Biochemical Pharmacology, Vol. 20, pp.1877–1882. Pergamon Press, 1971. Printed in Great Britain

QUININE: EFFECT ON *TETRAHYMENA PYRIFORMIS*—III

ENERGETICS OF ISOLATED MITOCHONDRIA IN THE PRESENCE OF QUININE AND OTHER ANTIMALARIAL DRUGS*

K. A. CONKLIN, S. C. CHOU and P. HEU

Department of Pharmacology, School of Medicine, Honolulu, Hawaii 96816, U.S.A.

(*Received* 2 *July* 1970; *accepted* 13 *November* 1970)

Abstract—The effect of quinine, primaquine, quinacrine and chloroquine on substrate oxidation and oxidative phosphorylation was studied in isolated *Tetrahymena pyriformis* mitochondria. Primaquine and chloroquine stimulated the oxidation of succinate and inhibited oxidative phosphorylation, which indicates that these antimalarials act as uncoupling agents. This activity apparently requires both the quinoline nucleus and the diamino alkane side-chain since neither quinine, which contains only the quinoline nucleus, nor quinacrine, which contains only the side-chain, had a significant effect on oxidative phosphorylation or the oxidation of succinate.

Primaquine, chloroquine and quinine inhibited the oxidation of fumarate, malate, β-hydroxybutyrate and glutamate, but had little effect when isocitrate, citrate, or α-ketoglutarate were used as substrates. Since primaquine and chloroquine stimulate electron transfer with succinate, these results indicate an inhibition of the enzyme systems which oxidize these substrates. Otherwise, an increased respiratory rate would have been observed upon addition of primaquine or chloroquine. The pattern of inhibition was similar for all three antimalarials and may be because of the quinoline moiety common to these drugs. Quinacrine had no significant effect on any of these reactions.

QUININE and quinacrine inhibit synchronized cell division in *Tetrahymena pyriformis*[1,2] while blocking the synthesis of DNA, RNA and protein.[1,3] Since neither drug produced selective inhibition of a single biosynthetic pathway, it was suggested that the drugs may exert their effect on energy-yielding reactions.

Other reports have also suggested that these drugs, as well as chloroquine, inhibit energy-yielding reactions of various cell types. Quinine and quinacrine inhibit oxygen consumption of *Plasmodium* sp.[4–6] Quinacrine inhibits succinate oxidation in *T. pyriformis*[7] and interferes with electron transport.[8,9] Quinine also inhibits succinate oxidation in *T. pyriformis*;[7] however, both quinine[10] and chloroquine[10,11] have been shown to stimulate this reaction in other systems. Chloroquine inhibits the oxidation of α-ketoglutarate,[10] malate,[11] β-hydroxybutyrate,[11] and glutamate,[12] as well as the coenzyme Q system.[13] Quinine has also been shown to inhibit α-ketoglutarate oxidation.[10]

The present study shows the effect of quinine, quinacrine, chloroquine and primaquine on oxidative phosphorylation and on the oxidation of tricarboxylic acid cycle

* Supported by a Fried Foundation Research Grant and a NIH Predoctoral Fellowship to K. A. Conklin.

intermediates, β-hydroxybutyrate and glutamate in isolated *T. pyriformis* mitochondria. The concentrations of each drug studied were those which inhibit synchronized cell division in this organism.

MATERIALS AND METHODS

Organism and growth conditions. Tetrahymena pyriformis, strain GL, was maintained in medium containing 1·0% proteose peptone (Difco), 0·1% sodium acetate, 0·1% K_2HPO_4, and 0·1% dextrose. For experimental purposes, 2-day-old cultures were used to inoculate 1250 ml of peptone-liver medium in 2800 ml Fernbach culture flasks. The medium contained 2·0% proteose peptone (Difco), 0·1% liver fraction (Wilson Laboratories), 0·5% dextrose, and sulfates and chlorides as described previously.[1] Cultures were incubated for 18–24 hr on a gyrotory shaker at 25–27°. The cells were harvested when the population reached approximately 200,000/ml. Cell counts were done as described previously.[1]

Preparation of mitochondria. Cells were harvested in conical 500-ml bottles in an International Centrifuge, Model PR-2. All of the following procedures were carried out at 0–4°. The supernatant was removed by aspiration and the cells were washed three times in 0·5% NaCl in 40-ml centrifuge tubes, with centrifugation for each wash at 250 *g* for 2 min. The washed cells (approximately 4 ml of packed cells) were suspended in 10 ml of a solution (solution A) containing 0·05 M tris–HCl (pH 7·4), 1 mM phosphate, 0·25% bovine serum albumin (Calbiochem, B grade), 4 mM $MgCl_2$ and 0·25 M sucrose. The cells were disrupted by homogenization for 5 min in a tight-fitting glass homogenizer. The homogenate was quickly diluted to 60 ml with solution A and centrifuged for 3 min at 8000 *g*. The supernatant was discarded and the pellet resuspended in 60 ml of solution A. Intact cells were removed by centrifugation at 250 *g* for 2 min. The remaining suspension was then centrifuged for 3 min at 1500 *g* and the resulting pellet was washed once more. The final pellet was resuspended in solution A for analysis. This procedure yielded a particulate preparation with a protein content of 10–50 mg/ml as determined by the method of Lowry *et al.*[14]

Measurement of oxidative processes. Oxidation of substrates was determined by measuring the rate of oxygen consumption of the mitochondria suspension in a Yellow Springs Instrument Co., Model 53, Biological Oxygen Monitor. Two reaction cells were used, each containing 2·0 ml of the mitochondria suspension (5–25 mg protein) in solution A plus 5 μmoles of ADP. The mitochondria suspension was allowed to equilibrate to 29°, and then the respiration was followed for 18 min. The first 3 min were used to determine the rate of oxidation of endogenous substrates. The appropriate substrate (5 μmoles) was then added, and 5 min later the drug was added to one cell while the other cell served as a control. In some experiments, ADP was omitted in order to investigate uncoupling activity.

Measurement of oxidative phosphorylation. The standard 2·0 ml reaction mixture contained 5–10 mg protein (the mitochondria preparation), 100 μmoles tris–HCl (pH 7·4), 8 μmoles $MgCl_2$, 2 μmoles phosphate, 5 mg bovine serum albumin, 500 μmoles sucrose, 2 μmoles ADP, 60 μmoles glucose, 25 units hexokinase (Calbiochem), 40 μmoles NaF, 5 μmoles succinate or isocitrate, and 2 μc ^{32}P orthophosphate (Schwarz BioResearch). Other additions were made as indicated. The reaction was started by the addition of the mitochondria suspension and the mixture was incubated

at 29°. Samples (0·5 ml) were taken at 0 and 20 min and placed in 0·1 ml of 24% trichloroacetic acid. Oxidative phosphorylation was determined by measuring the esterified ^{32}P as described by Nielson and Lehninger.[15] In brief, this procedure consists of extracting inorganic phosphate (as phosphomolybdic acid) into an isobutanol–benzene solution. Phosphorylation was then determined by placing 0·1-ml samples of the organic phosphate fraction on 2·3-cm discs of Whatman 3 MM filter paper, drying the discs at 60°, and counting the radioactivity in a Beckman LS 150 liquid scintillation counter. The scintillation fluid consisted of 5 g of 2,5-diphenyloxazole (PPO) per liter of toluene.

RESULTS

Properties of the mitochondria. The properties of the mitochondria are described in detail elsewhere.* In brief, the mitochondria are characterized as follows: potassium cyanide, amytal and rotenone (specific inhibitors of NADH oxidation), and thenoyltrifluroacetone (specific inhibitor of succinate oxidation) inhibited electron transport at concentrations comparable to those effective in mammalian mitochondria. The mitochondria, however, were quite insensitive to antimycin A. Oxidative phosphorylation was inhibited by the uncoupling agents, 2,4-dinitrophenol and pentachlorophenol; however, it was relatively insensitive to oligomycin as compared to that of mammalian mitochondria. If ADP was not added to the reaction mixture, incorporation of inorganic phosphate was almost completely inhibited. Respiratory control ratios were 2·0 with succinate as substrate and 1·7 with isocitrate as substrate. The P:O ratios were 1·0 and 1·4 with succinate and isocitrate respectively.

The effects of antimalarial drugs on the oxidation of tricarboxylic acid cycle intermediates, β-hydroxybutyrate, and glutamate. Table 1 shows the effect of the antimalarial drugs on the oxidation of various substrates. The concentrations of quinine, primaquine, and chloroquine are those which produce complete inhibition of synchronized cell division in *T. pyriformis*.[1,2]† Quinacrine completely inhibits cell division at $3{\cdot}6.10^{-5}$ M;[3] however, this drug was used at a concentration ($5{\cdot}0.10^{-4}$ M) comparable to that of the other drugs. Citrate oxidation was not significantly altered by any of the drugs, whereas isocitrate oxidation was affected only by chloroquine (12 per cent stimulation). Chloroquine was also the only drug significantly affecting α-ketoglutarate oxidation (8 per cent inhibition). The respiratory rate with succinate was stimulated 24 and 52 per cent by primaquine and chloroquine, respectively, but unaffected by quinine or quinacrine. Quinine, primaquine and chloroquine significantly inhibited the respiratory rate to varying degrees when fumarate, malate, β-hydroxybutyrate and glutamate were used as substrates; however, quinacrine had no significant effect on the respiratory rate with any of these substrates.

The effects of antimalarial drugs on the oxidation of succinate and isocitrate in the absence of ADP. Table 2 illustrates the effect of the antimalarial drugs on the respiratory rates with succinate and isocitrate in the absence of ADP. Succinate oxidation was significantly stimulated by chloroquine and primaquine, but not by quinine or quinacrine. The degree of stimulation observed with chloroquine (141 per cent) and primaquine (51 per cent) was considerably greater than that observed when ADP was

* K. A. Conklin and S. C. Chou, unpublished observations.
† Our unpublished results.

TABLE 1. EFFECT OF ANTIMALARIAL DRUGS ON THE RESPIRATORY RATE WITH TRICARBOXYLIC ACID CYCLE INTERMEDIATES, β-HYDROXYBUTYRATE AND GLUTAMATE AS SUBSTRATES*

Substrate	Respiratory rate μatoms O_2/min × 10^2/mg protein (mean ± S.E.)				
	Control	Quinine	Primaquine	Quinacrine	Chloroquine
Citrate	9·5 ± 0·6	9·1 ± 0·7	9·1 ± 0·3	9·0 ± 0·7	10·1 ± 0·7
Isocitrate	25 ± 1·0	24 ± 1·3	26 ± 1·7	24 ± 0·8	28 ± 0·7† (+12)
α-Ketoglutarate	5·0 ± 0·3	4·9 ± 0·5	5·0 ± 0·4	4·8 ± 0·3	4·6 ± 0·5
Succinate	21 ± 0·7	22 ± 1·2	26 ± 0·9† (+24)	20 ± 0·8	32 ± 2·5† (+52)
Fumarate	4·1 ± 0·4	3·2 ± 0·1† (−22)	2·7 ± 0·2† (−34)	3·9 ± 0·2	1·9 ± 0·2† (−54)
Malate	9·0 ± 0·4	7·1 ± 0·3† (−21)	6·2 ± 0·5† (−31)	8·6 ± 0·2	6·2 ± 0·5† (−31)
β-Hydroxybutyrate	6·3 ± 0·3	5·3 ± 0·3† (−16)	5·5 ± 0·2† (−12)	6·1 ± 0·4	4·6 ± 0·4† (−27)
Glutamate	6·8 ± 0·4	5·6 ± 0·3† (−18)	5·0 ± 0·2† (−27)	7·0 ± 0·4	5·4 ± 0·2† (−21)

* Assay conditions are as described in Materials and Methods with 5 μmoles of ADP added to each reaction cell. Drug concentrations are: quinine, $5{\cdot}01.0^{-4}$ M; primaquine, $5{\cdot}4.10^{-4}$ M; quinacrine $5{\cdot}0.10^{-4}$ M; and chloroquine, $1{\cdot}4.10^{-3}$ M. Numbers in parentheses are percentages inhibition (−) or stimulation (+) of the respiratory rate (when significant). Each value is from three determinations.

† P vs. control $<0{\cdot}05$; others not significant.

TABLE 2. EFFECT OF ANTIMALARIAL DRUGS ON THE RESPIRATORY RATE WITH SUCCINATE AND ISOCITRATE IN THE ABSENCE OF ADP*

Substrate	Respiratory rate μatoms O_2/min × 10^2/mg protein (mean ± S.E.)				
	Control	Quinine	Primaquine	Quinacrine	Chloroquine
Succinate	3·4 ± 0·2	3·6 ± 0·4	5·3 ± 0·6† (51)	3·2 ± 0·2	8·2 ± 0·3† (141)
Isocitrate	5·2 ± 0·5	4·6 ± 0·4	4·8 ± 0·4	5·1 ± 0·5	5·7 ± 0·8

* Assay conditions are as described in Materials and Methods, with ADP omitted. Drug concentrations are as described in Table 1. Numbers in parentheses are percentages stimulation of the respiratory rate (when significant). Each value is from three determinations.

† P vs. control $<0{\cdot}05$; others not significant.

present (Table 1). The oxidation of isocitrate was not significantly affected by any of the drugs.

The effects of antimalarial drugs on oxidative phosphorylation. Table 3 illustrates the effect of the antimalarial drugs on phosphate incorporation. With succinate as substrate, oxidative phosphorylation was inhibited 32 per cent by primaquine and 59 per cent by chloroquine, whereas with isocitrate these drugs produced 44 and 62 per cent inhibition respectively. Quinine and quinacrine had no significant effect on this reaction.

TABLE 3. EFFECT OF ANTIMALARIAL DRUGS ON OXIDATIVE PHOSPHORYLATION*

Substrate	Control	Phosphate incorporation counts/min $^{32}P \times 10^{-4}$/mg protein (mean ± S.E.) Quinine	Primaquine	Quinacrine	Chloroquine
Succinate	15·1 ± 0·18	14·5 ± 0·38	10·3 ± 1·04† (32)	14·7 ± 0·53	6·2 ± 0·24† (59)
Isocitrate	19·3 ± 0·23	19·0 ± 0·49	10·8 ± 0·53† (44)	18·8 ± 0·67	7·3 ± 0·31† (62)

* Assay conditions are as described in Materials and Methods, and drug concentrations are as described in Table 1. Numbers in parentheses are percentages inhibition of phosphate uptake (when significant). Each value is from three determinations.

† P vs. control $<0·05$; others not significant.

DISCUSSION

The results presented here indicate that quinine, primaquine and chloroquine, at concentrations which inhibit cell division, inhibit the energetics of *T. pyriformis*. Along with the interaction with DNA as others have suggested,[16–20] these actions may contribute to the inhibition of cell division. Quinacrine did not inhibit any of the reactions studied in isolated *T. pyriformis* mitochondria. Its primary mechanism may also be an interaction with DNA.[20]

Primaquine and chloroquine appear to act as uncoupling agents since they stimulated succinate oxidation and inhibited oxidative phosphorylation. Quinine and quinacrine apparently do not act in this manner. Primaquine and chloroquine have very similar side-chains (Fig. 1) in addition to a quinoline nucleus. Primaquine contains a diamino alkane side-chain on the 8 position, whereas chloroquine possesses a similar side-chain on the 4 position. Therefore, uncoupling activity appears to require both the quinoline nucleus and the side-chain since this effect is neither observed with quinine, which contains the quinoline nucleus but an appreciably different side-chain,

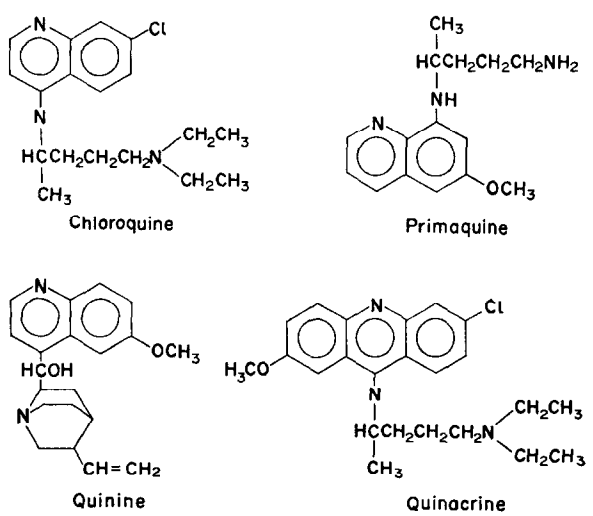

FIG. 1. Structures of the antimalarial drugs.

nor quinacrine, which contains the same side-chain as chloroquine but three fused aromatic rings instead of two.

The oxidation of malate, fumarate, β-hydroxybutyrate and glutamate was significantly inhibited by chloroquine, primaquine and quinine, whereas the oxidation of citrate, isocitrate and α-ketoglutarate was little affected. However, since chloroquine and primaquine act as uncoupling agents, the respiratory rate with these substrates should increase in the presence of these drugs. Except in the case of isocitrate oxidation in the presence of chloroquine, no significant increase was observed. Therefore, it must be concluded that the drugs have multiple inhibitory effects on the enzyme systems of respiration and phosphorylation. This interpretation is supported by the results of others which show stimulation of succinate oxidation[10,11] and inhibition of malate,[11] β-hydroxybutyrate,[11] α-ketoglutarate,[10] and glutamate[12] oxidation by chloroquine.

Inhibition of substrate oxidation may be because of a structural feature which is common to primaquine, chloroquine and quinine since the pattern of inhibition is similar for each drug. The quinoline nucleus is a major structural similarity between these three drugs (Fig. 1) and may be responsible for the inhibition observed.

Eichel[7] reported that quinine and quinacrine inhibit succinate oxidation in *T. pyriformis* homogenates at 2.10^{-3} M and 3.10^{-3} M respectively. Our results show that concentrations which inhibit cell division do not block this reaction. Both Hass[8] and Strittmatter[9] suggested that quinacrine blocks electron transport, but in both cases the drug concentration used (10^{-3} M) was higher than that employed by us.

REFERENCES

1. K. A. CONKLIN, S. C. CHOU and S. RAMANATHAN, *Pharmacology* **2**, 247 (1969).
2. S. C. CHOU, S. RAMANATHAN and W. C. CUTTING, *Pharmacology* **1**, 60 (1968).
3. S. C. CHOU and S. RAMANATHAN, *Life Sci.* **7**, 1053 (1968).
4. J. D. FULTON and S. R. CHRISTOPHERS, *Ann. Trop. Med. Parasitol.* **32**, 77 (1938).
5. L. T. COGGESHALL and J. MAIER, *J. infect. Dis.* **69**, 108 (1941).
6. M. SILVERMAN, J. CEITHMAL, L. G. TALIAFERO and E. A. EVANS, JR , *J. infect. Dis.* **75**, 212 (1944).
7. H. J. EICHEL, *Biochim. biophys. Acta* **22**, 571 (1956).
8. E. HASS, *J. biol. Chem.* **155**, 321 (1944).
9. C. F. STRITTMATTER, *J. biol. Chem.* **234**, 2794 (1960).
10. A. G. DATTA and U. P. BASU, *J. Sci. Ind. Research (India)* **14C**, 61 (1955)
11. G. A. ARDUESER and H. C. HEIM, *J. pharm. Sci.* **56**, 254 (1967).
12. U. GERLACH, *Klin. Wschr.* **36**, 376 (1958).
13. F. S. SKELTON, R. S. PARDINI, J. C. HEIDKER and K. FOLKERS, *J. Am. chem. Soc.* **90**, 5334 (1968).
14. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).
15. S. O. NIELSON and A. L. LEHNINGER, *J. biol. Chem.* **215**, 555 (1955).
16. R. D. ESTENSEN, A. K. KREY and F. E. HAHN, *Molec. Pharmac.* **5**, 532 (1969).
17. L. P. WHICHARD, C. R. MORRIS, J. M. SMITH and D. J. HOLBROOK, JR., *Molec. Pharmac.* **4**, 630 (1968).
18. S. N. COHEN and K. L. YIELDING, *J. biol. Chem.* **240**, 3132 (1965).
19. S. N. COHEN and K. L. YIELDING, *Proc. natn. Acad. Sci., U.S.A.* **54**, 521 (1965).
20. F. E. HAHN, R. L. O'BRIEN, J. CIAK, J. L. ALLISON and J. G. OLENICK, *Military Med.* **131** (suppl), 1071 (1966).