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THE EFFECT OF ANTIARTHRITIC DRUGS AND RELATED COMPOUNDS ON THE HUMAN NEUTROPHIL MYELOPEROXIDASE SYSTEM

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Summary: Human α-1-proteinase inhibitor is inactivated by human myeloperoxidase in the presence of hydrogen peroxide and chloride ion. Several antiarthritic drugs and related compounds, including many containing gold, were tested as inhibitors of the myeloperoxidase system. Of the twenty-six compounds used, twenty-two inhibited. The most important feature of these was the presence of a sulfhydryl group. The most effective compounds also were the most hydrophobic. The presence of gold, on the other hand, made little difference to the amount of inhibition. These drugs appear to have many effects, and their inhibition of the myeloperoxidase system suggests that this could be one of them.

Human rheumatoid arthritis and emphysema are diseases of chronic and acute inflammation of the joints and lungs with eventual proteolytic degradation of the affected tissues. An important feature of inflammatory lesions is the accumulation of various cell types, including phagocytic cells, which reach the site of inflammation in the circulating blood, pass through the vascular walls, and move into extracellular spaces. Polymorphonuclear leukocytes (PMN), which are phagocytic cells, contain both proteolytic (elastase and cathepsin G) and oxidative (myeloperoxidase) enzymes which are normally stored in cytoplasmic granules. However, upon encountering immune complexes, activated complement factors, or opsonized antigen, rapid degranulation associated with phagocytosis occurs and often leakage of the enzymes into the tissues themselves. Normally, the major plasma proteinase inhibitor, α-1-proteinase inhibitor (α-1-PI) which can also penetrate vascular walls, exerts control on connective tissue proteolysis. However, because of the massive infiltration of PMN leukocytes and the oxidative inactivation of α-1-PI by the myeloperoxidase-H_2O_2-Cl^- system (1,2) (operative during phagocytosis), the ability of tissue levels of this inhibitor

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to protect tissues from proteolysis becomes markedly reduced. Thus uncontrolled digestion of connective tissues may occur resulting in severe damage associated with diseases such as rheumatoid arthritis and emphysema. Indeed, evidence has been presented that this process does occur *in vivo*, since inactive, oxidized α-1-PI has been detected and isolated from both rheumatoid synovial fluid (3) and lung lavage fluid of patients with pulmonary emphysema (4).

Various drugs have been used for the treatment of rheumatoid arthritis. The approach has been a pragmatic one with attempts later made to improve the beneficial effect by modification of the original drug. Organic gold compounds and D-penicillamine are agents which have been useful in arresting the progress of the disease, unlike aspirin which acts only as an analgesic. Auranofin, a recently developed compound, appears to be less toxic than earlier antiarthritic drugs. The mechanism or site of action of these compounds, however, is unknown, though they appear to have many effects. Since they arrest the progress of the disease and thus presumably proteolytic degradation, hypothetically they might be acting as antioxidants inactivating the myeloperoxidase system, thereby sparing α-1-PI to continue to control proteolysis.

In the current paper, the effectiveness of twenty-six antiarthritic drugs and related compounds in inactivating the myeloperoxidase system *in vitro* is reported.

Materials and Methods

Materials

Human myeloperoxidase and α-1-PI were prepared as described previously (5,6). Porcine pancreatic elastase, glucose oxidase, tert-butoxy-carbonyl-L-alanyl p-nitrophenyl ester, and all inhibitor compounds except those containing gold were purchased from Sigma Chemical Co. The gold compounds were a gift from Dr. Blaine Sutton, Smith, Kline & French Laboratories, Philadelphia, PA.

Methods

The inactivation of α-1-PI by the myeloperoxidase system was performed essentially as described by Matheson et al. (1) except that glucose and glucose oxidase were used to generate H_2O_2. The standard reaction mixture contained 2.84 μM α-1-PI, 0.86 nM myeloperoxidase, 60 mM glucose, 1.25 U/ml glucose oxidase, 0.16 M NaCl, and 0.2 M Na phosphate buffer, pH 6.1 with a final volume of 0.5 ml. The treatment and subsequent assay of the aliquots removed were described before(1).

The gold and related compounds were prepared in Na phosphate buffer, pH 6.1, directly, or in neat dimethyl sulfoxide or acetone with subsequent dilution in

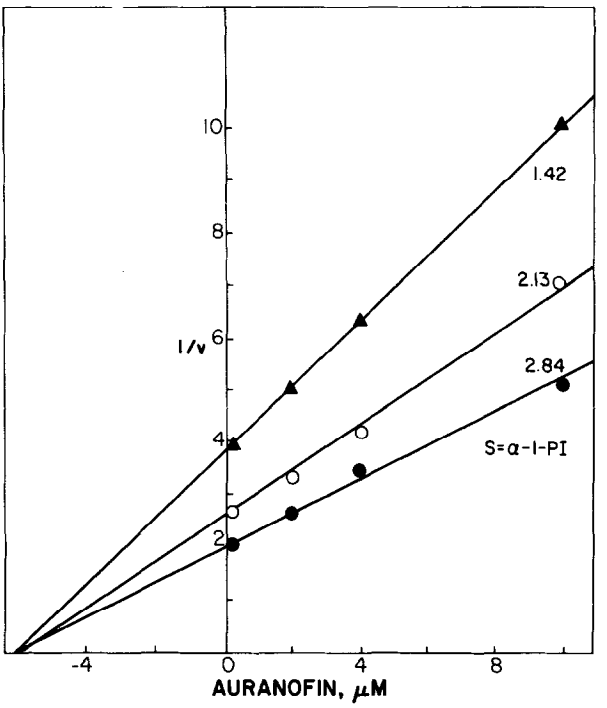


Figure 1: Kinetic plot of auranofin inhibiton of the myeloperoxidase system. The concentrations of auranofin indicated were incubated with the standard reaction mixture as described in Methods with three concentrations of α-1-PI: ●, 2.84 μM; ○, 2.13 μM; ▲, 1.42 μM.

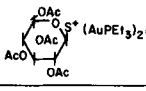
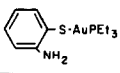
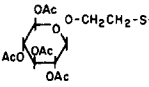
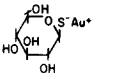
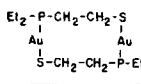
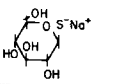
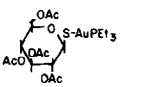
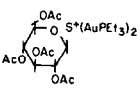
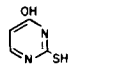
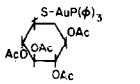
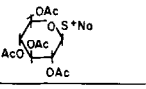
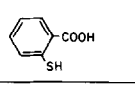
buffer to a final concentration of 0.5 to 5% of solvent. At these concentrations, the solvents themselves had no effect on any component of the reaction mixtures. Final concentrations (usually three) of the compounds in the reaction mixtures were such that the rates of inactivation of α-1-PI could be followed easily over the time of incubation.

Results and Discussion

When the myeloperoxidase-H_2O_2-Cl^- system is incubated with α-1-PI, the inhibitor rapidly loses activity (1). Addition of twenty-two of the compounds tested to the reaction mixtures resulted in reduction in the loss of α-1-PI activity. In order to determine which structures were important for inactivating the myeloperoxidase system, inhibition constants (K_i's) were calculated for several related compounds. A plot of 1/v versus i for auranofin at three concentrations of the substrate (α-1-PI) (Fig.1) indicate that it is a non-competitive inhibitor. Similar plots of the other compounds are in agreement.

The structures, and K_i's derived from the kinetic plots of the compounds, are listed in Table 1. It is obvious that the essential feature of these compounds is the presence of a sulfhydryl group. Four compounds which lack such a

TABLE 1: THE EFFECT OF ANTIARTHRITIC DRUGS AND RELATED COMPOUNDS ON THE MYELOPEROXIDASE SYSTEM

COMPOUND	K_i, μM	COMPOUND	K_i, μM	COMPOUND	K_i, μM
 1.	1.8	 8.	18.4	L-cysteine 14.	170
 2.	4.4	 9.	20.0	Glutathione 15.	175
 3.	4.9	 10.	33.0	Gold thiomalate 16.	180
 4.	6.2	 11.	41.0	Sodium thiomalate 17.	240
 5.	9.6	 12.	59.0	Triethylphosphine gold chloride 18.	272
 6.	11.6	Et = Ethyl Ac = Acetate ϕ = Phenol		bis(triethylphosphine) gold chloride 19.	287
 7.	13.2	D-or L-penicillamine 13.	66.0	5-thio-D-glucose 20.	1100
				NADH 21.	1500
				NADPH 22.	4100

1. [μ-(1 -thio- β-D-glucopyranose) 2,3,4,6-tetraacetato-S:S] bis (triethylphosphine) digold (1+) nitrate
2. (2,3,4,6-tetra-O-acetyl-1-thioethyl-β-D-glucopyranosato-S) gold
3. bis [μ- 2-(diethylphosphino) ethanethiolato-P:S] digold
4. (2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-S) (triethylphosphine) gold (auranofin)
5. 2-thiouracil
6. 2,3,4,6-tetraacetyl-β-D-thioglucose (thioglucose tetraacetate)
7. 2-mercaptobenzoic acid (thiosalicylic acid)
8. (2-aminobenzenethiolato-N,S) triethylphosphine gold
9. (1-thio-D-glucopyranosato) gold (gold thioglucose)
10. 1-thio-β-D-glucose (thioglucose)
11. [μ-(1-thio-β-D-glucopyranose) 2,3,4,6-tetraacetato-S:S] bis (triethylphosphine) digold (1+) chloride
12. (1,2,3,4-tetra-O-acetyl-6-thio-β-D-glucopyranosato-S) triphenylphosphine gold
13. bis [1,2-bis (diethylphosphino)-ethane] digold dichloride

group (gold sodium chloride, triethylphosphine oxide, sodium malate, and 2,2'-dithiodipyridine) (not shown) were totally ineffective. The compounds with the largest K_i's also lack a sulfhydryl group, although NADH and NADPH are also reducing agents.

Another important feature appears to be the presence of hydrophobic groups. Compounds with tetraaceylated glucose are more hydrophobic and thus more effective than compounds with glucose. Additional hydrophobic groups, including triethyl-

phines, are found in the compounds with the lowest K_i's in Table 1. Compounds with short carbon chains and hydrophilic carboxyl groups are much less effective than the hydrophobic compounds. Perhaps this requirement for hydrophobicity is involved in the binding of the compounds to myeloperoxidase.

The presence of gold, however, does not appear to be necessary to the inhibition of the myeloperoxidase system. Comparison of compounds which are similar except for the presence or absence of gold indicates that the K_i's are only slightly lower if gold is present.

Control experiments were also performed in which the effects of the compounds were determined for the partial reactions of the total multienzyme system. None of the partial reactions (porcine pancreatic elastase - tert-butoxycarbonyl-L-alanyl p-nitrophenyl ester, α-1-PI - porcine pancreatic elastase, or myeloperoxidase - glucose - glucose oxidase - guaiacol) were affected by the compounds tested. The lack of effect on myeloperoxidase alone may indicate that these compounds interfere only with an intermediate formed when myeloperoxidase, H_2O_2, and Cl^- react. Also since these are non-competitive inhibitors, they must be binding not at the active site but elsewhere on the enzyme.

The four antiarthritic drugs (auranofin, gold thioglucose, D-penicillamine, gold thiomalate) are all apparently equally effective in the treatment of individuals with rheumatoid arthritis although their in vitro inhibition of the myeloperoxidase system appears to be quite different. However, in vivo, these gold compounds bind to various proteins (7), and collect in various tissues, including the lysosomes of phagocytic cells (8,9). Thus, perhaps the local concentrations of these drugs may actually be high enough for all to be equally effective in inhibiting the myeloperoxidase system.

Various kinds of in vitro experiments indicate that gold compounds have a multitude of effects including suppression of formation or release of chemical mediators of inflammation (10), cell production in bone marrow (11), chemotaxis and cell migration (12), phagocytosis (13,14), and interference with immunologically-mediated processes (14,15). Many of these effects appear to require the presence of gold in the drug. However, the effect on enzymes apparently requires only the presence of a sulfhydryl group. As reported in the present paper, the

thiol moiety in the important feature, not the gold portion. It had previously been noted by others that the inactivation of serum elastase inhibitory capacity was prevented by D-penicillamine, gold thioglucose, and gold thiomalate (16). Gold thioglucose and gold thiomalate have also been shown to inhibit the lysosomal enzymes, β-glucuronidase and acid phosphatase, with reversal by cysteine (17). In fact, there is evidence that the gold is released from these compounds and is bound to various protein fractions, while the remaining portion of the molecules inhibits many enzymes by virtue of the thiol group (7).

The experiments detailed in this paper indicate that antiarthritic agents, in addition to their many other effects, might also be acting to prevent uncontrolled proteolysis through prevention of the oxidation and inactivation of α-1-PI, one of the major proteinase inhibitors from plasma. Since the etiology of emphysema is apparently the same, these antiarthritic drugs might be effective as antiemphysematic drugs as well, though this hypothesis has not yet been tested.

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