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Inhibition of Human Leukocyte Cathepsin G by NSAIDs (Non-Steroidal Anti-Inflammatory Drugs)

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ABSTRACT

Human leukocyte cathepsin-Gs are active participant in the active phase of inflammations like rheumatoid arthritis, emphysema and glomerular injury. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment of these inflammatory diseases. Mechanism of action of NSAIDs for treatment of inflammatory diseases, especially like rheumatoid arthritis, are known as the inhibitors of prostaglandin synthesis. Inhibitions of the activities of human leukocyte cathepsin-Gs by non-steroidal anti-inflammatory drugs, however, were not same as the known pharmacological effects (inhibition of cyclooxygenase) of these drugs. Among them, especially, sulindac, salicylate, phenylbutazone, oxyphenbutazone, and salicyluric acid inhibited human leukocyte cathepsin-Gs effectively. IC_{50}s of each drug were 4.3mM, 14.3mM, 6.5mM, 11mM and 15mM respectively. The drugs which have same chemical structure and same degree of inhibition effect on cyclooxygenase showed different degree or no effect on inhibition of cathepsin G. These inhibition effect might be, beside of inhibition of cyclooxygenase in the prostaglandin synthesis pathway, another benefitial antiinflammatory effect of NSAIDs by direct protection against tissue destruction in inflammatory diseases.

Key Words: Human Leukocyte Cathepsin G, Non-Steroidal Anti-Inflammatory Drugs

INTRODUCTION

Cathepsin Gs, cationic serine neutral proteases, are located in azurophil granule of human neutrophil (Deward *et al.*, 1975). Cathepsin Gs have antimicrobial activities by destroying the microorganism with their enzymatic properties (Casey *et al.*, 1985; Shafer *et al.*, 1986). However, imbalance between the activity of cathepsin G and inhibition effect of α_1-PI (α_1-protease inhibitor) or α_2-MG (α_2-macroglobulin), causes destruction of normal tissues and can cause rheumatoid arthritis and many other inflammatory diseases (Barrett, 1978). For treatment of these inflammatory events, NSAIDs have been used because of its analgesic, antipyretic and antiinflammatory effect. The mechanism of action of these drugs has been known that NSAIDs inhibit cyclooxygenase in prostaglandin synthesis pathway (Roderic *et al.*, 1985). But it may be possible that NSAIDs also inhibit cathepsin G activity which may partially be direct etiologic factor of these inflammatory process. In this report, we discribe the effect of inhibition of the activity of cathepsin G with various classes of NSAIDs.

MATERIAL AND METHODS

Materials

L-Benzoyl-DL-Phenylalanine-p-Naphthylamine (BPNE), N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (SAPNA), Fast Garnet GBC Base, Brij 35, N-Succinyl-L-Ala-L-Ala-L-Alanine-p-Nitroanilide (SANA), and NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) were purchased from Sigma and Ultrogel AcA54 was purchased from LKB. All other chemicals were of the highest quality of obtainable.

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Purification and identification of cathepsin G

Method for purification of human neutrophil cathepsin G from peripheral blood of healthy dornors was same as described previously (Kang *et al.*, 1987). Cathepsin Gs were purified by two step purification procedure involving ultrogel AcA54 gel filtration and CM-sephadex ion exchange chromatography. These enzymes were identified by their molecular weights after purification with PAGE and cross-reactions with anti-human cathepsin G antibody.

Cathepsin G assay

NSAIDs were preincubated with human neutrophil cathepsin G in the reaction medium containing 150mM NaCl, 100mM Tris-Cl (pH 7.3), and 12.5% DMSO for 30 minutes. The reaction was started by adding SAPNA (N-Succinyl-Ala-Ala- Pro-Phe-p-Nitroanilide). The activity was determined by measuring the quantity of the p-nitroanilide released from the synthetic substrate by monitoring absorbance at 410nm using spectrophotometer.

RESULTS

5mM of ibuprofen, one of the arylalkanoic acid derivatives, inhibited 48% of the activity of cathepsin G. However, ketoprofen and naproxen which also belong to the arylalkanoic derivatives, did not inhibit cathepsin G at all. Phenylbutazone and oxyphenbutazone, pyrazolone derivatives, inhibited the activity of cathepsin G (Fig. 1). IC_{50}s of phenylbutazone and oxyphenbutazone were 6.5mM and 11mM respectively. In the case of indol derivatives, sulindac inhibited the activity of cathepsin G very effectively, but indomethacine did not (Fig. 2.) IC_{50} of sulindac was 4.3mM, and 14mM of sulindac almost completely inhibited the activity of cathepsin G.

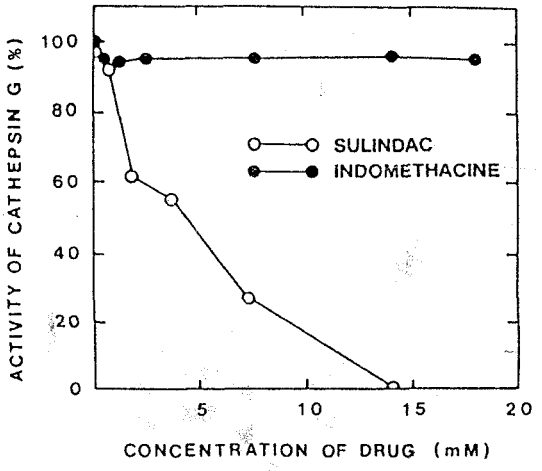

Fig. 2. Effects of Indole derivatives on activity of human leukocyte cathepsin G.

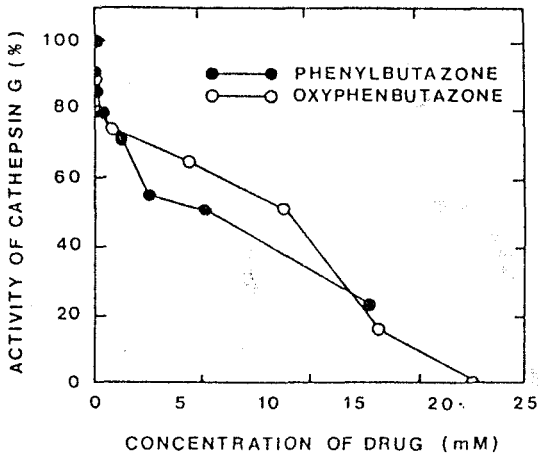

Fig. 1. Effects of Pyrazolone derivatives on activity of human leukocyte cathepsin G.

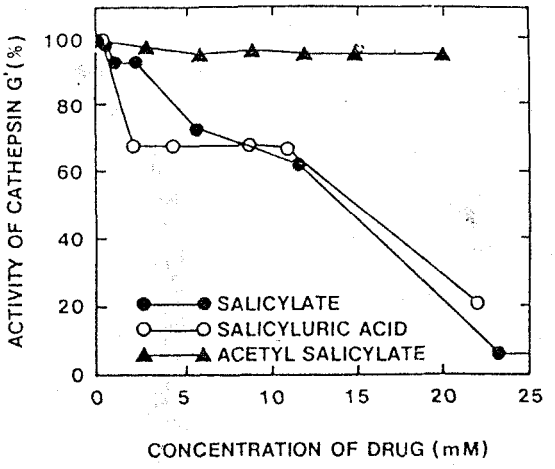

Fig. 3. Effect of Salicylates on activity of human leukocyte cathepsin G.

Among the salicylates, salicylic acid and salicyluric acid, the active metabolite of aspirin, inhibited the activity of cathepsin G moderately. IC_{50}s were 8.3mM and 15mM respectively (Fig. 3). However, aspirin, acetylated form of salicylate, did not inhibit the activity of cathepsin G.

DISCUSSION

NSAIDs has been used world wide for treatment of rheumatoid arthritis and non-suppurative inflammation. These agents not only relieve pain and inflammation but also help homeostasis of articular cartilage (Rederick *et al.*, 1985, Lentini *et al.*, 1987). It has been known that anti-inflammatory effect of NSAIDs are associated with inhibition of cyclooxygenase in prostaglandin synthesis pathway (Roderick *et al.*, 1985), but it may also be partially possible that inhibition of cathepsin G, which has been known as an enzyme for tissue destruction at the inflammatory sites, would be another possible mechanism of action of these drugs (Lentini *et al.*, 1987). Among these drugs, sulindac showed most powerful inhibition effect on human neutrophil cathepsin G with 4.3mM of IC_{50} (Fig. 2). Phenylbutazone, oxyphenbutazone, salicylate, salicyluric acid also were effective inhibitors of cathepsin G. The inhibition concentration of these drugs were higher than therapeutic plasma concentrations were, however it may be also effective in vivo since the concentration of drugs in inflammtory site may be maintained much higher than that of plasma. Salicylates, showed entirly different tendency on the activity of cathepsin G comparing with the inhibition of cyclooxygenase. Salicylate inhibited the activity of cathepsin G but aspirin did not (Fig. 3). Comparing the chemical structures of cathepsin G and salicylate, hydroxyl group of the ortho site of benzene ring of salicylate is intact but the hydoxyl group of the aspirin is acetylated. Thereby, we suggest that the hydroxyl group of salicylate may compete with the hydroxyl group of the active site of cathepsin G. This possible mechanism can be also applied to the salicyluric acid since this agent also have hydroxyl group on the ortho position of the aromatic ring. Even through aspirin did not show any effect on the inhibition of the activity of cathepsin G in vitro, it might have pharmacological effectiveness since aspirin can be converted to salicylate and salicyluric acid in plasma (Cleland *et al.*, 1980; Saklatvala & barrett, 1985). Indole derivatives, sulindac and indomethacine are well known agents as inhibitors of cyclooxygenase, but the inhibition effects on the activity of cathepsin G were different. Unlike to salicylates, it is difficult to interpretate the mechanism of action of these agents since the structure of both agents are much more complex than salicylates are. Over all, the fact that drugs belong to the same class showed different degree of inhibition on the activity of cathepsin G may implies another possibility of antiinflammatory effect of NSAIDs, ie inhibition of cathepsin G may partialy involves in protecting tissue destruction at inflammatory sites.

In conclusion, we suggest that the mechanism of anti-inflammtory effects of NSAIDs may partially be involved by inhibition of the activity of cathepsin G which may directly cause destruction of normal human tissue beside of the inhibition of cyclooxygenase in prostaglandin synthesis pathway.

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=국문초록=

NSAIDs (Non-Steroidal Anti-Inflammatory Drugs)에 의한 사람 중성구 Cathepsin G의 활성도 억제

고신의대 약리학교실

배성준 · 김사열 · 강구일

사람 중성구내의 azurophil granule 내에 존재하는 serine protease인 cathepsin G는 정상 반응에서는 항 박테리아 작용을 나타내는데 관여하지만, 이들의 효소활성이 비정상적으로 증가되었을 때는 오히려 인체 정상 조직을 파괴함으로써 rheumatoid arthritis를 비롯한 여러가지 염증성 질환을 야기시킨다고 알려져 있다. 항염증제로 작용하는데 있어서 prostaglandin 합성을 억제하는 작용 이외에 다른 작용 기전이 있는가 하는것은 대단히 흥미있는 연구 과제이었으므로 neutral protease 중의 하나인 cathepsin G와 이 염증반응에 직접적으로 관여하는지 알아보기 위하여 본 연구에서는 두 단계의 크로마토그라피를 거쳐 순수한 cathepsin G를 분리하고, 여러가지 비스테로이드성 항염증제를 이용하여 cathepsin G에 대한 억제 정도를 관찰하였다. 이중 sulindac, salicylate, phenylbutazone, oxyphenbutazone 그리고 salicyluric acid가 각각 4.3mM, 14.3mM, 6.5mM, 11mM, 15mM의 IC_{50}로써 cathepsin G의 활성도를 억제하였다. 따라서 NSAIDs의 항염증 작용 기전은 기존에 알려지고 있는 cyclooxygenase 억제에 따른 prostaglanndin 합성, 분비 억제 기전이외에 rheumatoid arthritis 부위에 직접적 원인으로 작용할 가능성이 있는 cathepsin G를 억제 함으로써 조직 파괴를 막는 역할을 하고 있을 가능성이 있는 것으로 사료된다.