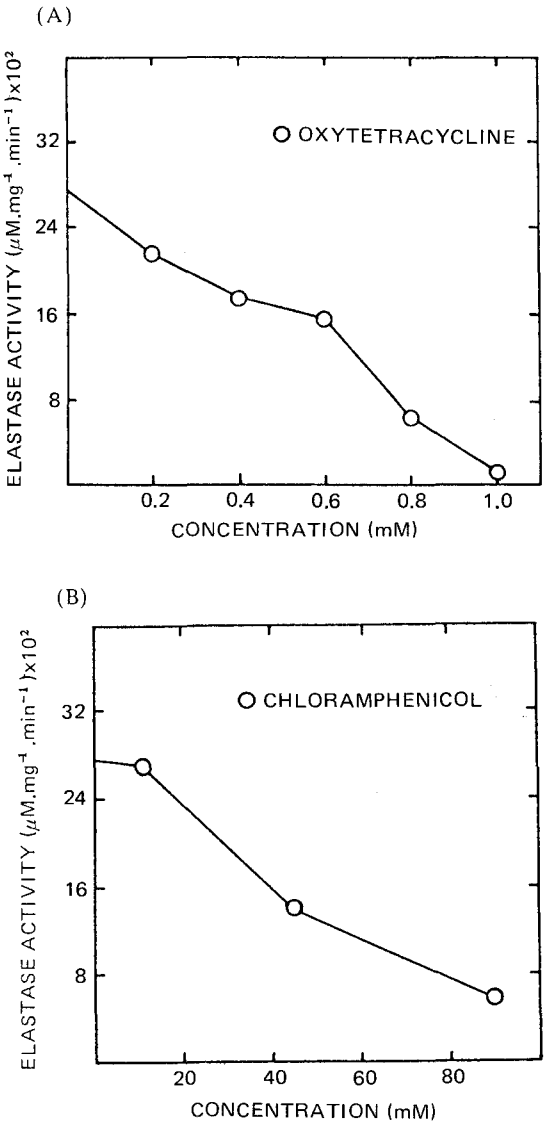

Fig. 3. Effects of (A) oxytetracycline and (B) chloramphenicol on activity of hunan neutrophil elastase.

are known as inhibitors of protein synthesis in microorganisms, inhibited HNE up to 96% and 4 % respectively (Table 1). Oxytetracycline showed most significant inhibition effect on the activity of HNE. Less than 1 mM of oxytetracycline inhibited 96% of control activity of HNE, and the inhibition was dose dependent (Fig. 3-(A). IC_{50} of oxytetracycline was 0.3 mM (Table 2).

DISCUSSION

Penicillins and cephalosporins are known as a inhibitors of cell wall synthesis against microorganisms to reveal their antibiotic effects. However, Doherty, J.B. and his colleagues (1986) reported that the activity of human leukocyte elastase could 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 the activity of human leukocyte elastase with compound XIII in vitro. We also could demonstrate the modification of HNE activity up to 50% with 10 mM of methicillin and cefamandole which belong to penicillins and cephalosporins respectively. However, penicillin G, cloxacillin, ampicillin, cefazolin, amikacin, kanamycin and chloramphenicol inhibited HNE less than 10 % 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, with same concentrations of these drugs, but they showed different degree of inhibition effect on HNE in vitro. Therefore we suggest that the mechanisms of action of these antibiotics to microorganisms and to elastase would be different.

We could demonstrate similar experimental result in other antibiotics, i.e., chloramphenicol and oxytetracycline. 10 mM of oxytetracycline inhibited 95.7% of HNE, however, same concentration of chloramphenicol inhibited only 4.3% of HNE. IC_{50} of oxytetracycline (0.3 mM) revealed extremely potent inhibitor on HNE and it was very impressive result in controlling the activity of HNE. In aminoglycosides, amikacin and gentamicin showed moderate effect on the activity of HNE (IC_{50}:31.0 and 9.0 respectively).

It is interesting point of view seeing different inhibition effect of some class of antibiotics on HNE. Overall, we suggest that oxytetracycline, cefamandole and methicillin are strong inhibitors of HNE. We suggest that the mechanism of action of these antibiotics may be not same as antimicrobial effects, inhibition of cell wall synthesis and protein synthesis. Their mechanisms of action would be same as inhibition on the active site of serine proteases. We also suggest the mechanism of action of tetracycline could be removal or freeing of structural metal ion, Zn, since tetracycline is powerful chelating characteristics. Strong inhibitors, oxytetracycline, cefamandole and methicillin may be related to the inhibition of HNE which was known as a major causative of destruction of extracellular matrix in various organs and may be a drug of choice in acute inflammatory diseases.

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=국문초록=

Methicillin, Cefamandole, Oxytetracycline에 의한 사람 호중구 Elastase의 변화

고신의대 약리학교실

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사람 혈액속의 elastase와 관련된 질병에 대한 연구는 다양한 저해제의 개발을 동반해 왔으며, 최근 항생제도 그 관심대상이 되고 있다. 두 단계의 액체 크로마토그래피를 거쳐 얻은 고순도의 elastase에 12종의 항생제를 처리하였다. 세포벽합성 저해제로 알려져 있는 penicillin계와 cephalosporin계 항생제를 각각 3종씩 처리한 결과, methicillin과 cefamandole은 10 mM 농도에서 elastase 활성을 50% 이상 저해하였지만, 나머지는 거의 10% 미만이었다. 단백질합성 저해제 중 oxytetracycline의 elastase에 대한 저해효과는 10 mM 농도에서 95% 이상으로 매우 탁월하였으며 (IC_{50} = 0.3 mM), gentamicin 도 50 % 이 상 저 해 하 였 으 나, 다 른 aminoglycoside 나 chloramphenicol은 역시 10%미만이었다.

실험해 본 항생제 가운데, oxytetracycline, cefamandole, methicillin, gentamicin 등은 elastase에 대한 강력한 저해제였으며, 그 작용기전은 항생제의 알려진 약리학적 기전과는 다른 차원의 모델임이 분명하였다.