Chapter 4
Treatment with Antimalarials Adversely Effects The Oxidative Energy Metabolism in Rat Liver Mitochondria

Introduction

Majority of the antimalarial drugs are quinoline derivatives or substituted synthetic quinolines (1). Chloroquine (CQ), primaquine (PQ) and quinine (Q) are the three widely used antimalarials in the tropical regions of the world (2). These drugs are used not only for the treatment of malaria but also for the prevention and prophylaxis, as well as for treatment of other diseases (1). Although these drugs belong to the quinoline groups, their mode of action and the stage of *Plasmodium* at which they act differs. One common property of the three drugs is that they accumulate in the *Plasmodium* and the cells which harbor the parasite (3,4). Reports in the literature suggest that CQ and PQ affect the structure and function of the mitochondria in *Plasmodium* as well as in heart (5-8). Based on these observations it has been suggested that affecting the mitochondrial function could be one of the mechanism of antimalarial action (7,8). *Plasmodium* develops and remains dormant for months in the liver to cause relapse (1). The antimalarials are known to be lysosomotropic and accumulates in large quantities in the liver (3). The tissue concentration can be as high as 75 mM (9). Concentration up to 125 mM have been reported in fibroblast cell cultures (2). It is possible that the accumulation of antimalarials in the liver can affect the mitochondrial functions in the host tissue i.e liver. To illustrate this point, the effect of in vivo treatment with the three commonly used antimalarials i.e. CQ, PQ and Q on oxidative energy metabolism in rat liver mitochondria was studied. The results of these investigations are summarized in this chapter.

Materials and Methods

Chemicals

L- glutamic acid was obtained from E Merck, Germany. Sodium salts of succinic acid, pyruvic acid, L- malic acid, ADP and rotenone and BSA were purchased from Sigma Chemical Co. USA. TMPD was purchased from British Drug Houses, Dorset, Poole, England. Ascorbic acid was from Sarabhai Chemicals, Vadodara. Chloroquine diphosphate, primaquine diphosphate and quinine hydrochloride were purchased from Sigma- Aldrich. USA All other chemicals were purchased locally and were of analytical-reagent grade

Animals and treatment with antimalarials

Male albino rats of Charles-Foster strain weighing between 250-300 g were used. The animals received daily injections of antimalarials intraperitonially (i.p.) for 7 or 14 days The solutions of antimalarials were prepared in saline and the doses of the three antimalarials CQ, PQ and Q were 10 mg, 0.21 mg and 30 mg /Kg body wt respectively(2) The controls received equivalent volume of saline. The animals were killed on 8^{th} and 15^{th} day of treatment. In the control groups the results of 7 day and 14 day saline injected rats were in close agreement and hence the data were pooled. Thus the control is given only as one group in all the Tables.

Isolation of mitochondria

At the end of the experimental period the animals were killed by decapitation and the livers were quickly removed and placed in beakers containing chilled isolation medium for liver mitochondria. which consisted of 250 mM sucrose containing 10 mM Tris-HCl buffer pH 7 4. 1 mM EDTA and 0 25 mg BSA/ml (10). Liver was minced with a fine pair

of scissors and was washed repeatedly with the isolation medium to free it from blood. The tissue was then homogenized using a Potter Elvehjem type glass-Teflon homogenizer to obtain 10 % (w/v) homogenate. After removal of nuclei and cell debris at 650Xg for 10 min, the mitochondria were sedimented at 7500Xg for 10 min, washed once and were finally suspended in the isolation medium to give a protein concentration in the range of 25-30 mg/ ml. All the steps in the isolation procedure were carried out at 0-4 °C.

Oxidative phosphorylation

Measurements of oxidative phosphorylation were carried out at 25 °C using a Clark type oxygen electrode as described previously (10,11). Briefly, the respiration medium (total volume 1.6 ml) consisted of 225 mM sucrose, 20 mM KCl, 10 mM Tris-HCl buffer pH 7.4, 5 mM potassium phosphate buffer pH 7.4, 0.2 mM EDTA and 160 μg of BSA(i.e. 0 1 mg/ml)

Final concentrations of the substrates used were: glutamate (10 mM), pyruvate + malate (10 mM +1 mM), succinate (10 mM) and ascorbate +TMPD (10 mM + 0.1mM). Measurements with the latter two substrates were performed in the presence of 1 μM rotenone. State 3 respiration rates initiated by the addition of 80-200 nmoles of ADP and state 4 rates ensued after the depletion of added ADP were recorded. Calculations of ADP/O ratio and ADP phosphorylation rates were as described previously (10,12).

Assay of ATPase

ATPase activities were measured in the assay medium (total volume 0 4 ml) containing 50 mM Tris-HCl buffer pH 7.4, 75 mM KCl and 0.4 mM EDTA. The assays were performed in the absence and presence of $MgCl_2$ (6mM) and 100 μM DNP, or a combination thereof. After pre-incubating the mitochondrial protein (Ca 1 mg) in the assay medium at 37 °C, the reaction was initiated by the addition of ATP at a final concentration of 5 mM. The reaction was carried out for 10 min and then terminated by the addition of 0.1 ml of 5%(w/v) SDS (10) and the amount of liberated inorganic phosphorus was estimated by the method of Fiske and Subba Row (13)

Cytochrome content

The contents of cytochromes (aa_3, b, and $c+c_1$) were calculated from the difference spectra as described previously (14,15). Briefly, 6-8 mg mitochondrial protein was taken up in potassium phosphate buffered sucrose and solubilized by adding 0.25 ml of 10 % Triton X 100 The total volume was made up to 2 5 ml The sample was then transferred to two cuvettes The sample in the reference cuvette was oxidized by adding small amount of potassium ferricyanide and the sample in the experimental cuvette was reduced by adding a few mg of sodium dithionite. From the difference spectra contents of $c+c_1$, b and aa_3 were calculated using the wavelength pairs 540-552, 562-575 and 605-625 respectively. The spectra were recorded using a Shimadzu UV 160 spectrophotometer.

Protein estimation was by the method of Lowry *et al.* with bovine serum albumin used as the standard (16)

Results

The effects of treatment with the three antimalarials on rat liver mitochondrial oxidative phosphorylation are summarized in Tables 1-3. Thus following CQ treatment the state3 respiration showed an overall 75% decrease in both the treatment groups (i.e 7 day and 14 day treatment). A similar 60-70 % decrease was noted also for state 4 respiration. The ADP/O ratio decreased by 27 % in both the treatment groups. The ADP phosphorylation rates decreased by 83 and 72 %. Similar effect was observed even with pyruvate + malate as the substrate pair. However, the extent of inhibition of state 3 and state 4 respiration rates was comparatively higher (90 to 95 % inhibition). The decrease in ADP/O ratios was also somewhat of a greater magnitude (35 % decrease). As a consequence the ADP phosphorylation rate also decreased to a greater extent (96 % decrease) compared to glutamate When succinate was used as the substrate the extent of inhibition of state 3 and state 4 respiration rates was somewhat lower in the range of 75 to 80 %. However the extent of uncoupling was much higher compared to the former two substrates. Thus the ADP/O ratios decreased by about 65 %. The overall effect was a decrease in ADP phosphorylation rate by about 95 %. With ascorbate + TMPD as the substrate the effect on state 3 and state 4 respiration was the least, and the inhibition ranged from 60 to 70 %. The ADP/O ratios decreased progressively (52 and 73 % respectively in the two groups). Due to greater uncoupling, the ADP phosphorylation rates decreased by 80 and 93 % respectively

The effects of PQ treatment on liver mitochondrial energy metabolism are summarized in Table 2 From the data presented it can be noted that PQ treatment resulted in an overall 70 to 75 % inhibition of state 3 and state 4 respiration rates with glutamate as the substrate The ADP/O ratios decreased to the extent of 20 % and ADP phosphorylation

Table 1. Effect of chloroquine treatment on oxidative phosphorylation in rat liver mitochondria.

Substrate	Animals	ADP/O ratio	Rate of respiration (n moles O_2 /min /mg protein) + ADP	- ADP	ADP phosphorylation rate (n mole/min/mg/protein)
Glutamate	Control (46)	2.88±0.21	23.5±1.29	10.8±0.92	129.3±9 70
	CQ-7(15)	2.10±0.11[a]	5.9±1.25[d]	3.4±0.65[d]	21 9±3.44[d]
	CQ-14(12)	2.09±0.13[a]	6.8±0.51[d]	4.4±0.31[d]	36.8±3.65[d]
Pyruvate + malate	Control (41)	2.74±0.23	24.4±1.24	12.6±0.44	128.2±11.52
	CQ-7 (15)	1.83±0.22[a]	1.2±0.11[d]	0.8±0.09[d]	3.9±0.27[d]
	CQ-14 (24)	1.86±0.16[d]	2.2±0.17[d]	1.5±0.14	7.4±0.44[d]
Succinate	Control (44)	1.44±0.09	82.8±7.00	43.2±3.18	249.8±28.34
	CQ-7(17)	0.44±0.04[d]	18.0±0.95[d]	13.5±0.88[d]	15.2±1.00[d]
	CQ-14(21)	0.56±0.04[d]	14.4±0.69[d]	8.9±0.61[d]	15.8±0.97[d]
Ascorbate + TMPD	Control (42)	0.66±0.06	94.6±9.91	50.2±5.08	106.2±8.95
	CQ-7(13)	0.35±0.02[d]	31.0±2.17[d]	21.6±1.59[d]	21.0±1.59[d]
	CQ-14(18)	0 18±0 02[d]	24.2±2.29[d]	17.6±2.11[d]	7.4±0.39[d]

The experimental conditions are as described in the text. The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis.
[a] $p< 0.02$; [b] $p<0.01$; [c] $p<0.002$ and [d] $p<0\ 001$ as compared with the corresponding control.

Table 2. Effect of primaquine treatment on oxidative phosphorylation in rat liver mitochondria.

Substrate	Animals	ADP/O ratio	Rate of respiration (n moles O_2/min /mg protein)		ADP phosphorylation rate (n mole/min/mg protein)
			+ ADP	- ADP	
Glutamate	Control (46)	2.88±0.21	23.5±1.29	10.8±0.92	129.3±9.70
	PQ-7(17)	2.39±0.15	5.5±0.88[c]	3.5±0.44[c]	25.8±4.55[c]
	PQ-14(24)	2.25±0.09[b]	5.2±0.17[c]	3.8±0.17[c]	23.2±1.07[c]
Pyruvate + Malate	Control (41)	2.74±0.23	24.4±1.24	12.6±0.44	128.2±11.52
	PQ-7(16)	2.33±0.14[c]	1.8±0.23[c]	1.5±0.21[c]	8.6±1.36[c]
	PQ-14(17)	2.41±0.18	2.1±0.24[c]	1.5±0.17[c]	10.5±1.70[c]
Succinate	Control (44)	1.44±0.09	2.8±7.00	43.2±3.18	249.8±28.34
	PQ-7(13)	0.94±0.07[c]	18.0±1.80[c]	13.0±1.39[c]	34.5±5.06[c]
	PQ-14(13)	0.81±0.16[c]	18.2±1.04[c]	14.3±1.59[c]	27.4±3.60[c]
Ascorbate + TMPD	Control (42)	0-66±0.06	4.6±9.91	50.2±5.08	106.2±8.95
	PQ-7(8)	0.50±0.05[a]	28.6±2.28[c]	22.8±2.29[c]	29.5±5.43[c]
	PQ-14(16)	0.41±0.03[a]	33.0±0.15[c]	23.4±1.70[c]	26.5±2.41[c]

The experimental conditions are as described in the text. The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis.
[a] $p< 0.05$; [b] $p<0$ 02 and [c] $p<0.001$ as compared with the corresponding control.

Table 3. Effect of quinine treatment on oxidative phosphorylation in rat liver mitochondria

Substrate	Animals	ADP/O ratio	Rate of respiration (n moles O_2 /min /mg protein) + ADP	- ADP	ADP phosphorylation rate (n mole/min/mg protein)
Glutamate	Control (46)	2.88±0.21	23.5±1.29	10.8±0.92	129 3±9 70
	Q-7(22)	2.28±0.12[a]	5 2±0.40[b]	3.1±0.22[b]	23.0±1.81[b]
	Q-14(11)	2.12±0.24[a]	3 8±0.35[b]	2 5±0.30[b]	23.3±2.07[b]
Pyruvate + malate	Control (41)	2.74±0.23	24.4±1.24	12.6±0.44	128.2±11.52
	Q-7(15)	2.37±0.21[b]	2.8±0.29[b]	1.9±0.27[b]	12.8±1.37[b]
	Q-14(10)	2.27±0.45	1 1±0.13[b]	0.4±0.12[b]	4 0±0.48[b]
Succinate	Control (44)	1.44±0.09	82.8±7.00	43.2±3.18	249.8±28.34
	Q-7(19)	0.75±0.07[b]	11.0±0.88[b]	7.9±0.86[b]	15.9±1.74[b]
	Q-14(14)	0.51±0.03[b]	13.9±0.87[b]	8.5±0.71[b]	13.8±0.59
Ascorbate + TMPD	Control (42)	0.66±0.06	94.6±9.91	50 2±5 08	106.2±8.95
	Q-7(14)	0.28±0.03[b]	15.4±1.36[b]	12.6±0.83[b]	8.4±0.93[b]
	Q-14(10)	0 38±0 04[b]	25.2±1.89[b]	19.5±1.93[b]	18.4±1.43[b]

The experimental conditions are as described in the text. The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis.

[a] $p < 0.02$ and [b] $p < 0.001$ as compared with the corresponding control.

rates were lower by 80 % With pyruvate + malate as substrate the extent of inhibition of respiration rates was about 90 %. The net overall effect on ATP synthesis was about 95 % decrease. As in the case of CQ (Table1) with succinate as the substrate PQ treatment also affected the respiration rates to a lesser extent and the inhibition ranged from 70 to 78 %. Likewise the extent of uncoupling was 35 and 44 % respectively in the two treatment groups and the ADP phosphorylation rate showed an overall 90 % decrease. Even with ascorbate + TMPD the inhibition of respiration rates was of lower magnitude and ranged from 55 to 70 %. Consequently the ADP phosphorylation rates were lowered only to the extent of 70 %.

When the animals were treated with Q the pattern was somewhat different in that the state 3 and state 4 respiration rates were affected maximally with pyruvate + malate and succinate as the substrate; extent of inhibition with glutamate and ascorbate + TMPD as the substrates was lower (Table 3). The pattern of uncoupling was similar to that seen for PQ The ADP phosphorylation rate decreased maximally with pyruvate + malate and succinate

Since FoF1 ATPase (complex V) plays an important role in ATP synthesis (17) we next evaluated the effect of antimalarials treatment on mitochondrial ATPase activity under different conditions The data are given in Table 4. As can be noted, in the controls addition of Mg^{2-} stimulated the ATPase activity, with addition of DNP showing the maximum stimulation. Activity was intermediate in the presence of Mg^{2+} and DNP. CQ treatment in one week group caused stimulation of basal (- Mg^{2+} and – DNP) and Mg^{2+} - stimulated activities but DNP stimulated activity was lower Treatment with CQ for a prolonged period restored the basal and Mg^{2+}-stimulated activities PQ treatment elevated

Table 4. Effect of antimalarial treatment on liver mitochondrial ATPase.

Addition	Control (16)	CQ-7 (8)	CQ-14 (8)	PQ-7 (8)	PQ-14 (8)	Q-7 (8)	Q-14 (8)
-- --	0.77±0 11	3.78±0 37[c]	0.78±0 15	2 08±0.22[c]	1.95±0.07[c]	4.17±0.35[c]	2 24±0.26[c]
+ Mg	6.03±0.48	14.60±1 13[c]	6.76±1.30	7 70±0.70	7.31±0.78	14.55±1.05[c]	23.21±1.62[c]
+ DNP	20.49±0.57	11.88±1.60[c]	2.21±0.25[c]	11.85±1.82[c]	12.01±1.47[c]	11.85±1.45[c]	9.72±1.82[c]
+Mg + DNP	13.53±0.41	17.27±1.08[a]	9.03±0.41[c]	10.28±0.78[a]	9.17±0.53[c]	17.42±1.06[a]	15.29±0.29[b]

The experimental conditions are as described in the text. ATPase activity in umole Pi liberated / hr / mg protein. The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis.

[a] $p< 0.01$; [b] $p<0.002$ and [c] $p<0.001$ as compared with the corresponding control.

the basal and Mg^{2+} activities but the DNP and Mg^{2+} and DNP stimulated activities were lower Q treatments also stimulated the basal ATPase activity and Mg^{2+} stimulated activity. The latter activity was elevated to a very great extent. The DNP stimulated activities were low (about 50 %) and Mg^{2+} and DNP stimulations were comparable to the controls but somewhat higher.

The effect of antimalarial treatment on cytochrome contents in the mitochondria was also evaluated. The typical cytochrome spectra are shown in Fig 1 and the contents of the respective cytochromes are given in Table 5. Our values for the cytochrome contents in the control groups are comparable with previously reported data (12,15). CQ treatment resulted in about 38 % decrease in cytochrome aa_3 and cytochrome b contents. However, in the animals treated for 14 days, the values were normalized. PQ treatment had resulted in 23 % decrease in cytochrome aa_3 content with a reciprocal 28 % increase in the cytochrome b content. Following Q treatment for 14 days there was a substantial decrease in cytochrome aa_3 content whereas cytochrome b content remained elevated by 20 %

No appreciable changes in cytochrome $c+c_1$ content were seen under any of the experimental conditions

Discussion

The present investigations were undertaken to find out if long-term administration of antimalarials for treatment, management and prophylaxis could have adverse effects on the biochemical functions of the host tissue itself. Inhibition of the mitochondrial functions in *Plasmodium* by antimalarials has been suggested as one of the possible

Fig 1. Typical difference spectra of cytochromes of liver mitochondria from control and antimalarial treated rats. Mitochondria were solublized-using Triton X-100. Samples in the reference cuvette were oxidized with potassium ferrycyanide and those in the experimental cuvette were reduced with sodium dithionite. The difference spectra of reduced minus oxidized cytochromes were recorded for a wavelength span of 650-500 nm. Other experimental details are as given in the text. Final concentrations of liver mitochondria in (a) were 6.69, 3.05 and 3.55 mg /ml for control, CQ-7 and CQ-14 groups, (b) 6.69, 5.59 and 5.59 mg /ml for control PQ-7 and PQ-14 groups and (c) 6.69, 4.97 and 4.27 mg/ml for control, Q-7 and Q-14 groups.

Fig.1

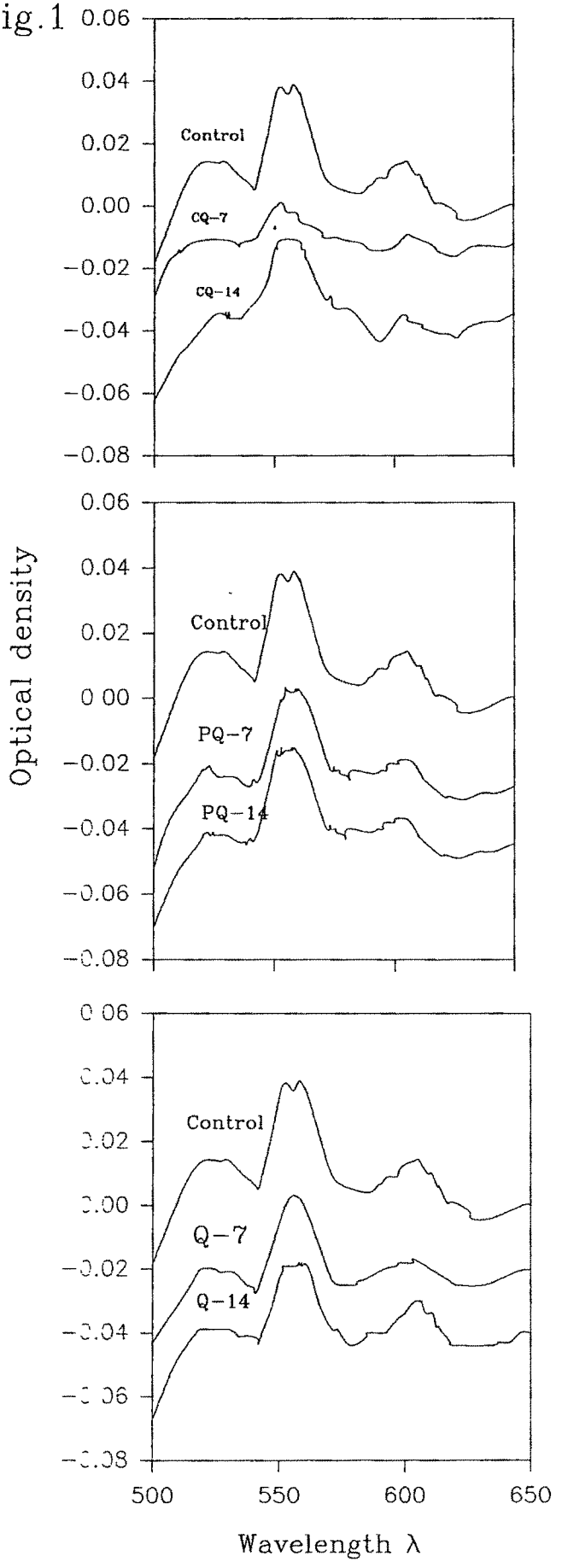
0.06
0.04
0.02
0.00
−0.02
−0.04
−0.06
−0.08
Control
CQ−7
CQ−14
Optical density
Control
PQ−7
PQ−14
Control
Q−7
Q−14
500
550
600
650
Wavelength λ

Table 5.
Effect of antimalarial treatment on liver mitochondrial cytochrome contents.

Group	Cytochrome		
	aa_3	b	$c+c_1$
Control (16)	80.2±4.48	191.8±5.75	276.3±7.17
CQ-7 (8)	50.2±5.94[d]	118.8±9.82[d]	254.4±12.95
CQ-14 (8)	77.7±4.62	182.9±7.18	270.7±8.12
PQ-7 (8)	69.6±5.42	167.9±9.04[a]	252.9±5.61
PQ-14 (8)	109.0±8.53[b]	229.7±10.77[b]	288.2±6.83
Q-7 (8) 296.4±11.28		61.8±6.45[a]	246.1±5.98[d]
Q-14 (8)	128.5±9.32[d]	229.8±8.68[c]	283.6±9.18

The experimental conditions are as described in the text. Cytochrome contents are given as pmoles / mg protein

The results are expressed as mean ± SEM of the number of observations indicated in the parenthesis.

[a] $p< 0.05$; [b] $p<0.01$, [c] $p<0.002$ and [d] $p<0.001$ as compared with the corresponding control

mechanism of action of antimalarials (7-8). Hence we focused our attention to find out if prolonged treatment with antimalarials adversely affected mitochondrial functions in liver- the tissue which harbors the parasite.

Indeed the present results have shown that the mitochondrial respiration rates were drastically inhibited by the treatment with CQ, PQ and Q. Interestingly the extent of inhibition was relatively higher when glutamate, pyruvate + malate and succinate were used as the substrates than that observed with ascorbate + TMPD as the electron donor system. Inhibition of $NAD(P)^+$-dependent dehydrogenases by antimalarials is well documented, which is attributed to electron extraction by the antimalarials (18,19) However, we find that even oxidation of succinate and ascorbate + TMPD was severely impaired by treatment with the three antimalarials. The results with succinate would suggest that the antimalarials interact not only at the level of NAD^+ but also at the level of flavins. Such an assumption is also supported by our observation that the extent of inhibition of respiration was always higher for pyruvate + malate compared to glutamate (Tables 1-3): pyruvate dehydrogenase complex requires both NAD^+ as well as FAD as the coenzymes (20). Ascorbate + TMPD oxidation does not involve any dehydrogenase system. Despite this a substantial inhibition of respiration, albeit of lesser magnitude compared to that seen for the NAD^+- and FAD- linked substrates was noted even for this system The results would therefore suggest that antimalarials may be interacting directly with components of electron transport chain other than NAD^+ and flavins which are involved in ascorbate +TMPD mediated electron transport. We also see a significant inhibition of DNP stimulated ATPase activity. (Table 4) which would also substantiate the assumption that the antimalarials interact with other sites which do not involve NAD^+ and/or flavins

Interestingly, the basal and Mg2+ stimulated ATPase activities had increased following antimalarial treatment (Table 4) which is suggestive of increased mitochondrial fragility.

One interesting observation which emerged from these studies was that the antimalarials also acted as uncouplers of oxidative phosphorylation. The maximum uncoupling effect was seen on the second and the third site of phosphorylation; apparently the first site was not affected.

Although the data on site-specific uncoupling effects are not given, these can be easily computed from ADP/O ratios given in Tables 1-3.

Analysis of the cytochrome contents revealed that the antimalarials treatment did not significantly affect the cytochrome $c+c_1$ content. Antimalarial treatment for 7 days resulted in the lowering of cytochrome aa_3 content but longer treatment for 14 days resulted either in restoration or increased content of cytochrome aa_3. Similar trend was noted for cytochrome b content following CQ and PQ treatments. With quinine, in both treatment groups the content of cytochrome b was elevated by 20 to 28 %. The alterations in cytochrome profile may represent both response to antimalarial treatment as well as compensatory mechanisms at a latter stages when increase in cytochrome aa_3 and cytochrome b is noted. Cytochrome c is known to be synthesized on cytoribosomes whereas cytochrome b and three large subunits of cytochrome aa_3 are mitochondrial gene products (14) It may hence be inferred that the antimalarials could act at the level of mitochondrial DNA. The effects on ATPase were of opposite nature i.e. the DNP stimulated activities were always low in antimalarials treatment groups (Table 4). Since

the functional crucial polypeptides of FoF1 ATPase are also mitochondrial gene product (14), it is possible that the antimalarials may inhibit their synthesis.

The routes of the catabolism of three antimalarials are different. CQ and PQ are mainly metabolized by mitochondrial monoamine oxidase whereas Q is metabolized by the cytochrome P_{450} system in the microsomes (21,22). It has also been reported that the metabolites of the antimalarials could have different antimalarial and toxicity properties (1,23) This is consistent with the variability of the effects of the three antimalarials that are observed here on the mitochondrial functions.

Thus the results of the present studies show that prolonged treatment with antimalarial can adversely affect the mitochondria functions. However, at this stage it is not possible to comment on whether the effect observed are the effect per say due to antimalarial actions or are to be attributed to the actions of their metabolites or to both. The question can partly be clarified by performing in vitro experiments with antimalarials and/or metabolites.

The decreased respiration rates and ADP phosphorylation rates are a matter of concern and deserve some comment It may be anticipated that the energy restriction following antimalarials treatment would affect the metabolic activities of the tissue i.e. liver and the organism as a whole. Paradoxically, however, treatment with PQ or Q had practically no effect on body and liver weights (Table 6). CQ treatment lowered the body weight marginally and drastically reduced the liver weights after one-week treatment; in two week group there was some recovery in liver weights (Table 6). We also found that the antimalarials treated animals became sluggish and lethargic.

Table 6.
Effect of antimalarials treatment on body and liver weight of the rats.

Treatment liver wt	Group	Body wt, g	Liver wt, g	Relative
7 Day	Control	272.9±11.33	6.94±0.52	2.56±0.20
	Chloroquine	236.3±5.14[b]	4.57±0.12[d]	1.93±0.04[c]
	Primaquine	281.0±13.14	6.67±0.28	2.36±0.04
	Quinine	276.4±3.00	6.56±0.33	2.38±0.10
14 Days	Control	275.5±11.70	7.03±0.31	2.55±0.05
	Chloroquine	243.7±6.20[a]	5.51±0.10[c]	2.27±0.06[c]
	Primaquine	290.5±9.63	6.97±0.23	2.40±0.39
	Quinine	283.8±7.60	7.21±0.19	2.30±0.06[c]

The results are expressed as mean ± SEM of eight independent observations.

[a] $p< 0.05$; [b] $p<0.02$; [c] $p<0.01$ and [d] $p<0.001$ as compared with the corresponding control.

The respiratory activity of mitochondria and the ATP synthesis rates in situ are under rigorous control of various factor which include concentration of ADP and Pi, NADH/ NAD^+ ratio, phosphorylation potential, adenylate energy charge and calcium levels (24). However, ATP concentration is cosidered as the major controling factor (24). In the context of this, it may be mentioned that in the isolated mitochondria the respiration rates and ADP phosphorylation rates are determined under optimized conditions; the rates in situ need not be optimum at all the times and will very depending on energy requirements (24). Hence it may be suggested the residual ATP synthesizing activities that we see after antimalarials treatment may be just about sufficient to maintain tissue functions under constraint of antimalarials treatment. This becomes apparent especially from experiments with CQ where reduction in body and liver weight was apparent(Table 6). It is possible that given sufficient time similar effects could have been observed with PQ and Q. However, in these instances we did not extend the experiments beyond 14 days. Nevertheless the results of the present study caution against prolonged use of antimalarials which can result in severe energy impairment and metabolic activities.

It is also possible that the antimalarials effects may be restricted to the tissue such as liver since as mentioned above, the liver accumulates the antimalarials in very high concentration of about 75 mM (9). Hence it is likely that the energy metabolism in other tissues may not be affected. However, this point needs to be verified experimentally.

In parallel studies it was also found that CQ and PQ treatment alters the mitochondrial lipid/ phospholipid composition. The level of acidic phospholipids i e. phosphotidyl serine and phosphotidyl inositol increased while phosphotidyl choline levels decreased

(Chapter 7). The altered phospholipid composition could have a bearing on the decreased respiration rates, which are observed here (Tables 1-3).

Reference

1. Tracy J W and Leslie T W Jr. Drugs used in the chemotherapy of protozoal infections- Malaria, in The pharmacological basis of therapeutics, edited by A G Gilmann, T W Rall, A S Nies and P Taylor, 10th ed, Pergamon press, New York 2001.1069-1095.
2. Thabrew M I, Nashiru T O, Emerole G O. Drug induced alterations in some rat hepatic microsomal components: a comparative study of four structurally different antimalarials. Comp Biochem Physiol C. 1985;81(1):133-138.
3. De Duve C, De Barsy T, Poole B, Trouet A, Tulkens P and Van Hoof F. Lysosomotropic agents, Biochem Pharmacol, 23 (1974) 2495-2568.
4. Foley M and Tilley L. Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. Pharmacol Ther. 1998; 79(1):55-87.
5. Essien E E and Ette E I. Effects of chloroquine and didesethylchloroquine on rabbit myocardium and mitochondria. J Pharm Pharmacol. 1986;38(7):543-546.
6 Ayitey-Smith E, Gbewonyo A J. Ultrastructural changes in the heart of rats treated with chloroquine. West Afr J Pharmacol Drug Res. 1977;4(1):7-15.
7 Krungkrai J, Burat D, Kudan S, Krungkrai S and Prapunwattana P. Mitochondrial oxygen consumption in asexual and sexual blood stages of the human malarial parasite, *Plasmodium falciparum*. Southeast Asian J Trop Med Public Health. 1999;30(4):636-642.
8. Lanners H N. Effect of the 8-aminoquinoline primaquine on culture-derived gametocytes of the malaria parasite *Plasmodium falciparum*. Parasitol Res 1991;77(6).478-481.

9. Hostetler K Y, Reasor M and Yazaki P J. Chloroquine-induced phospholipid fatty liver. Measurement of drug and lipid concentrations in rat liver lysosomes. J Biol Chem. 1985,260(1):215-219

10. Swegert C V, Dave K Rand Katyare S S. Effect of aluminium-induced Alzheimer like condition on oxidative energy metabolism in rat liver, brain and heart mitochondria. Mechanisms of Ageing and Development. 1999;112:27-42.

11. Satav J G and Katyare S S. Effect of experimental thyrotoxicosis on oxidative phosphorylation in rat liver, kidney and brain mitochondria. Molec Cell Endocrinol. 1982;28.173-189.

12. Katyare S S, Joshi M V, Fatterpaker P and Sreenivasan A. Effect of thyroid deficiency on oxidative phosphorylation in rat liver, kidney and brain mitochondria. Arch. Biochem Biophys. 1977;182:155-163.

13. Fiske C H and Subba Row Y, Colorometric determination of phosphorous, J Biol Chem. 1925;66:375-381.

14 Poyton R O and McEwen J E. Crosstalk between nuclear and mitochondrial genomes. Annu Rev Biochem. 1996;65:563-607.

15 Katyare S S and Rajan R R. Enhanced oxidative phosphorylation in rat liver mitochondria following prolonged in vivo treatment with imipramine Br. J. Pharmacol 1988,95 914-922

16. Lowry O H, Rosebrough N J, Farr A L and Randall R J, Protein measurement with folin-phenol reagent, J Biol Chem. 1951;193: 265-272.

17. Lehninger A L. Biochemistry, 1st ed, Worth Publishers, New York, 1970;520

18. Deepalakshmi P D, Parasakthy K, Shanti S and Devrajan N S, Effect of chloroquine on rat liver mitochondria, Ind J Exp Biol. 1994;32:797-802.

19. Jarzyna R, Lenarcik E and Bryla J. Chloroquine is a potent inhibitor of glutamate dehydrogenase in liver and kidney-cortex of rabbit. Pharmacol Res. 1997;35(1):79-84

20. Zubay J. Biochemistry, 3rd edn. WmC Brown Publishers, Dubuque, Iowa. 1993.

21. Zhao X-J and Ishizaki T. The in vitro hepatic metabolism of quinine in mice, rats and dogs: comparison with human liver microsomes, J Pharmacol Exp Ther. 1997;283:1168-1176.

22. Ni Y C, Xu Y Q and Wang M J. Rat liver microsomal and mitochondrial metabolism of primaquine in vitro. Zhongguo Yao Li Xue Bao. 1992;13(5):431-435.

23. Constantino L, Paixao P, Moreira R, Portela M J, Do Rosario VE, Iley J. Metabolism of primaquine by liver homogenate fractions. Evidence for monoamine oxidase and cytochrome P450 involvement in the oxidative deamination of primaquine to carboxyprimaquine. Exp Toxicol Pathol 1999;51(4-5):299-303.

24 Brown C G Control of respiration and ATP synthesis in mammalian mitochondria and cells. Biochem J 1992;284:1-13.

Summary

Effects of in vivo treatment with the three antimalarials chloroquine, primaquine and quinine on rat liver mitochondrial energy transduction functions were examined. Treatment with all the three antimalarials decreased the state 3 and state 4 respiration rates drastically. The extent of inhibition was higher with pyruvate + malate as the substrate than with glutamate. The antimalarials also acted as uncouplers; the uncoupling effect was seen on site II and site III of phosphorylation; site I was unaffected. As a consequence the ADP phosphorylation rates also decreased significantly. Following antimalarials treatment the basal and Mg^{2+} stimulated activities of mitochondrial ATPase increased while the DNP stimulated – ATPase activity was reduced by half. Treatment with chloroquine resulted in a decrease in contents of cytochromes aa_3 and b; primaquine and quinine treatments increased the contents of the two cytochromes in 14 day groups.