INHIBITION OF LYSOsomAL ENZYME RELEASE FROM RAT LEUKOCYTES BY AURANOFIN¹

A New Chrysotherapeutic Agent²

M. J. DIMARTINO and D. T. WALZ

Smith Kline & French Laboratories,
1500 Spring Garden Street,
Philadelphia, Pennsylvania 19101

Abstract—Auranofin (SK&F D-39162), a new antiarthritic gold compound reported to be orally effective in animal (adjuvant rat) and human (rheumatoid) arthritic conditions, is a potent in vitro inhibitor of the release of lysosomal enzymes from phagocytizing rat leukocytes. Auranofin, at micromolar concentrations (1–10 μM), produced a dose-dependent reduction in extracellular levels of lysosomal enzyme markers (β-glucuronidase and lysozyme) which are selectively released from rat leukocytes during phagocytosis of zymosan particles. The reduction in extracellular levels of lysosomal enzymes appears to be caused by inhibition of their selective cellular release, since effective concentrations of auranofin did not produce leukocyte cytotoxicity or inhibition of cell-free lysosomal enzyme activity. Morphologic and biochemical evidence indicated that auranofin also interferes with phagocytosis of zymosan particles. The potent in vitro activity of auranofin appears to result from its unique gold complex, since neither structurally related nongold compounds nor clinically used gold compounds (gold sodium thiomalate and gold thioglucose) were potent inhibitors of lysosomal enzyme release. The results of this investigation suggest that the antiarthritic activity of auranofin may be caused at least in part, by inhibition of lysosomal enzyme release and/or cellular processing of antigens.

INTRODUCTION

Alkylphosphine gold coordination complexes have been reported to exhibit antiinflammatory properties when administered orally to adjuvant rats (1). One of the most potent examples in this chemical series is auranofin (SK&F D-39162), whose structure is illustrated in Figure 1.

1 SK&F D-39162 (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosato-S) (triethylphosphine) gold.
2 Presented in part at the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 11, 1974 (4).
Recently, auranofin was shown to be orally absorbed, free of major side effects, well tolerated, and clinically effective in rheumatoid arthritic patients (2, 3).

Although the mechanism by which auranofin produces its therapeutic effect is not known, it has been suggested (4, 5) that its antiarthritic activity may be caused, at least in part, by inhibition of lysosomal enzyme release, which is believed to be involved in the pathogenesis of inflammatory arthritis (6-8).

The present report describes the in vitro activity of auranofin on inhibiting selective lysosomal enzyme release from rat leukocytes during phagocytosis of zymosan particles.

Clinically used gold compounds (gold thioglucose and gold sodium thiomalate) and nongold compounds structurally related to auranofin were also evaluated for comparative purposes.

**MATERIALS AND METHODS**

The leukocyte preparation, reaction media, and phagocytic system used in this investigation are based on methods reported by Henson (9).

Adjuvant arthritis was induced in Charles River Wistar rats by previously described methods (10).

**Leukocyte Preparation.** Peripheral blood leukocytes from adjuvant arthritic rats were obtained 18–23 days after adjuvant injection. Differential centrifugation and gelatin sedimentation procedures consistently yielded a mixed leukocyte population of approximately 60% PMN leukocytes and 40% lymphocytes. Leukocyte preparations containing \( \geq 94\% \) PMNs were obtained by Hypaque–Ficoll gradient centrifugation and dextran sedimentation.

**Reaction Media.** Tyrode's solution containing 0.1% glucose was prepared with 0.01 M TRIS buffer (pH 7.3). Bovine serum albumin was added (0.25%) as a source of protein. Drug solutions were prepared in the reaction media at appropriate concentrations, and pH adjustments were performed when required.

Purified zymosan (S-cerevisiae yeast) was incubated (37°C for 30 min) with fresh rat