Research note

EFFECT OF CHLOROQUINE ON OXYGEN RADICAL PRODUCTION BY KUPFFER CELLS, BLOOD MONOCYTES AND PERITONEAL MACROPHAGES OF NORMAL GUINEA PIGS

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Abstract: The study was conducted to determine whether chloroquine has any effect on the oxygen radical production of Kupffer cells, blood monocytes and peritoneal macrophages. Exposure of these cells to chloroquine, using therapeutic concentrations, for 30 min at 37°C did not alter the viability of cells. The chemiluminescence (CL) response of all the cell populations was not effected after 10 min of exposure to drug. However, the CL response of all the three types of cells was significantly inhibited after 20 min exposure to chloroquine. The effect of chloroquine was reversible after washing out the drug from cuvetes after 20 min of treatment. But when the cells were washed after 30 min it was only partially reversible. The effect of chloroquine on CL response of the cells have been discussed.

INTRODUCTION

Mononuclear phagocytic cells (MPC) play an important role in the host defense mechanism (Nelson, 1982). However, the over activation of these cells and the subsequent release of their secretory products can lead to various pathological consequences (Clark, 1987). In the recent years many workers have emphasized that elevated release of free oxygen radicals by immunologically activated MPC is responsible for tissue damage in a variety of disease (Bulkley, 1983; Allen and Egui, 1983). The reactive oxygen radicals have also been shown to cause malaria pathology by damaging vascular endothelium and erythrocytes (Clark and Hunt, 1983; Weisiger, 1986). Chloroquine is the widely used antimalarial drug for the treatment of malaria. It has been shown that chloroquine inhibits the phagocytic capacity of monocytes in normal animals whereas in the infected animals it controls the hyperactivation of these cells (Prasad *et al.*, 1986). However, its effect on the oxygen radical production capacity of phagocytic cells is not well understood. Since the oxygen radical production by leukocytes is dependent upon their phagocytic capacity (Allen *et al.*, 1972), it is very much

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important to study the effect of chloroquine on the oxygen radical production capacity of these cells. The present study was, therefore, conducted to determine whether chloroquine has any effect on the oxygen radical production of Kupffer cells, blood monocytes and peritoneal macrophages.

MATERIALS AND METHODS

Three to four weeks old normal healthy guinea pigs of either sex were obtained from Central Animal House of the Postgraduate Institute of Medical Education and Research, Chandigarh.

Kupffer cells and blood monocytes were separated by pronase digestion and Ficoll hypaque density gradient methods respectively as described elsewhere (Virk *et al.*, 1987). Peritoneal macrophages were obtained from peritoneal large without any prior activation with slight modifications as described in our previous publication (Virk *et al.*, 1987).

The concentration of chloroquine which is attainable during treatment (Prasad *et al.*, 1985) of malaria (230 $\mu\text{g/ml}$) was added to the cuvettes containing cells. The cells were exposed to chloroquine for 0, 10, 20 and 30 min before taking the chemiluminescence reading. In some of the experiments the drug was removed from cuvettes by washing the cells with minimum essential medium without phenol red.

The oxygen radical production by MPC was studied through chemiluminescence. Chemiluminescence (CL) was measured, after culturing the blood monocytes and peritoneal macrophages for 1 hr and Kupffer cells for 3 hrs, in LKB luminometer (Model, 1250). Latex particles were used for the stimulation of cells (Virk *et al.*, 1987).

RESULTS

Exposure of cells to chloroquine, using therapeutic concentrations, for 30 min at 37°C did not alter the viability of cells. The CL response of Kupffer cells, blood monocytes and peritoneal macrophages recorded before and after chloroquine treatment is shown in Table 1. There was no significant effect on the CL response of all the cell populations after 10 min of exposure to drug. However, the CL response of all the three types of cells was significantly

Table 1 Effect of chloroquine on chemiluminescence response of Kupffer cells, blood monocytes and peritoneal macrophages

Time of chloro- quine treatment in min	Peak CL response (mV)					
	Kupffer cells		Blood monocytes		Peritoneal macrophages	
	A	B	A	B	A	B
0	5.56 $\pm$ 1.23	5.16 $\pm$ 1.20	6.50 $\pm$ 1.17	6.86 $\pm$ 0.75	8.50 $\pm$ 0.81	8.50 $\pm$ 0.55
10	5.67 $\pm$ 0.45	5.30 $\pm$ 1.04	5.70 $\pm$ 0.50	6.46 $\pm$ 0.61	6.10 $\pm$ 1.37	7.46 $\pm$ 0.45
20	2.65 $\pm$ 0.32*	4.20 $\pm$ 0.30	3.30 $\pm$ 0.80*	5.86 $\pm$ 0.31	1.95 $\pm$ 0.37*	6.90 $\pm$ 0.26
30	0.66 $\pm$ 0.11*	2.83 $\pm$ 0.91*	1.34 $\pm$ 0.81*	4.96 $\pm$ 0.15	0.91 $\pm$ 0.14*	5.56 $\pm$ 0.66*

A = In the presence of chloroquine

B = In the absence of chloroquine, i.e. after washing

* = (P < 0.01)

inhibited after 20 min of exposure to chloroquine ($P < 0.01$). The effect of chloroquine, was reversible, as no significant inhibition was noticed when the CL response was measured after washing out the drug from cuvettes after 20 min of treatment. When the cells were washed after 30 min of treatment, the inhibitory effect of drug on the CL response was only partially reversible.

DISCUSSION

The data presented here shows that chloroquine inhibits the CL response of Kupffer cells, blood monocytes and peritoneal macrophages of normal guinea pigs. The depressed CL response of MPC may be due the prevention of recycling of surface receptors of macrophages by chloroquine (Brodsky, 1984). Toxic oxygen radical are important effector molecules in the microbicidal activity of MPC (Badwey and Karnovsky, 1980). *Plasmodium* species are sensitive and can be killed within erythrocytes and mononuclear cells by oxidant stress. But why the parasite is not eliminated at the time of peak oxygen metabolite production *in vivo* is still not well understood (Brinkmann *et al.*, 1984). It appears that parasite escapes from the toxic effect of oxygen radical produced by MPC (Allen and Egui, 1983). However, the enhanced release of these matabolites may cause extensive tissue damage in their immediate environment (Clark, 1987; Marx, 1987). Apart from this production of oxygen radicals by activated MPC at a higher quantus can effect the generating phagocytic cells also (Weisiger, 1986). Under these circumstance inhibition of oxygen radical production of MPC by chloroquine seems to be beneficial in protecting the host tissue from oxygen radical mediated damage. Antinflammatory and antiphagocytic activity of chloroquine, apart from its parasitocidal action, have already been shown to be helpful in the resolution of disease (Prasad *et al.*, 1986).

It is hypothesised that this inhibitory effect of the chloroquine might be protecting the host from the toxic effect of the oxygen radicals during malaria infection as enhanced release of these radicals is known to cause malarial pathology.

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