HYPOXANTHINE DEPLETION INDUCED BY XANTHINE OXIDASE INHIBITS MALARIA
PARASITE GROWTH IN VITRO

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INTRODUCTION

While Plasmodium falciparum, cause of the most virulent form of human malaria, synthesizes its pyrimidine requirements de novo, it lacks the ability to synthesize purines de novo, and depends on the erythrocyte host for its purine supply (1). Although ATP forms about 90% of the total erythrocyte purine pool, there is considerable evidence that the purine base, hypoxanthine, is the immediate purine precursor utilized by these parasites (2). Previous studies by ourselves (3) and others (4), have shown that the steady state concentration and nett rate of formation of hypoxanthine under physiological conditions is relatively low, and, apparently, inadequate to support parasite nucleic acid synthesis. We showed, however, that hypoxanthine formed in erythrocytes was recycled via a three component oxypurine cycle, comprising hypoxanthine, IMP and inosine, and that such recycling limited the rate of hypoxanthine release (5). Irreversible oxidation of hypoxanthine to uric acid by xanthine oxidase interrupted the cycle and enhanced the nett rate of ATP catabolism. During the course of these experiments, we noted that xanthine oxidase markedly decreased intracellular hypoxanthine levels. We therefore investigated whether xanthine oxidase could deplete intracellular hypoxanthine to the point where it could no longer sustain the growth and replication of malaria parasites in vitro.

METHODS

A chloroquine resistant strain of P. falciparum, FCR-3, was maintained in continuous culture in human O+ erythrocytes, suspended in RPM1-1640 medium supplemented with 10% human serum, in an atmosphere of 4% O2, 4% CO2, and 92% N2 (6). Parasite growth was assessed microscopically on Giemsa-stained blood films, and quantitated by incorporation of [14C]glycine into parasite protein, or by incorporation of [14C]purine, in cells in which the ATP pool had been prelabelled with [14C]adenine (5), into parasite nucleic acid. Infected erythrocytes were lysed in 0.15% saponin, liberated parasites trapped on glass-fibre discs, washed, and the radioactive content measured by liquid scintillation photometry. Nucleic acid content of infected erythrocytes was determined by lysing the cells and digesting the parasite pellet with Proteinase K. Residual protein was ‘salted out’ with saturated sodium acetate, nucleic acid isolated by isopropanol precipitation, and quantitated by its absorbance at 260 nm.
The nucleic acid extract was electrophoresed on 2% agarose, and stained with ethidium bromide to assess its composition and provide visual semiquantitation. Xanthine oxidase, superoxide dismutase and catalase were used at concentrations of 0.1, 0.4 and 0.15 mg/ml respectively. Chloroquine sulphate was added at the concentrations indicated, and 5 mM hypoxanthine used in the 'rescue' experiments. L-lactate was measured by a spectrophotometric end-point assay, using NAD+ and lactate dehydrogenase.

RESULTS

As can be seen in Fig. 1A and B, xanthine oxidase markedly impaired both incorporation of [14C]glycine and [14C]ATP derived purine into parasite protein and nucleic acid respectively. Heating the xanthine oxidase at 100°C for 10 minutes abolished inhibitory activity, while inclusion of superoxide dismutase and catalase had no effect on growth inhibition, as assessed by [14C]glycine uptake.

![Fig. 1. Time course of [14C]glycine (A) and [14C]ATP derived purine (B) incorporation into parasite protein and nucleic acid, respectively (● infected erythrocytes, ▲ infected erythrocytes plus xanthine oxidase, ○ non-infected erythrocytes).](image)

Fig. 2 shows the effect of chloroquine and xanthine oxidase on nucleic acid content of infected erythrocytes. Non-infected erythrocytes contain negligible nucleic acid, while infected cells show a progressive increase in their nucleic acid content. This accumulation is markedly inhibited by xanthine oxidase, but can be restored by addition of excess hypoxanthine. The effect of chloroquine is shown for comparison. $10^{-7}$M chloroquine is insufficient to inhibit nucleic acid synthesis, while $5 \times 10^{-7}$M chloroquine is effective. This confirms a degree of chloroquine resistance. Agarose electrophoresis (Fig. 3) provides direct visual confirmation of these findings, and indicates that the predominant nucleic acid isolated is high molecular weight DNA. Microscopic examination of parasites showed that xanthine oxidase caused arrest of parasite maturation in the late trophozoite stage, that is, at the commencement of replication. Hypoxanthine restored the normal distribution of parasite stages, with ring forms predominating.