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TOXICITY OF CERTAIN PRODUCTS OF LIPID PEROXIDATION TO THE HUMAN MALARIA PARASITE *PLASMODIUM FALCIPARUM*

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Abstract—Aldehydes generated during radical-induced lipid peroxidation, in particular 4-hydroxynonenal, are known to inhibit growth of certain cells. To extend our arguments that free radicals might be involved in the host response against malaria parasites we tested 26 carbonyls (*n*-alkanals, C_6–C_{11}; 2-alkenals, C_3–C_9; 2,4-alkadienals, C_7, C_9, C_{10}; 4-OH-2-alkenals, C_6, C_8, C_9; 2-alkanones, C_3–C_9; and malonyldialdehyde) against *Plasmodium falciparum in vitro*. We had previously detected many of these substances in oxidant-stressed, malaria-infected erythrocytes. Three 2,4-alkadienals (C_7, C_9 and C_{10}) and three 4-OH-2-alkenals (C_6, C_8 and C_9), at 20–100 μM concentrations, markedly inhibited incorporation of [^{3}H]-hypoxanthine by *P. falciparum*. Acrolein had low effect, and none of the other compounds (12 aldehydes and 7 ketones) were active at concentrations up to 100 μM. Malonyldialdehyde was without effect at concentrations up to 450 μM. The aldehydes found to be inhibitory against *P. falciparum* could contribute to both the non-antibody host responses against this parasite and the antimalarial effects of radical-generating compounds such as *t*-butyl hydroperoxide, hydrogen peroxide, alloxan, isouramil, divicine and primaquine

When agents that generate free oxygen radicals are injected into *Plasmodium vinckei*-infected mice [1–3] or included in cultures of *Plasmodium falciparum*, the organism that causes the most important type of human malaria [4], the parasites degenerate inside red cells. Since stimulated myeloid leukocytes can secrete the superoxide anion radical, we have suggested that these chemical generators of reactive oxygen species might be mimicking one mechanism of cell-mediated immunity to malaria [1, 2].

While it is convenient to visualize parasite damage arising from direct contact with free radicals, it seemed possible that toxic aldehydes, generated when these radicals oxidize lipids, mediate much of the injury. These toxic products have half-lives much longer than those of oxygen radicals, and can thus cause cellular damage at some distance from their source [5]. Some of these molecules are known to harm tumour cells [6, 7], bacteria [8], and certain mammalian cells [9, 10], but their effects on malaria parasites are unknown. We therefore tested a series of carbonyl compounds, most of which were detected in *P. vinckei*-infected red cells exposed to *in vitro* oxidative stress (G. D. Buffinton *et al.*, manuscript submitted), against *P. falciparum* in culture.

MATERIALS AND METHODS

The *n*-alkanals, *trans*-2-alkenals, *trans*, *trans*-2,4-alkadienals and 2-alkanones were purchased from EGA-CHEMIE, F.R.G. 4-OH-2-hexenal, 4-OH-2-octenal and 4-OH-2-nonenal were generous gifts from Dr H. Esterbauer, University of Graz, Austria. Malonyldialdehyde was prepared as described by Marnett and Tuttle [11].

The FCQ27 strain of *Plasmodium falciparum*, originally isolated in Papua New Guinea, was maintained in culture using a modified Trager and Jensen technique [12]. Briefly, RPMI 1640 medium (Gibco Ltd) was supplemented with 20 mM HEPES (*N*-2-hydroxethylpiperazine-*N*-2-ethane-sulphonic acid) buffer, 26 mM sodium bicarbonate and 10% v/v normal human blood group O serum. The parasites were maintained in group O erythrocytes, from the local blood bank, in flasks containing 5–8 ml of cell suspension at 5% haematocrit and gassed with a 5% oxygen, 5% carbon dioxide and 90% nitrogen mixture. Medium was replaced daily and the cultures split every 2–3 days.

Inhibition of parasite growth was assessed in 96 well micro-culture trays (Nunc), starting with parasitized erythrocytes suspended at 1% haematocrit, in supplemented medium. In the experiments to test the inhibitory activity of various lipid peroxidation products in combination, a stock mixture was prepared. This comprised the following 10 carbonyls, the figure being the amount of each, in μmoles, contained in 1 ml ethanol: propanone, 80; propanal, 120; hexanal, 16; octanal, 3.2; undecanal, 1; dode canal, 1; 2,4-nonadienal,‡ 3.2; 2,4-decadienal 3.2; 4-OH-2-octenal, 4.8; 4-OH-2-nonenal, 8.0. This mixture was first serial diluted in 12 equal steps, from 100 to 1000-fold, in phosphate-buffered saline. A 12 μl aliquot of each dilution was placed in triplicate wells, along with 100 μl of red cell suspension and 20 μl diluted [^{3}H]-hypoxanthine (1 in 100 dilution of a 1 mCi/ml, 2.8 Ci/mmol stock solution from Amersham Radiochemicals). Samples were processed 18–24 hr later on a Titertek cell harvester, using distilled water as the washing fluid. Filters were counted in

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‡ Substituted for 2,4 octadienal, which was unavailable.

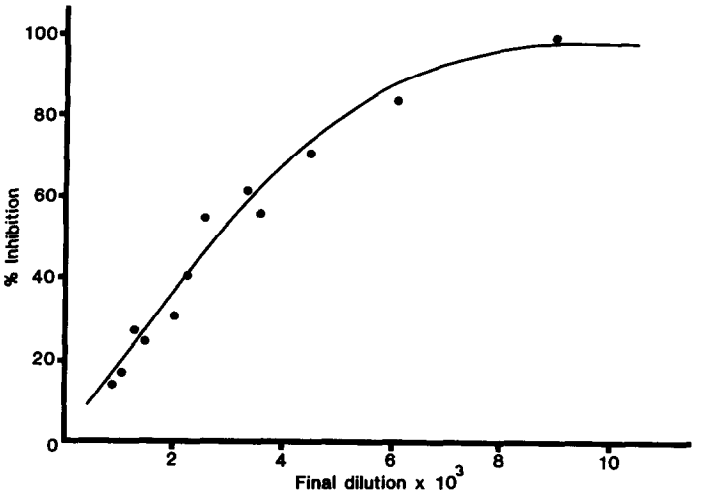

Fig. 1. Inhibition of [^{3}H]-hypoxanthine uptake by *P. falciparum* cultured in the presence of increasing dilutions of a mixture of 10 carbonyls. A quadratic regression curve was calculated ($Y = -4.394 + 22.762X - 1.269X^2$; $R^2 = 0.969$). The drawn line was constructed from 12 points predicted from this equation.

2.5 ml PCS liquid scintillant (Amersham Radiochemicals) in a β counter. Growth inhibition was calculated as previously described [13].

In one experiment parasites were previously synchronised using 5% D sorbitol [14] and the culture began with the early "ring" forms of the parasite, harvesting 18 hr later when the parasite had developed to the late trophozoite stage.

RESULTS

In the first set of experiments *P. falciparum* was exposed to a mixture of 10 carbonyls. This combination (see Materials and Methods) was chosen because it reconstituted, in their detected proportions, the dominant carbonyls in oxidant-stressed, malaria-infected erythrocytes (G. D. Buffinton *et al.*, manuscript submitted). The outcome of one of three experiments that used a range of dilutions of this mixture, with very similar results, is shown in Fig. 1. The concentrations of each component in the dilution (1 in 3636) that inhibited growth of *P. falciparum* by 50% were propanone 22, propanal 33, hexanal 4.4, octanal 0.88, 4-OH-2-octenal 1.3 and 4-OH-2-nonenal 2.2 μM.

To see which components of this mixture were active we conducted a second series of experiments in which 18 aldehydes and 7 ketones, all from classes of carbonyls detected in other *in vitro* models of lipid peroxidation [15], were tested individually against *P. falciparum*. All compounds in the inhibitory mixture were included. Only six, three 4-OH-2-alkenals and three 2,4-alkadienals (Table 1), comprising four of those in the mixture and another two varying from these four only in length of carbon chain, were active at final concentrations up to 100 μM (Fig. 2; combined results of three experiments). Fifty per cent inhibition of *P. falciparum* with a single aldehyde (e.g. 4-OH-2-nonenal) used alone required much higher concentrations than if the same aldehyde was part of the biogenic test mixture (Fig. 1). Inhibition of [^{3}H]-hypoxanthine uptake was similar in cultures containing synchronous or asynchronous parasites (data not shown). When we examined Giemsa-stained blood smears made from the contents of wells containing the inhibitory aldehydes, the erythrocytes contained degenerate pyknotic parasites. There was no obvious haemolysis.

Table 1. Lipid peroxidation products tested *in vitro* against *P. falciparum*

Effective at 20–100 μM (see Fig. 2)		
4-OH-2-alkenals		2,4-alkadienals
hexenal		heptadienal
octenal		nonadienal
nonenal		decadienal
Small effect below 100 μM		
2-propenal (acrolein)		
No effect up to 100 μM		
n-alkanals	2-alkenals	2-alkanones
hexanal	butenal	propanone
heptanal	hexenal	butanone
octanal	heptenal	pentanone
nonanal	octenal	hexanone
decanal	nonenal	heptanone
undecanal		octanone
		nonanone
No effect up to 450 μM		
malonyldialdehyde		

The 19 other compounds tested individually (*n*-alkanals, 2-alkenals and 2-alkanones) did not inhibit [^{3}H]-hypoxanthine uptake by *P. falciparum* when present at concentrations up to 100 μM (Table 1). This list includes malonyldialdehyde (MDA). Because of its reported effects on lipids [16] and

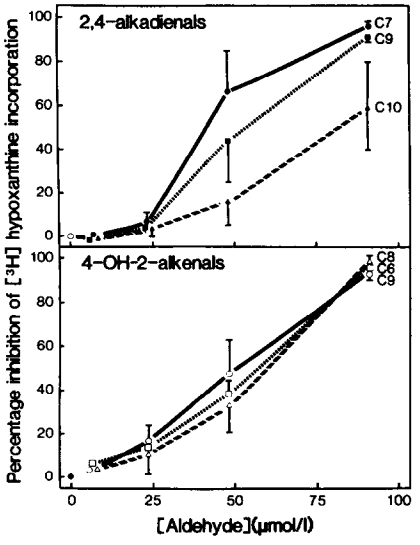

Fig. 2. Concentration-dependent inhibition of uptake of [^{3}H]-hypoxanthine by *P. falciparum* by three 2,4-alkadienals (C_7, C_9, C_{10}) and three 4-OH-2-alkenals (C_6, C_8, C_9). Standard errors of the mean are indicated.

proteins [17] this aldehyde was rigorously tested, but was without detectable inhibitory activity at a series of concentrations up to 450 μM. In addition acrolein (2-propenal) was tested, and gave a response curve that warrants further study. Although reasonably active at higher concentrations, its activity at 50 μM was 5–10-fold lower than that of the six compounds shown in Fig. 2.

DISCUSSION

Reactive oxygen species damage many types of cells, rapidly initiating changes that generate products of lipid peroxidation [15]. These products are harmful to bacteria [7] and various mammalian normal [9, 10] and tumour [6, 7] cells. We have previously shown MDA production in oxidatively-stressed, malaria-infected red cells *in vitro* [2, 3] and *in vivo* (unpublished data). The work described in this paper is the first demonstration, to our knowledge, that any of the carbonyls generated during lipid peroxidation are toxic to malaria parasites.

The higher potency of an aldehyde when it was part of a mixture of products of lipid peroxidation (Fig. 1) than when tested alone (Fig. 2) could have biological significance, since these compounds are not generated in isolation. Possible explanations include an additive or synergistic effect between aldehydes. Alternatively, the non-toxic compounds may protect the toxic ones from degradation by acting as a substate for aldehyde or alcohol dehydrogenases [18] or reacting with thiol groups, such as reduced glutathione [19]. In addition, if MDA was to prove toxic it should have done so at the concentrations tested (Table 1), since the highest (450 μM) was detected in parasitized erythrocytes exposed to oxidative stress *in vitro* in experiments where damage to parasites was severe [2, 3].

Since these compounds are detectable in *in vitro* models of lipid peroxidation [15], and in parasitized red cells exposed to oxidant stress (Buffinton *et al.*, manuscript submitted), it is feasible that they mediate much of the intra-erythrocytic parasite death noted when H_2O_2 [1, 20], alloxan [1], *t*-butyl hydroperoxide [2, 4] or divicine [3] are injected into malaria-infected animals. Since primaquine has been reported to generate hydroxyl radical inside erythrocytes [21], these products of lipid peroxidation may also be a final mediator of its antimalarial activity.

Products of lipid peroxidation can be toxic to endothelial cells [10] and alter red cell membranes [22]. Both of these sites are damaged in malaria (reviewed in ref. 23). Therefore it is worth exploring whether these aldehydes, generated by reactive oxygen species released from leukocytes during cell-mediated responses, could thus contribute to the immunopathology of this disease. The longer *in vivo* half life of these compounds compared to free radicals [18] makes it feasible for them to mediate injury at sites distant from their origin [5], allowing inflammatory damage without local leukocytic accumulation [24]. In addition, through their chemotactic ability [25] these compounds could magnify the cellular inflammatory response, such as that seen in the small pulmonary blood vessels in severe cases of this disease [24].

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