Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 101-108

# INHIBITION OF HUMAN NEUTROPHIL COLLAGENASE BY GOLD(I) SALTS USED IN CHRYSOTHERAPY

Satish K. Mallya and Harold E. Van Wart

Institute of Molecular Biophysics and
Department of Chemistry,
Florida State University,
Tallahassee, Florida 32306

Received January 30, 1987

SUMMARY: Six gold(I) salts, some of which are used as drugs in chrysotherapy, are shown to be inhibitors of two forms of human neutrophil collagenase. The $IC_{50}$ values vary over six orders of magnitude, the lowest being 3.5 nM for Myocrisin. Thus, inhibition is greatly affected by the identity of the ligands to the gold(I) atom. The inhibition of collagenase by these gold(I) salts may be a partial basis for their anti-arthritic action. 

Chrysotherapy, the use of gold(I) salts to treat rheumatoid arthritis, has been a useful clinical tool for over 50 years (1). The earliest chrysotherapeutic drugs were gold(I)-thiol complexes such as Sanocrisin, Myocrisin and Solganol that were administered parenterally (2). The recent development of orally active gold(I) salts with one or more phosphine ligands such as Auranofin (3-5) has stimulated renewed interest in the chemistry, pharmacology and clinical action of these drugs.

The mechanism by which gold(I) salts induce arthritic remission is generally believed to involve alteration of inflammatory cell function (6-9). Neutrophils constitute over 90% of the cells in the synovial fluid of arthritic joints (10) and are found at the panus-cartilage junction (11). Since neutrophils contain proteinases capable of connective tissue destruction (12-15), these cells may

0006-291X/87 $1.50

contribute markedly to the pathophysiology of arthritic joints. Indeed, one often postulated mechanism of action of gold(I) salts is the inhibition of neutrophil proteinase release and/or activity (16-24). In this study, we demonstrate that six gold(I) drugs are inhibitors of human neutrophil collagenase (HNC).

## MATERIALS AND METHODS

Solganol (aurothioglucose), thioglucose, Myocrisin (sodium gold thiomalate), thiomalic acid and 1-Thio-β-D-glucopyranose 2,3,4,6-tetraacetate (thioglucose tetraacetate) were purchased from Sigma Chemical Company, Sanocrisin (sodium gold thiosulfate) was obtained from Pfaltz and Bauer, triethylphosphine oxide from Aldrich and sodium thiosulfate from J. T. Baker. Auranofin [(1-Thio-β-D-glucopyranose 2,3,4,6-tetraacetato-S)(triethylphosphine) gold, SKF D-39162], chloro(triethylphosphine) gold (SKF 36914) and bis(triethylphosphine) gold chloride (SKF 80544) were gifts of Smith, Kline and French Laboratories. Latent HNC was purified from a crude human neutrophil extract that had been treated with phenylmethylsulfonylfluoride (PMSF) by sequential chromatography over Reactive Red-120 Agarose (Sigma), Sephacryl S-200 and an affinity resin consisting of Pro-Leu-Gly-NHOH immobilized on CH-Sepharose 4B (25). The active enzyme was isolated by the same procedure, except that PMSF was not added to the neutrophil extract to inhibit proteolysis.

Collagenase activity was measured by monitoring the initial rate of hydrolysis of soluble [$^3$H]acetylated type I rat tail tendon collagen in solution (26,27). Assays were carried out at 30°C in 50 mM Tricine, 10 mM $CaCl_2$, 0.5 μM $ZnSO_4$, 0.2 M NaCl, pH 7.5 at a substrate concentration of 100 μg/ml. Latent HNC was activated with 0.1 mM p-chloromercuribenzoate (PCMB) for 30 minutes prior to the inhibition experiments. Samples of HNC were incubated with the gold(I) compounds in 960 μl of assay buffer for five minutes after which the assay was started by addition of 40 μl of collagen stock solution. Auranofin and SKF 36914 were dissolved in methanol and the inhibition studies performed so that the final methanol concentration in the assay was 2% v/v, conditions under which HNC is over 90% active. The inhibition results are expressed as percentages of the activity of control assays in which no gold(I) compounds were present.

## RESULTS

The structures of the six gold(I) salts examined in this study, as suggested from their molecular formulas, are shown in Figure 1. Gold(I) is usually bidentate and recent EXAFS

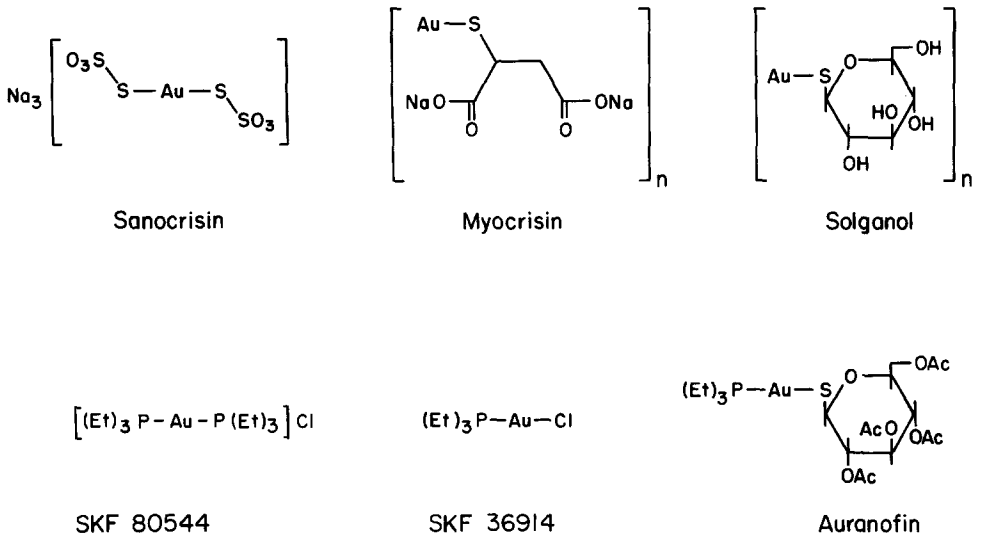


Figure 1. Structures, as indicated from their molecular formulas, of six gold(I) salts.

data suggest that the gold(I) atoms of both Myocrisin and Solganol are, in fact, coordinated to a second bridging sulfur atom in both solid and solution (28,29). The inhibition of two forms of HNC by these gold(I) salts has been investigated. The first is latent HNC, which is inactive as isolated, but which can be reversibly activated by PCMB. The second form will be referred to as active HNC, since it is already active on isolation, presumably by virtue of limited proteolysis of latent HNC that occurred during extraction from the neutrophils.

All six compounds inhibit PCMB-activated latent HNC, but their potencies vary markedly. The variations in inhibition observed as a function of the gold(I) salt concentrations are shown in Figure 2. The $IC_{50}$ values are summarized in Table 1 and vary from 0.79 mM for SKF 80544 to 3.5 nM for Myocrisin. Sanocrisin, Solganol and Myocrisin all have $IC_{50}$ values below 11 nM. The possibility was considered that the inhibition observed by these salts was the result of the

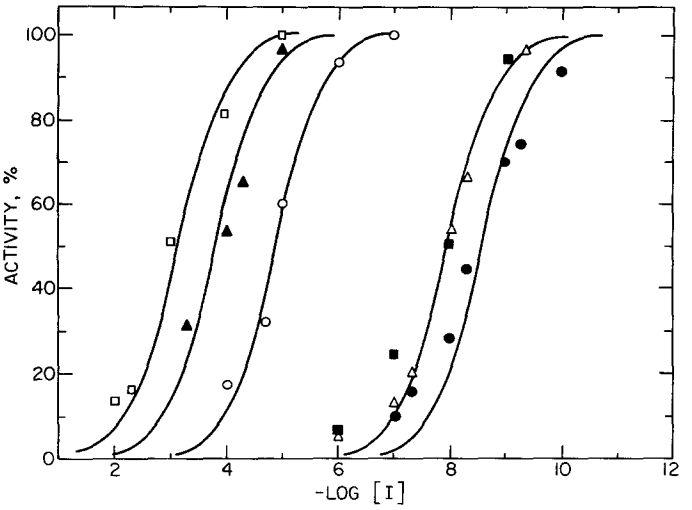


Figure 2. Variation in the activity of PCMB-activated latent HNC as a function of the concentration of (□) SKF 80544, (▲) Auranofin, (○) SKF 36914, (■) Sanocrisin, (△) Solganol and (●) Myocrisin. Collagenase assays were carried out in 50 mM Tricine, 10 mM $CaCl_2$, 0.2 M NaCl, 0.5μM $ZnSO_4$, pH 7.5 at 30°C.

Table 1. Inhibition of Human Neutrophil Collagenase by Gold(I) Drugs and Their Ligands

| Gold Drug (ligand) | $IC_{50}$ (μM) Latent HNC + PCMB | Active HNC | Active HNC + PCMB |
|---|---|---|---|
| SKF 80544 (triethylphosphine oxide) | 790<br>>10,000 | 1,300<br>>10,000 | 1,300<br>>10,000 |
| SKF 36914 (triethylphosphine oxide) | 16<br>>10,000 | 28<br>>10,000 | 28<br>>10,000 |
| Auranofin (thioglucose tetraacetate) | 160<br>40 | >500<br>50 | >500<br>150 |
| Sanocrisin (thiosulfate) | 0.011<br>>1,000 | >1,000<br>>1,000 | 0.063<br>>1,000 |
| Solganol (thioglucose) | 0.011<br>25 | >1,000<br>390 | -<br>- |
| Myocrisin (thiomalate) | 0.0035<br>100 | 130<br>>100 | 0.050<br>>100 |

gold(I) ligands, since HNC is a metalloenzyme and many of the ligands can chelate metal ions. Only thiomalate, thioglucose and thioglucose tetraacetate inhibit appreciably at concentrations below 1 mM. Thiosulfate and triethylphosphine oxide (the expected oxidation product of triethylphosphine after dissociation from gold(I)) are very poor inhibitors. Only in the case of Auranofin is the $IC_{50}$ of the ligand similar to that of the gold(I) drug. Thus, at least for the other five compounds, the gold(I) atom is ultimately responsible for the inhibition observed here.

Different results were found for HNC that had been isolated in active form. For this enzyme, PCMB is not required for activation and was initially omitted from the assays. Under these conditions, only SKF 36914 and Myocrisin have $IC_{50}$ values below 200 μM. With the exception of SKF 36914 and SKF 80544, all of the $IC_{50}$ values are markedly higher than those for PCMB-activated latent HNC. When the inhibition of active HNC was carried out in the presence of 0.1 mM PCMB, the $IC_{50}$ values for Sanocrisin and Myocrisin dropped markedly, but were still not as low as the values for PCMB-activated latent HNC. The presence of the PCMB did not lower the $IC_{50}$ values for SKF 80544, SKF 36914 or Auranofin while anomalous results (not shown) were found for Solganol. As with latent HNC, the inhibition observed for active HNC is not attributable to the ligands of the gold(I) drugs.

No time dependence was observed for the inhibition of either form of HNC by any of these compounds. Although the assay takes five hours, there was no evidence from the assay curves that inhibition was increasing with time. Thus, the kinetics of interaction of these drugs with HNC are probably complete within one hour.

## DISCUSSION

With the aid of a new, quantitative collagenase assay (26,27), the present study demonstrates that all six gold(I) salts examined here inhibit PCMB-activated latent HNC. Only SKF 80544, SKF 36914 and Myocrisin, however, inhibit HNC that had been isolated in active form. The fact that PCMB greatly accentuates the inhibition of active HNC by Sanocrisin and Myocrisin strongly implies that it interacts with their thiol ligands to "free" the gold(I) atom for transfer to the enzyme. Inhibition by SKF 80544, SKF 36914 and Auranofin, all of which have at least one triethylphosphine ligand, is not affected by PCMB. The $IC_{50}$ values for latent HNC vary over a remarkable six orders of magnitude. Apparently, the identity of the ligands to the gold(I) critically affects its reactivity or ability to interact with HNC. The $IC_{50}$ value of 3.5 nM for Myocrisin presently makes it the best known synthetic inhibitor of any collagenase.

The inhibition of HNC by these gold(I) salts suggests that this may be a basis for their anti-arthritic action. Cartilage is composed of type II collagen which is susceptible to attack by HNC. It is therefore possible that inhibition of HNC or of collagenases from other inflammatory or endogenous cells in synovium could be an important mechanism for chrysotherapy. The very potent inhibition of HNC by Myocrisin and Sanocrisin in the presence of PCMB suggests further that the inhibition may be aided by agents that liberate the gold(I) atom from its ligands. While it is known that gold(I) salts like Auranofin penetrate the synovial fluid of patients with rheumatoid arthritis, the identity of the ligands to the gold(I) at this site remain unknown (30). If the gold(I) atom in synovial fluid resides

in an environment that allows its efficient binding to HNC, or a similar collagenase, the results described herein could be clinically significant.

ACKNOWLEDGEMENTS

The authors thank Smith, Kline and French Laboratories for their gift of Auranofin, SKF 80544 and SKF 36914. This work was supported by NIH Research Grant GM-27939 and Research Career Development Award (to HEVW) AM-01066.

REFERENCES

1. Forestier, J. (1935) J. Lab. Clin. Med. 20, 827-840.
2. Brown, D.H., and Smith. W. E. (1980) Chem. Soc. Rev. 9, 217-240.
3. Sutton, B. M., McGusty, E., Walz, D. T., and DiMartino, M. J. (1972) J. Med. Chem. 15, 1095-1098.
4. Walz, D. T., DiMartino, M. J., Sutton, B., and Misher, A. (1972) J. Pharmacol. Exp. Ther. 181, 292-297.
5. Finkelstein, A. E., Walz, D. T., Batista, V., Mizraji, M., Roisman, F., and Misher, A. (1976) Ann. Rheum. Dis. 35, 251-257.
6. Leibfarth, J. H., and Persellin, R. H. (1981) Agents and Actions 11, 458-472.
7. Harth, M., and Davis, P. (1979) J. Rheumatol. (Suppl. 5) 6, 164.
8. Vernon-Roberts, B. (1979) J. Rheumatol. (Suppl. 5) 6, 120-129.
9. Lewis, A. J., Cottney, J., White, D. D, Fox, P. K., McNeillie, A., Dunlop, J., Smith, W. E., and Brown, D. H. (1980) Agents and Actions 10, 63-77.
10. Menninger, H., Putzier, R., Mohr, W., Wessinghage, D., and Tillman, K. (1980) J. Rheumatol. 39, 145-156.
11. Mohr, W., Westerhellweg, H., and Wessinghage, D. (1981) Ann. Rheum. Dis. 40, 396-399.
12. Lazarus, G. S., Daniels, J. R., Brown, R. S., Bladen, H. A., and Fullmer, H. M. (1968) J. Clin. Invest. 47, 2622-2629.
13. Barrett, A. J. (1978) Agents and Actions 8, 11-18.
14. Starkey, P. M., Barrett, A. J., and Burleigh, M. C. (1977) Biochim. Biophys. Acta 483, 386-397.
15. Janoff, A. (1975) Ann. New York Acad. Sci. 256, 402-408.
16. Rohozkova', D., and Steven, F. S. (1983) Br. J. Pharmac. 79, 181-189.
17. Paltemaa, S. (1968) Acta Rheum. Scand. 14, 161-168.
18. Janoff, A. (1970) Biochemical Pharmacology 19, 626-628.
19. Wojtecka-Lukasik, E., and Dancewicz, A. M. (1974) Biochemical Pharmacology 23, 2077-2081.
20. DiMartino, M. J., and Walz, D. T. (1977) Inflammation 2, 131-142.
21. Finkelstein, A. E., Roisman, F. R., and Walz, D. T. (1977) Inflammation 2, 143-150.
22. Fox, P. K., Lewis, A. J., McKeown, P., and White, D. D. (1979) Br. J. Pharmac. 66, 141P-142P.

23. Stancikova, M., and Trnavsky, K. (1980) Fysiat. Revmatol. Vestn. 58, 189-192.
24. Baici, A., Salgam, P., Fehr, K., and Boni, A. (1981) Biochemical Pharmacology 30, 703-708.
25. Moore, W. M., and Spilburg, C. A. (1986) Biochemistry 25, 5189-5195.
26. Mookhtiar, K. A., Mallya, S. K., and Van Wart, H. E. (1986) Anal. Biochem. 158, 322-333.
27. Mallya, S. K., Mookhtiar, K. A., and Van Wart, H. E. (1986) Anal. Biochem. 158, 334-345.
28. Mazid, M. A., Razi, M. T., Sadler, P. J., Greaves, G. N., Gurman, S. J., Koch, M. H. J., and Phillips, J. C. (1980) J. C. S. Chem. Comm., 1261-1263.
29. Elder, R. C., Ludwig, K., Cooper, J. N., and Eidsness, M. K. (1985) J. Am. Chem. Soc. 107, 5024-5025.
30. Chaffman, M., Brogden, R. N., Heel, R. C., Speight, T. M., and Avery, G. S. (1984) Drugs 27, 378-424.