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Oxygen- and Time-Dependent Effects of Antibiotics and Selected Mitochondrial Inhibitors on *Plasmodium falciparum* in Culture†

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Several antibiotics which inhibit protein synthesis on 70S ribosomes, including clindamycin, pirlimycin, 4′-pentyl-*N*-demethyl clindamycin, four tetracyclines, chloramphenicol, thiamphenicol, and erythromycin, had antimalarial effects against *Plasmodium falciparum* in culture which were greatly influenced by the duration of drug exposure and by oxygen tension. In 96-h incubations, potency was increased by a factor of up to 10^6 over the first 48-h period and by a factor of up to 10^4 in 15% O_2 versus 1% O_2. Two aminoglycosides, kanamycin and tobramycin, had no antimalarial activity. Rifampin and nalidixic acid, which inhibit nucleic acid synthesis, were not similar to the 70S inhibitors. The mitochondrial inhibitors Janus Green, rhodamine 123, antimycin A_1, and 8-methylamino-8-desmethyl riboflavin had activities which were influenced by the duration of exposure and oxygen tension. Quinoline-containing antimalarial agents, ionophores, and other antimalarial drugs were influenced to a minor extent by the duration of exposure but were not affected by oxygen tension. These data can best be explained by the hypothesis that antimalarial 70S ribosome-specific protein synthesis inhibitors are toxic to the parasites by acting on the mitochondrion.

The increasing spread of chloroquine-resistant strains of *Plasmodium falciparum* (30, 55) has evoked considerable effort toward the identification and characterization of new antimalarial drugs. Except for antifolates, most of this effort has been directed toward drugs which are modifications of the quinoline-containing antimalarial agents quinine and chloroquine (29, 38, 47), which may not escape the problems of resistance that currently plague the prototypes.

Antimalarial activity is present in some antibiotics, many of which are active against *P. falciparum* in vitro at relevant pharmacological concentrations (16). Rifampin (2), chloramphenicol (12, 39), and erythromycin (53) have demonstrable antimalarial effects in animal models, whereas tetracyclines (11, 13, 32, 36, 54) and clindamycin (7, 10, 26, 37) have been shown to be effective against *P. falciparum* in humans. Although no data are available on the mechanism(s) of antimalarial action of these drugs, it has been suggested that they act on parasite mitochondria, which, like all eucaryotic mitochondria, probably contain 70S ribosomes (16; J. J. Blum, A. Yayon, F. Friedman, and H. Ginsburg, J. Protozool., in press). Recently, we have shown that *P. falciparum* contains a functional mitochondrion which, by virtue of its ability to concentrate rhodamine 123, actively maintains a high transmembrane potential (A. A. Divo, G. Geary, H. Ginsburg, and J. B. Jensen, J. Protozool., in press). However, since no information has yet been obtained on the metabolic role (if any) of this organelle in the erythrocytic stages of this parasite (45), it is difficult to directly test the hypothesis that antimalarial antibiotics act at the mitochondrial level.

It has been shown that the antimalarial potency of some antibiotics is influenced by the duration of exposure in culture (16; D. J. Krogstad, submitted for publication) and, for clindamycin, by oxygen tension (Krogstad, submitted for publication). We indirectly tested the mitochondrial toxin hypothesis by using these two variables to compare the effects of various antibiotics, mitochondrial inhibitors, and other drugs on *P. falciparum* in vitro.

MATERIALS AND METHODS

Stock cultures were maintained in candle jars (19, 49). In most experiments, the FCR_{3TC} isolate (18) was used, although the Viet Nam Smith, $FCMSU_1$/Sudan (manuscript in preparation), and FCR_8 (28) strains were tested to control for strain differences in sensitivity.

Experiments were conducted in 96-well microtiter plates (Linbro) as described previously (15); [^{3}H]hypoxanthine (10 Ci/mmol; New England Nuclear Corp., Boston, Mass.) incorporation was used as a measure of drug effects (14). Each well contained 2 μl of infected erythrocytes, 200 μl of RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with HEPES (*N*-2-hydroxy-ethylpiperazine-*N*′-2-ethanesulfonic acid) (Sigma Chemical Co., St. Louis, Mo.), sodium bicarbonate, and 10% pooled human serum (RP-10), and various drug concentrations. Drug exposure occurred for 48 or 96 h. In 48-h experiments, initial parasitemias were 1 to 2% schizonts synchronized as described previously (56) by a combination of sorbitol lysis (22) and gelatin flotation (17). Each well received 2 μCi of [^{3}H]hypoxanthine. After 48 h, the plates were harvested onto glass fiber filters with a Bellco cell harvester. The filters were dried, added to scintillation fluid, and counted with a Beckman LS 7500 scintillation spectrometer (15).

In 96-h experiments, parasitemias were initially 0.2 to 0.3% schizonts. After 48 h, the supernatant was carefully aspirated from each well and replaced with medium containing identical drug concentrations and 10 μCi of [^{3}H]hypoxanthine per ml. After an additional 48 h, the microtiter plates were harvested as described above. 8-Methylamino-8-desmethyl riboflavin was tested exactly as described previously, except RPMI 1640 medium was replaced with a semi-defined minimal medium which lacks riboflavin (A. A. Divo, T. G. Geary, N. L. Davis, and J. B. Jensen, J. Parasitol., in press).

Experiments were conducted in a tissue culture chamber (Billups-Rothenburg, Inc.) gassed with a mixture of 1%

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O_2–3% CO_2–96% N_2 (low oxygen) or in candle jars; the gas mixture in these containers was reported to be approximately 15% O_2, 2% CO_2, and 84% N_2 (43). Gas phases were changed daily.

All drugs were initially dissolved in either 100% ethanol or in triple-distilled water at concentrations ranging from 10^{-1} to 10^{-3} M. These solutions were sterilized by filtration through 0.45-μM (pore size) membrane filters (Schleicher & Schuell, Inc., Keene, N.H.) when necessary.

All data points represent means of 3 to 12 experimental observations; standard errors were ≤10% of the mean. When potencies are compared, values for 50% inhibitory concentrations (IC_{50}s) are defined as the drug concentration resulting in a 50% decrease in [^{3}H]hypoxanthine incorporation compared with drug-free controls; values were obtained from simple graphic extrapolation and, as such, are only estimates.

Drugs used and sources were as follows: tetracycline, oxytetracycline, minocycline, erythromycin, chloramphenicol, thiamphenicol, kanamycin, tobramycin, nalidixic acid, Janus Green, antimycin A_1, cycloheximide, anisomycin, aminopterin, valinomycin, gramicidin, nigericin, monensin, tetraethylthiuram disulfide, actinomycin D, chloroquine, and quinine from Sigma; rifampin from Boehringer-Mannheim Biochemicals, Indianapolis, Ind.; halofuginone from Roussel Laboratories, Ltd., Middlesex, England; and rhodamine 123 from Eastman Kodak Co., Rochester, N.Y. The following were generous gifts: clindamycin, pirlimycin, and 4′-pentyl-*N*-demethyl clindamycin from S. Folz and R. Westerman, The Upjohn Co., Kalamazoo, Mich.; 8-methylamino-8-desmethyl riboflavin from E. F. Rogers, Merck Sharp & Dohme, Rahway, N.J.; mefloquine from the Walter Reed Army Institute of Research, Washington, D.C.; amodiaquine from L. Werbel, Parke-Davis/Werner Lambert, Inc., Ann Arbor, Mich.; and desethylchloroquine from Stirling-Winthrop, Inc., New York, N.Y.

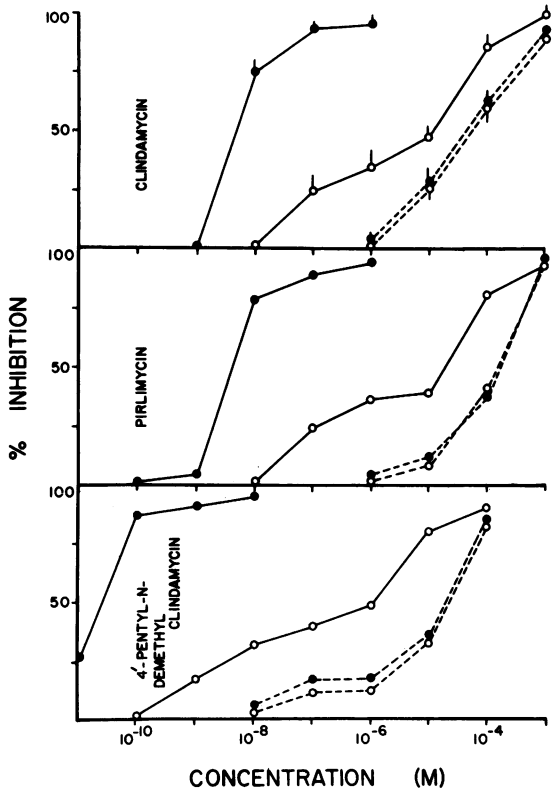

FIG. 1. Concentration-response curves describing the inhibition of *P. falciparum* by clindamycin and related derivatives. Data were obtained with the FCR_{3TC} isolate. -----, Cultures exposed for 48 h; ——, cultures exposed for 96 h. ●, Experiments in candle jars (high oxygen); ○, experiments done at 1% O_2. Vertical bars represent standard errors and are drawn in the clindamycin figure for illustrative purposes; in every case, these values were ≤10% of the mean. Each point represents the mean of 3 to 12 observations. Data are presented as the percent reduction in [^{3}H]hypoxanthine incorporation compared with drug-free controls.

RESULTS

Antibiotics which inhibit protein synthesis on 70S ribosomes, except for the aminoglycosides tobramycin and kanamycin, which were essentially inactive, showed marked dependence on exposure time and O_2 tension (Fig. 1; Table 1). The most potent were clindamycin, pirlimycin, and 4′-pentyl-*N*-demethyl clindamycin; at high O_2, IC_{50}s at 96 h were 5.1×10^{-9}, 2.2×10^{-9}, and 3.3×10^{-11} M, respectively. These values are between 10^4 and 10^6 times lower than those observed for the same drugs at 48 h in either high or low oxygen. The shape of the concentration-response curves for these drugs was extremely sensitive to O_2 tension at 96 h, although curves were identical in both atmospheres at 48 h.

Similar results were obtained for erythromycin, four tetracyclines, chloramphenicol, and thiamphenicol (Fig. 2; Table 1). IC_{50}s at 96 h in high oxygen were generally about 100 times lower than those observed at 48 h. O_2 tension did not affect potency or efficacy at 48 h but had profound influence in 96-h incubations.

The effects of anisomycin and cycloheximide, which inhibit protein synthesis on 80S ribosomes, were identical in high and low O_2 and were not increased by prolonged exposure (Fig. 3). Antibiotics which inhibit nucleic acid synthesis, including actinomycin D, nalidixic acid, and rifampin, did not resemble the 70S inhibitors (Table 1). Responses to actinomycin D and rifampin were unaffected by O_2 tension or incubation time; although nalidixic acid was more potent at 96 h, it had greater activity in low O_2.

Several compounds which can act as mitochondrial inhibitors had antimalarial effects which were time dependent to a variable degree. The influence of O_2 tension was variable and never as pronounced as for the 70S inhibitors (Fig. 4). Rhodamine 123, like nalidixic acid, was somewhat more potent in low oxygen. Janus Green and 8-methylamino-8-desmethyl riboflavin were somewhat more potent in high oxygen. The effect of antimycin A_1 was not affected by oxygen; however, the effects were reversed to some extent, particularly at low antimycin A_1 concentrations, by ascorbate (data not shown).

A variety of other drugs, including the ionophores valinomycin, gramicidin, monensin, and nigericin; the quinoline-containing antimalarial agents quinine, chloroquine, mefloquine, amodiaquine, and desethylchloroquine; and miscellaneous compounds with antimalarial activity, including halofuginone, tetraethylthiuram disulfide, and aminopterin; showed only minor increases in potency in prolonged incubations and were not affected by O_2 tension (Table 1).

The effects of the antibiotics and mitochondrial inhibitors were essentially identical in the FCR_{3TC}, Viet Nam Smith, $FCMSU_1$/Sudan, and FCR_8 strains of *P. falciparum* (data not shown).

TABLE 1. Influence of time and oxygen on potency of antimalarial agents in cultures of *P. falciparum* (FCR_{3TC} isolate)

Drug	IC_{50} (M) at following time and O_2 tension[a]:			
	48 h		96 h	
	High O_2	Low O_2	High O_2	Low O_2
Oxytetracycline	3.2×10^{-5}	3.9×10^{-5}	3.3×10^{-7}	2.8×10^{-6}
Chlortetracycline	2.6×10^{-5}	3.3×10^{-5}	7.6×10^{-7}	7.7×10^{-6}
Minocycline	3.0×10^{-6}	5.6×10^{-6}	2.9×10^{-8}	3.9×10^{-7}
Thiamphenicol	9.1×10^{-5}	1.0×10^{-4}	2.9×10^{-6}	2.8×10^{-5}
Tobramycin[b]	2.51 ± 0.22	1.21 ± 0.21	15.82 ± 1.44	11.69 ± 1.00
Kanamycin[b]	19.34 ± 1.71	20.53 ± 1.36	36.38 ± 3.25	34.56 ± 2.99
Rifampin	1.9×10^{-7}	2.4×10^{-7}	3.0×10^{-7}	1.4×10^{-7}
Nalidixic acid	3.1×10^{-4}	5.2×10^{-4}	1.3×10^{-4}	1.8×10^{-5}
Actinomycin D	9.1×10^{-10}	1.1×10^{-9}	3.1×10^{-10}	4.0×10^{-10}
Valinomycin	3.2×10^{-9}	5.9×10^{-9}	1.3×10^{-9}	1.3×10^{-9}
Gramicidin	6.2×10^{-11}	1.1×10^{-10}	1.7×10^{-11}	2.8×10^{-11}
Monensin	3.2×10^{-10}	3.9×10^{-10}	1.1×10^{-10}	1.7×10^{-10}
Nigericin	3.8×10^{-11}	5.2×10^{-11}	1.0×10^{-11}	2.2×10^{-11}
TETD[c]	2.5×10^{-6}	2.5×10^{-6}	3.2×10^{-7}	9.0×10^{-7}
Halofuginone	5.8×10^{-9}	5.9×10^{-9}	2.4×10^{-9}	3.0×10^{-9}
Aminopterin	4.5×10^{-6}	3.3×10^{-6}	1.4×10^{-7}	4.0×10^{-8}
Chloroquine	1.4×10^{-7}	1.4×10^{-7}	4.2×10^{-8}	5.0×10^{-8}
Quinine	1.7×10^{-7}	2.8×10^{-7}	4.7×10^{-8}	1.0×10^{-7}
Amodiaquine	2.7×10^{-8}	3.3×10^{-8}	5.3×10^{-9}	4.1×10^{-9}
Mefloquine	2.2×10^{-8}	3.4×10^{-6}	9.2×10^{-9}	4.0×10^{-9}
Desethylchloroquine	5.6×10^{-7}	4.9×10^{-7}	4.1×10^{-7}	3.1×10^{-7}
8-Methylamino-8-desmethyl riboflavin	4.0×10^{-7}	2.0×10^{-6}	1.8×10^{-10}	5.0×10^{-10}

[a] Values are means of 3 to 12 observations. In every case, standard errors were ≤10% of the mean.
[b] Values are mean percent inhibition ± standard error of the mean at 10^{-4} M, the highest concentration tested.
[c] TETD, Tetraethylthiuram disulfide.

DISCUSSION

Malaria parasites are sensitive to O_2 tension (43); in our experiments, *P. falciparum* grown in 1% O_2 incorporated about 30% more [^{3}H]hypoxanthine than those grown in candle jars (15% O_2). These organisms are extremely susceptible to oxidant stress (3). This sensitivity has been invoked to explain the antimalarial effects of certain chelators which may inhibit parasite or red cell enzymes involved in the detoxification of oxygen radicals (41). This finding is consistent with observations that buthionine sulfoximine, an inhibitor of glutathione synthetase, has antimalarial activity in *P. falciparum* cultures (manuscript in preparation). In both cases, toxicity was related to O_2 tension.

Oxygen dependence has been demonstrated for the antimalarial effects of the imidazoles. Potency was increased by roughly 10-fold when the O_2 tension was increased from 0.3 to 18% (31). These drugs inhibit fungal sterol synthesis at concentrations at least 100-fold lower than those which are antimalarial (about 5 nM versus approximately 1 μM); at the higher concentrations, these drugs affect membranes and inhibit membrane-bound oxidases (51). Significantly, mitochondrial function is inhibited (51).

Recently, the antimalarial activity of clindamycin was reported to be O_2 dependent (Krogstad, submitted for publication). Our findings extend this observation to a variety of other antibiotics whose only common mechanism of action is inhibition of protein synthesis on 70S ribosomes (35). Some of these drugs have been shown to have antimalarial effects in vivo and in vitro (16 and references therein) and included clindamycin, pirlimycin (6), 4′-pentyl-*N*-demethyl clindamycin (24, 25, 33, 34, 39), tetracycline, minocycline, oxytetracycline, chloramphenicol, thiamphenicol, and erythromycin. The influence of O_2 was generally not evident at 48 h but was profound at 96 h; IC_{50}s were up to 10^6 times lower for 4′-pentyl-*N*-demethyl clindamycin in high O_2. The unusual pattern seen in the concentration-response curves of the clindamycin derivatives at 96 h in low O_2, as previously reported (Krogstad, submitted for publication), was not evident for the other drugs. Pirlimycin has not been shown to have antimalarial effects before. However, it presents no advantage over clindamycin in potency. On the other hand, 4′-pentyl-*N*-demethyl clindamycin has been shown to be more potent than clindamycin in several animal models (24, 25, 33, 34, 39) and was recommended as more promising than clindamycin (44). This compound is more potent than clindamycin in high oxygen by a factor of nearly 100. It may be that slight modifications of other antibiotics could generate a variety of potent antimalarial agents.

Of the tetracyclines, minocycline was the most potent. It is also more potent than other tetracyclines as an antibacterial agent (40) and is apparently more potent than tetracycline, oxytetracycline, and chlortetracycline against *P. falciparum* (13, 36, 54) and *P. berghei* (21, 50) in vivo. Enhanced potency may be due to the increased lipophilicity of minocycline, facilitating its entry into cells (40). Against *P. falciparum*, chlortetracycline was the least active; however, in *P. berghei*, chlortetracycline has been found to be about twice as potent as oxytetracycline (48).

Chloramphenicol is considered to be too toxic for routine use (40), and this drug has no advantages over other antibiotics for the treatment of malaria. It is interesting that thiamphenicol, a derivative which shares the antibacterial effects but not the toxicity of chloramphenicol (4), is some-

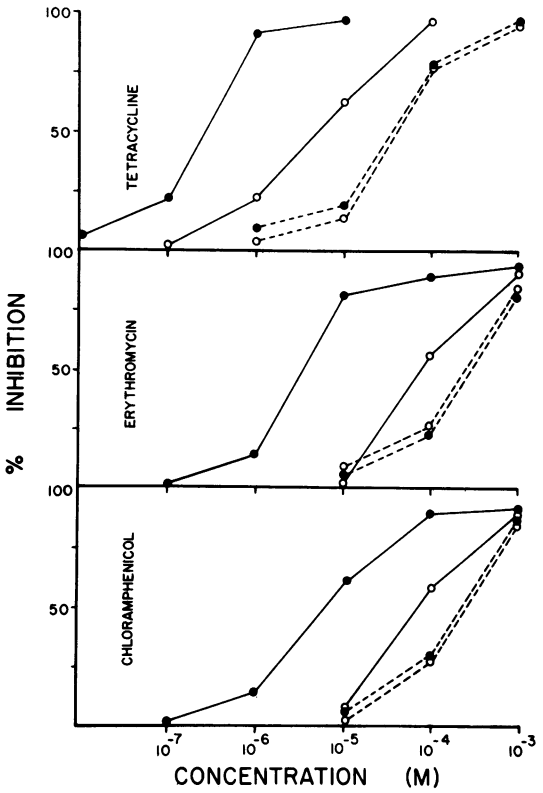

FIG. 2. Concentration-response curves describing the effects of other 70S ribosome inhibitors on *P. falciparum* in culture. Data are presentation as described in the legend to Fig. 1.

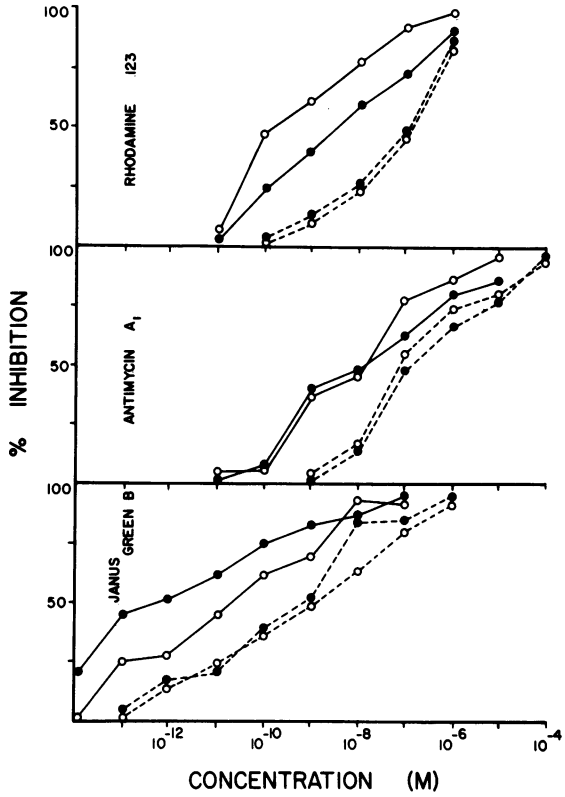

FIG. 4. Concentration-response curves describing the effects of the mitochondrial inhibitors Janus Green, rhodamine 123, and antimycin A on *P. falciparum* in culture. Data are presented as described in the legend to Fig. 1.

what more potent as an antimalarial agent in vitro. The aminoglycosides tobramycin and kanamycin, which also inhibit protein synthesis on 70S ribosomes (35), were essentially inactive, similar to gentamicin and streptomycin (16). Whether this lack of effect is due to inadequate uptake or represents a true ribosomal resistance is not known.

Oxygen tension also influenced the activity of other compounds. Nalidixic acid, which inhibits mitochondrial and procaryotic DNA gyrase (8), was somewhat more potent (at 96 h) in low oxygen. A similar effect was observed for the mitochondrial-specific fluorescent dye rhodamine 123 (9, 20), which is under investigation as an anticancer drug because of the markedly enhanced killing effect observed in transformed cells (5, 23). Rhodamine 123 is believed to function as a mitochondrial inhibitor (27) since it is highly concentrated in these organelles (9, 20). This drug is effective in vitro against *P. falciparum* at concentrations even lower than those which kill transformed cells and far lower than those which are toxic to normal mammalian cells (23).

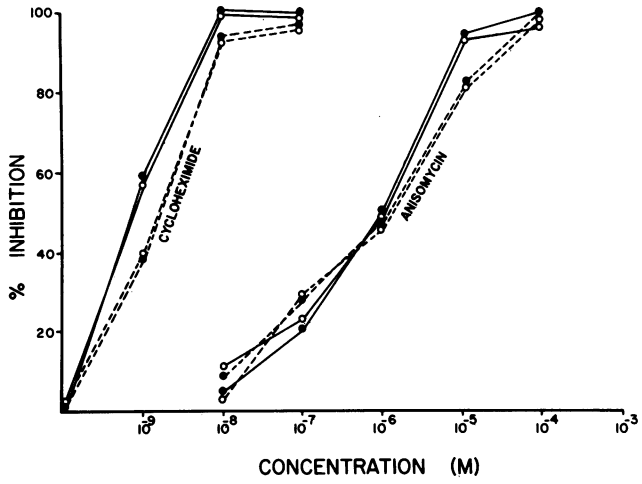

FIG. 3. Concentration-response curves describing the effects of the 80S ribosome inhibitors anisomycin and cycloheximide on *P. falciparum* in culture. Data are presented as described in the legend to Fig. 1.

Another mitochondrial-specific dye, Janus Green (50), was found to inhibit *P. falciparum* with extraordinary potency; these effects were enhanced in high oxygen (IC_{50} at 96 h, approximately 10^{-12} M). The antimalarial riboflavin antagonist 8-methylamino-8-desmethyl riboflavin (T. G. Geary, A. A. Divo, and J. B. Jensen, J. Protozool., in press) also showed increased potency at 96 h in the high-oxygen atmosphere. Riboflavin functions as a cofactor in many enzymes which may be presumed to be important to malaria parasites, such as glutathione reductase or orotic acid dehydrogenase; orotic acid could partially reverse the toxicity of riboflavin antagonists in culture (manuscript in preparation). Neither of these compounds had concentration-response curves which resembled those of the 70S inhibitors. That of Janus Green in particular was extremely gradual, resembling that of the electron transport inhibitor antimycin A_1 (50). Antimycin A_1 was unaffected by oxygen tension but was partially reversed by ascorbate; this observation adds credence to the hypothesis that antimycin A_1 acts on parasite mitochondria since, in other cells, ascorbate can partially

reverse the inhibition of electron transport by directly reducing cytochrome *b* bypassing the drug blockade (50).

The specificity of these effects can be seen by the lack of influence of time and oxygen on the potency of a wide variety of other antimalarial agents. These drugs include the nucleic acid synthesis inhibitors actinomycin D and rifampin (35); the ionophores valinomycin, gramicidin, monensin, and nigericin (Geary et al., in press); halofuginone (15); aminopterin (17); tetraethylthiuram disulfide (42) and a variety of quinoline-containing antimalarial agents, including desethylchloroquine (1). The ionophores were extremely potent antimalarials agents, with IC_{50}s as low as 10^{-11} M. It would be interesting to determine the biochemical basis for this exceptional potency. Most importantly, the effects of the 80S-specific protein synthesis inhibitors cycloheximide and anisomycin were not affected by oxygen or duration of exposure.

The inhibition of parasite growth by the 70S-specific drugs cannot be explained by actions on the 80S ribosome. This is a critical distinction, given the observation that 80S ribosomes of coccidia (closely related to *Plasmodium* spp.) have some characteristics of 70S ribosomes, including sensitivity to some antibiotics (52). The complete dissimilarity in oxygen and time dependence between 80S- and 70S-specific antibiotics clearly demonstrates that the cytoplasmic ribosomes of malaria parasites are not the targets of the 70S inhibitors. This distinction extends to the tetracyclines as well, which are know to inhibit protein synthesis on both 80S and 70S ribosomes (35). Selective toxicity to bacteria is achieved because these drugs penetrate most eucaryotic cells poorly (35). However, their antimalarial potency, which is at least as great in vitro as their antibacterial potency (16), indicates that these drugs do enter *P. falciparum*. It is reasonable to expect that cytoplasmic 80S ribosomes would be affected so that these drugs should resemble anisomycin and cycloheximide. Instead, they had patterns of activity identical to those of the other 70S-specific drugs. One possible explanation of this phenomenon could be selective localization. Tetracyclines are chelators with high affinity for Ca^{2+} (35), a cation known to be accumulated by *P. falciparum* (46). It is reasonable to assume that the organelle primarily responsible for this accumulation is, as in other cells, the mitochondrion (50). Since Ca^{2+}-tetracycline complexes cannot freely pass membranes (40), this might result in the accumulation and trapping of tetracycline in the parasite mitochondrion.

Recent evidence from this laboratory demonstrates that *P. falciparum* possesses a single mitochondrion which undergoes a complex pattern of growth, development, and replication during erythrocytic schizogony (Divo et al., in press). This process was visualized with the fluorescent dye rhodamine 123, which accumulates specifically in metabolically active mitochondria (9, 20). The presence of a functional mitochondrion has been demonstrated by the ability of a variety of mitochondrial toxins to inhibit rhodamine 123 accumulation (Divo et al., in press) and to kill the parasites (H. Ginsburg et al., unpublished observations). The data presented here demonstrating the extraordinary antimalarial toxicity of Janus Green, rhodamine 123, antimycin A_1, and 8-methylamino-8-desmethyl riboflavin further illustrate this point.

Our experiments were initiated with parasites synchronized to the schizont stage. In concentrations of 70S inhibitors which were not deleterious at 48 h, parasites appeared to be morphologically normal (16). Continuation of exposure to such concentrations (e.g., 10^{-7} M clindamycin) led to complete inhibition. The progeny of parasites which developed in the first 48-h period were much more sensitive to the antibiotics than the parent. We would like to term this phenomenon the "second-cycle effect." Although we do not yet understand the pharmacological basis for this effect, we propose that it is based on antimitochondrial actions. This hypothesis is currently being tested.

This proposal is somewhat limited by the lack of similarity of the parasite response to the 70S inhibitors and to rifampin and nalidixic acid, both of which can inhibit nucleic acid synthesis in mitochondria (8, 35). Both could be expected to inhibit mitochondrial growth and development as proposed for the 70S inhibitors, but their effects are quite different. We have as yet no data to explain this discrepancy. Our proposal does not explain the mechanism(s) by which oxygen increases the toxicity of these drugs for malaria parasites. Much further work will be required to fully characterize oxygen metabolism in these organelles. Nonetheless, given the facts that the 70S inhibitors are very much alike in their patterns of parasite inhibition and that the only common mechanism of action of this structurally disparate group of drugs is inhibition of protein synthesis on 70S ribosomes, the proposal that they act as antimalarial agents by inhibiting mitochondrial protein synthesis is prudent.

With regard to the potential chemotherapeutic value of the antibiotics, several points should be made. Rifampin is an effective antimalarial agent in vitro at concentrations which are at or below those achieved during chemotherapy of tuberculosis (16), appears to work faster than the other drugs, and is not affected by oxygen. Although this drug is expensive and not without toxicity, it may be of use as an alternative for the treatment of multiply drug-resistant falciparum malaria.

Tetracyclines and clindamycin have been used in combination with other drugs for the treatment of falciparum malaria (13, 26, 54). Tetracyclines, particularly doxycycline, are also effective when used alone as antimalarial agents (11, 32, 36). Clindamycin, which is the most potent antimalarial antibiotic currently available, has demonstrated good clinical results when given alone for the treatment of malaria (7, 10, 37), and further clinical trials are under way. Although this drug clearly does not act rapidly enough for use in critically ill patients, it may be of use in treating some drug-resistant infections. It is important that antibiotics be reserved for infections which cannot be controlled by other antimalarial agents since routine use may generate resistant bacterial strains.

The demonstration that these organisms contain functional mitochondria which are vulnerable to selective chemotherapy opens a new area for investigation. Antimitochondrial compounds may provide a new source of antimalarial agents, and characterization of the role of mitochondrial metabolism in the erythrocytic stages of *P. falciparum* may identify other metabolic pathways which are targets for chemotherapy.

ACKNOWLEDGMENT

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