EFFECT OF AURANOFIG^1 ON ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC) MEDIATED BY RAT PERIPHERAL BLOOD POLYMORPHONUCLEAR LEUKOCYTES AND MONONUCLEAR CELLS

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Abstract—Auranofin and other clinically used gold compounds were evaluated in vitro for effects on antibody-dependent cellular cytotoxicity (ADCC) of L929 fibroblast target cells mediated by adjuvant rat peripheral blood PMNs or mononuclear cells. Auranofin (10 μM) was found to be a potent inhibitor of PMNADCC. In contrast, gold sodium thiomalate (10-100 μM), gold thioglucose (10-1000 μM), and nongold substructures of auranofin (10 μM) were not inhibitory. In continuous culture, gold sodium thiomalate and relatively low concentrations of auranofin (<1 μM) significantly enhanced PMNADCC. Results of pretreatment studies indicate that auranofin's inhibitory activity of PMNADCC is caused by a noncytotoxic effect on PMN function which is not associated with alteration of PMN-target cell contact. In contrast to its inhibitory activity on PMNADCC, auranofin pretreatment of mononuclear cells resulted in enhanced target cell destruction which appeared to correlate with increased mononuclear cell-target cell contact.

INTRODUCTION

Auranofin, a new antiarthritic gold compound reported to be orally effective in animal (1) and human (2-4) arthritic conditions, is presently undergoing multiclinical evaluations. Although the mechanism by which auranofin produces its therapeutic effect is not known, the compound exhibits a variety of relevant pharmacological activities including effects on antibody production (1, 5), cellular release of lysosomal enzymes (6, 7), and cell-mediated hypersensitivity reactions (8, 9). Auranofin appears to be a unique thera-

^1SK&F D-39162 (2,3,4,6-tetra-O-acetyl-l-thio-β-d-glucopyranosato-S)triethylphosphine gold.

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therapeutic gold compound since its physical, chemical, pharmacological, and pharmacokinetic properties differ from those of other clinically used gold compounds (1, 5-13).

The present report extends the pharmacological profile of auranofin to include in vitro effects on antibody-dependent cellular cytotoxicity (ADCC) mediated by adjuvant rat peripheral blood polymorphonuclear (PMN) leukocytes and mononuclear cells. Other clinically used gold compounds (gold sodium thiomalate and gold thioglucose) and nongold compounds structurally related to auranofin were also evaluated for comparative purposes.

MATERIALS AND METHODS

Antibody-dependent cellular cytotoxicity (ADCC) reactions were performed utilizing previously described materials and methods (5, 14) with minor modifications.

Incubation Media. ADCC reactions were performed in Eagle's minimum essential media (MEM) with Earle's balanced salt solution supplemented with NaHCO₃ (22.5 mg/ml), heat inactivated fetal calf serum (10%), L-glutamine (2 mM), lactalbumin hydrolysate (0.5%), penicillin (50 units/ml), and streptomycin (50 μg/ml).

ADCC Components. L929 fibroblast target cells were harvested and labeled with chromium-51 (Amersham/Searle) as described (14) with minor modifications. To decrease the levels of spontaneous ⁵¹Cr release, the [⁵¹Cr]L929 fibroblasts were preincubated in MEM for 18-23 h prior to ADCC studies. Anti-L929 fibroblast immune serum (i.e., antibody source) was obtained from male Wistar-Lewis rats 15 days following a single intraperitoneal injection of 50 × 10⁶ L929 fibroblasts. Immune and nonimmune (control) rat sera were heat inactivated at 56°C for 1 h. Adjuvant arthritic rat peripheral blood leukocyte preparations containing 89-96% PMNs and 94-97% mononuclear cells were obtained by Hypaque-Ficoll gradient centrifugation (15). Adjuvant arthritis was induced in male Wistar rats by previously described methods (16).

Antibody-Dependent Cellular Cytotoxicity. ADCC was determined by measuring extracellular release of chromium-51 (⁵¹Cr) from 10⁵ ⁵¹Cr-labeled target cells (L929 fibroblasts) incubated with heat-inactivated immune sera (rat anti-mouse L929 fibroblast sera) and adjuvant arthritic rat peripheral blood leukocytes (100:1 target cell ratio) for 4-5 h at 37°C. Nonspecific ⁵¹Cr release was determined utilizing heat-inactivated normal (nonimmune) rat sera. Total releasable ⁵¹Cr was determined following H₂O lysis of the target cells. Extracellular ⁵¹Cr levels were measured with a Searle Analytical (1185 series) automatic gamma counting system. All assays were performed in triplicate unless otherwise noted.

Statistics. The results of ADCC experiments are expressed as “% specific lysis” of the ⁵¹Cr-labeled mouse L929 fibroblast target cells.

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\text{% specific lysis} = \frac{E - C}{T - C} \times 100
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where mean ⁵¹Cr release (cpm/ml); E = experimental sample; C = control (nonimmune sera); and T = total releasable (H₂O lysis).

The average T/C ratio derived from experiments reported in this investigation is 5.56 ± 1.43 (SD).