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Inhibition of human granulocyte elastase by gold sodium thiomalate*

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THE EFFECT of gold salts and other anti-inflammatory drugs on the release and activity of lysosomal hydrolases has been the subject of several recent studies. Uptake of colloidal gold salt by phagocytosis has been reported to result in inhibition of acid-phosphatase and β-glucuronidase in guinea pig peritoneal macrophage lysosomes.[1] Gold compounds also inhibit acid-phosphatase, β-glucuronidase and cathepsin obtained from rabbit liver lysosomes and synovial fluids of the knee joints of patients with rheumatoid arthritis.[2] On the basis of these findings, it has been suggested that a possible mechanism of gold action in rheumatoid arthritis is the inhibition of intra-articular hydrolases, either within the joint fluid[2] or after uptake of the drug into lysosomes of phagocytic cells in the inflamed synovial tissue.[1]

Human neutrophil lysosomes contain neutral proteases[3] capable of breaking down basement membrane,[4] protein-polysaccharide,[5] elastin[6] and collagen.[7] We recently obtained evidence that the elastolytic protease of human granulocyte lysosomes, rather than the collagenase of these cells, is primarily responsible for effective solubilization of basement membrane and production of vascular damage.† Thus, in addition to a probable role in elastic fiber degeneration in acute arteritis, the elastolytic protease of human leukocytes may also play a broad role in inflammation.

Accordingly, the present experiments were undertaken to test several anti-inflammatory drugs, including gold salt, as possible inhibitors of human granulocyte elastase. Drugs tested were as follows: hydrocortisone sodium succinate (Solu-Cortef, Upjohn Co.); acetyl salicylic acid, phenylbutazone (Butazolidin, Geigy Pharmaceuticals); indomethacin (Indocin, Merck, Sharp & Dohme Research Lab., Division of Merck & Company); and gold sodium thiomalate (Merck & Company). In each case, the pure, active ingredient was obtained in powder form, free of inert carrier material and preservatives.

Human peripheral blood leukocytes were collected and their granules harvested and extracted as described in an earlier report.[6] Assays of elastolytic activity of granule extracts were performed using orcein-dyed elastin substrate (Worthington Biochemical Corp.) according to the method of Sachar *et al.*,[8] with modifications as described before.[6] Hydrolysis of *p*-nitrophenyl-*t*-boc-l-alaninate (NBA) a synthetic substrate for pancreatic[9] and leukocyte[10] elastases, was performed by techniques published elsewhere.[10]

The method of testing each drug for inhibition of elastolysis and esterolysis was as follows. Compounds were dissolved directly in the buffers normally employed for enzyme–substrate incubation (buffers are described in Table 1). The resultant solutions maintained the pH of the original buffer, except for indomethacin (free acid) which persisted as a suspension until neutralized with added base (NaOH). Phenylbutazone was not soluble in phosphate buffer and was therefore dissolved initially in acetonitrile and subsequently diluted in buffer (final concentration of acetonitrile, 2·5%; v/v). Extracts of leukocyte granules were prepared in the same buffers and mixed with aliquots of the drug stock solutions to achieve the final inhibitor concentrations shown in the table. Incubation (37°) of the enzyme–drug mixtures was carried out for various times, as shown in Table 1, prior to the addition of substrates. In elastase assays, aliquots of enzyme–drug mixture containing 300 μg of granule protein were added to 5 mg of elastin-orcein substrate and incubation was allowed to proceed for 2 additional hr at 37°. Elastolytic activity of leukocyte granules was previously shown to remain linear during the first 2 hr of incubation.* Under these conditions, about 0·6 mg of elastin protein (12 per cent of the starting weight of substrate) is solubilized in the absence of drug inhibition. Esterase activity was measured by removing aliquots (25 μg of granule protein) from the preincubation mixtures at regular intervals and assaying against NBA. This amount of granule protein normally hydrolyzes the synthetic ester at a rate equivalent to that given by 10 μg of crystalline pan-

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† A. Janoff, unpublished observations.

TABLE 1. EFFECT OF GOLD SODIUM THIOMALATE ON ELASTASE AND NBA-ESTERASE ACTIVITIES OF HUMAN LEUKOCYTE GRANULES

Drug concn. in pre-incubation (mg/ml)	Pre-incubation time (min)	Elastolysis (orcein-elastin)			Esterolysis (NBA)		
		No. assays	Δ E 590/2 hr*	% Inhibition	No. assays	Δ E347·5/30 sec†	% Inhibition
0·0	0	20	0·259 ± 0·041				
0·0	15	6	0·246 ± 0·043				
0·4	15	2	0·165	33			
1·0	15	4	0·091 ± 0·014	63			
0·0	0				10	0·023 ± 0·002	
0·0	60–180‡				5	0·014 ± 0·001	
0·2	60–180				7	0·010 ± 0·001	20–30
0·8	60				2	0·008	40
1·0	120				1	0·008	40
1·4	60				2	0·007	50
2·0	60				2	0·007	50

* Final increase in absorbance of orcein solubilized from the substrate (measured after 2 hr of incubation, during which enzyme activity remains linear with time).
† Average increase in absorbance of *p*-nitrophenol released from the substrate (measured at 30-sec intervals over 2 min of incubation, during which enzyme activity remains linear with time).
‡ NBA-esterase activity of control enzyme (no drug) decreased to value shown after 1 hr of preincubation at 37°. Thereafter, no further decrease occurred up to 3 hr.
Buffers: elastolysis . . . tris-HCl, 0·2 M, pH 8·5; esterolysis . . . Na phosphate, 0·05 M, pH 6·5.

creatic elastase. Enzyme solutions preincubated without drugs and solutions of drugs alone were included in the elastase and esterase assays for control purposes. Siliconized glassware was used throughout these procedures.

Hydrocortisone, phenylbutazone and indomethacin, in concentrations ranging from 0·1 to 1·0 mg/ml and in two different buffer systems (described in Table 1), failed to inhibit elastolysis by leukocyte granules in my experiments. Phenylbutazone and indomethacin were also ineffective against esterolysis of NBA by granule extracts. Preincubation times were 15 min in the case of the hydrocortisone and ranged from 15 min to 3 hr in the case of the nonsteroidal agents. The same results were obtained with acetylsalicylic acid, except that the latter was tested in concentrations of 0·01 to 0·1 mg/ml. On the other hand, gold salt proved to be an effective inhibitor of both elastolysis and esterolysis by leukocyte granules. The results of experiments with this agent are summarized in Table 1.

The concentration range of gold salt used to demonstrate inhibition in these experiments was approximately 5×10^{-4} to 5×10^{-3} M. It is unlikely that gold compounds reach this concentration in synovial or other tissue fluids of patients treated with the drug. However, such concentrations may be attained within the lysosomes of leukocytes which have phagocytosed and sequestered gold salts within their cytoplasmic granules. Thus, an effective concentration of the inhibitor could be reached at the sites of enzyme storage in inflammatory cells.

In conclusion, five anti-inflammatory agents were tested for possible inhibition of the elastolytic and esterolytic activities of human leukocyte granules. Hydrocortisone, acetylsalicylic acid, phenylbutazone and indomethacin were ineffective. Of the compounds tested, only gold sodium thiomalate possessed inhibitory activity against these granule-mediated reactions.

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Effects of hydroxamic acids on L-histidine carboxy-lyase*

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THE HYDROXYLAMINE derivative, 4-bromo-3-hydroxybenzyloxyamine (NSD-1055), is a potent inhibitor of L-histidine carboxy-lyase (histidine decarboxylase, HD),[1] and reduces tissue histamine levels in

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