RESEARCH NOTE

INDIRECT INHIBITION BY ANTIBIOTICS OF NUCLEOTIDE AND DEOXYNUCLEOTIDE BIOSYNTHESIS IN *PLASMODIUM FALCIPARUM*

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Abstract. The effects of the antibiotics, doxycycline, azithromycin, ciprofloxacin and chloramphenicol, upon levels of nucleoside-5'-triphosphates (NTPs) and 2'-deoxynucleoside-5'-triphosphates (dNTPs) have been compared in the malarial parasite, *Plasmodium falciparum*, and in human CCRF-CEM leukemia cells. All 4 antibiotics had more severe effects upon levels of NTPs and dNTPs in *P. falciparum* compared with leukemia cells providing an explanation for their selective toxicity against malaria and their utility as antimalarial drugs. In bacteria, the first 3 drugs inhibit protein synthesis while ciprofloxacin inhibits topoisomerase II. The observed depletions of NTPs and dNTPs would be a secondary effect of the drug but may result in death of the parasite.

The antibiotic, tetracycline, depresses the activity of dihydroorotate dehydrogenase of the pyrimidine pathway in *Plasmodium falciparum* (Prapunwattana *et al*, 1988), presumably due to inhibition of enzyme protein synthesis. Chloramphenicol is a specific inhibitor of bacterial protein synthesis binding to the 50S subunit of the ribosome, blocking peptidyl transfer (Gale *et al*, 1981) and also inhibits cytochrome P450 isozymes (Miller and Halpert, 1986). Ciprofloxacin inhibits topoisomerase II in bacteria (Hooper and Wolfson, 1988) but its mechanism of action in *P. falciparum* is unknown. Azithromycin, an azalide antibiotic, is effective against gram-negative organisms inhibiting protein synthesis, blocking transpeptidation and translocation reactions by reversibly binding to the 50S ribosomal sub-unit (Sturgill and Rapp, 1992).

The growth and division of *P. falciparum* is dependent upon an adequate supply of 2'-deoxynucleoside 5'-triphosphates (dNTPs) required for replication of DNA. Comparison of the effects of a drug upon levels of dNTPs and nucleoside 5'-triphosphates (NTPs) in *P. falciparum* and human CCRF-CEM leukemia cells may indicate the mechanism of action and selective toxicity for *P. falciparum*. In this paper, malarial parasites and human leukemia cells have been grown under similar conditions and the effects of some antibiotics on the levels of NTPs and dNTPs have been compared.

Azithromycin was from Pfizer (Sydney, Australia), chloramphenicol was from Parke Davis (Sydney, Australia), ciprofloxacin was from Bayer (Sydney, Australia), and doxycycline was from Faulding Pharmaceuticals (Adelaide, Australia). LPLF RPMI was obtained from Gibco (Grand Island, NY, USA). A gas mixture of 90% N_2, 5% CO_2 and 5% O_2 was obtained from BOC gases (Sydney, Australia). Flat bottom 96-well microtiter plates were obtained from Flow (McLean, VA, USA) and flasks were obtained from Corning (Corning, NY, USA). Erythrocytes were obtained from healthy donors screened negative for HIV, hepatitis B and C, and human serum also screened for the same viral pathogens was obtained from the Red Cross Blood Bank, Sydney.

The multidrug resistant K1 strain of *P. falciparum* (Thaithong and Beale, 1981) was maintained in low PABA low folate, LPLF-RPMI medium (0.0005 mg/l PABA and 0.01 mg/l folate), 25 mM K•Hepes pH 7.2, 32 mM $NaHCO_3$, gentamicin (40 μg/ml) in 10% (v/v) human serum (Trager and Jensen, 1976). Cultures were maintained at 37.5°C in a gas mixture of 5% CO_2, 5% O_2 and 90% N_2 and were synchronized with 5% (w/v) sorbitol (Lambros and Vanderberg, 1979).

Concentrations of the antibiotics used in culture were several-fold higher than their IC_{95} values de-

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termined against *P. falciparum* in erythrocytic culture. The concentrations used were: azithromycin 25 μM (IC95 = 8.3 μM at 48 hours; Yeo and Rieckmann, 1995), chloramphenicol 600 μM (IC95 > 310 μM at 48 hours; Yeo and Rieckmann, 1994a), ciprofloxacin 200 μM (IC95 = 82 μM at 48 hours; Yeo and Rieckmann, 1994b) and doxycycline 200 μM (IC95 > 40 μM at 48 hours; Yeo and Rieckmann, 1995). Malarial extracts were prepared and nucleotides separated and quantified by HPLC as described by Seymour *et al* (1994).

Experimental procedures for culture and extraction of human CCRF-CEM leukemia cells were as described by Sant *et al* (1992). The procedure for harvesting leukemia cells was similar to that for *P. falciparum* except that the saponin lysis step was omitted. Conditions for cell culture, concentrations of drugs and the exposure time (6 hours) for leukemia cells duplicated conditions for *P. falciparum*. Levels of NTPs in perchloric acid extracts of human leukemia cells were determined by gradient anion exchange HPLC (Sant *et al*, 1992) and dNTPs were quantified by HPLC after oxidation of cell extracts with periodate (Crisp *et al*, 1996). Nucleotide levels in control cultures are expressed as amol/cell and in drug-treated cultures as a fraction of control cells.

The effects of antibiotics on cellular levels of NTPs and dNTPs are summarized in Table 1. Azithromycin induced marked depletion of NTPs and dNTPs in *P. falciparum* without affecting leukemia cells, suggesting selective toxicity for malaria. Chloramphenicol induced decreases in NTPs and dNTPs in *P. falciparum*, leukemia cells were not affected (Table 1). Ciprofloxacin induced marked depression of NTPs and dNTPs in *P. falciparum* and had a marginal effect on leukemia cells (Table 1) again suggesting selective toxicity. Doxycycline reduced NTP levels to approximately 50% of control values in *P. falciparum*, dTTP and dCTP were depressed to 20% and dATP to 6.8% (Table 1). For leukemia cells, doxycycline induced moderate depression of NTP and dNTP levels.

The antibiotics tested appear to have a selective toxicity against the malarial parasite as indicated by selective reduction of levels of malarial NTPs and dNTPs, consistent with their utility as antimalarial drugs. While the primary mechanism of cytotoxicity for the antibiotics (except ciprofloxacin) would be inhibition of protein synthesis, the parasites may die due to an imbalance in dNTP

Table 1

Effects of antibiotics upon nucleotides in human CCRF-CEM leukemia cells and *P. falciparum*.

Nucleotide	Leukemia cells control (amol/cell) (n=9)	*P. falciparum* control (amol/pe)* (n=14)	Doxycycline (200 μM, 6h)		Azithromycin (25 μM, 6h)		Ciprofloxacin (200 μM, 6h)		Chloramphenicol (600 μM, 6h)	
			Leukemia	Malaria	Leukemia	Malaria	Leukemia	Malaria	Leukemia	Malaria
GTP	667 ± 56	5.49 ± 0.90	0.711	0.554	1.04	0.458	0.828	0.495	1.10	0.585
ATP	3,320 ± 236	20.2 ± 3.0	0.635	0.578	1.09	0.452	0.891	0.516	1.12	0.598
UTP	745 ± 122	5.65 ± 0.67	0.901	0.604	1.13	0.435	0.897	0.540	1.22	0.572
CTP	351 ± 60	2.24 ± 0.38	1.23	0.756	0.979	0.436	1.42	0.676	1.00	0.527
dATP	60.6 ± 5.0	0.302 ± 0.057	0.753	0.0679	0.945	0.356	0.641	0.486	0.955	0.502
dTTP	60.1 ± 4.7	0.377 ± 0.053	0.884	0.193	0.997	0.348	1.10	0.530	1.09	0.501
dCTP	31.8 ± 2.4	0.543 ± 0.093	0.993	0.226	1.03	0.720	1.24	0.689	1.21	0.499

Levels of nucleotides were determined by analysis of cell extracts by HPLC and are expressed as fractions of control values.
Experimental procedures are described in the text.
*Data from Yeo and Christopherson, manuscript submitted.
#Data from Yeo *et al.* (1997). Reprinted with permission.

pools resulting from inhibition of the biosynthesis of enzymes catalyzing reactions in the pathways for biosynthesis of NTPs and dNTPs. An imbalance in dNTPs could result in genetic miscoding in the parasite or a deficiency of a dNTP could lead to arrest of DNA replication.

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REFERENCES

Crisp LB, Smith SM, Mathers MAG, Young GAR, Lyons SD, Christopherson RI. Effects of cytosine arabinoside on human leukemia cells. *Int J Biochem Cell Biol* 1996; 28 : 1061-9.

Gale EF, Cundliffe E, Reynolds PE, Richmond MH, Waring MJ. The molecular basis of antibiotic action. New York: Wiley, 1981.

Hooper DC, Wolfson JS. Mode of action of the quinolone antimicrobial agents. *Rev Infect Dis* 1988; 10 (Suppl 1) : S514-21.

Lambros C, Vanderberg JP. Synchronisation of *Plasmodium falciparum* erythrocytic stages in culture. *J Parasitol* 1979; 65 : 418-20.

Miller NE, Halpert J. Analogues of chloramphenicol as mechanism based inactivators of rat liver cytochrome P-450. Modifications of the propanediol side chain, the p-nitro group, and the dichloromethyl moiety. *Mol Pharmacol* 1986; 29 : 391-8.

Prapunwattana P, O'Sullivan WJ, Yuthavong Y. Depression of *Plasmodium falciparum* dihydroorotate dehydrogenase activity in *in vitro* culture. *Mol Biochem Parasitol* 1988; 27 : 119-24.

Sant ME, Lyons SD, Phillips L, Christopherson RI. Antifolates induce inhibition of amido phosphoribosyltranferase in leukemia cells. *J Biol Chem* 1992; 267 : 11038-45.

Seymour KK, Lyons SD, Phillips L, Rieckmann KH, Christopherson RI. Cytotoxic effects of inhibitors of *de novo* primidine biosynthesis upon *Plasmodium falciparum. Biochemistry* 1994; 33 : 5268-74.

Sturgill MG, Rapp RP. Clarithromycin: Review of a new macrolide antibiotic with improved microbiologic spectrum and favourable pharmacokinetic and adverse effect profiles. *Ann Pharamacother* 1992; 26 : 1099-108.

Thaithong S, Beale GH. Resistance of ten Thai isolates of *Plasmodium falciparum* to chloroquine and pyrimethamine by *in vitro* tests. *Trans R Soc Trop Med Hyg* 1981; 75 : 271-3.

Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science* 1976; 193 : 673-5.

Yeo AET, Rieckmann KH. The *in vitro* antimalarial activity of chloramphenicol against *Plasmodium falciparum. Acta Tropica* 1994a; 56 : 51-4.

Yeo AET, Rieckmann KH. Prolonged exposure of *Plasmodium falciparum* to ciprofloxacin increase antimalarial activity. *J Parasitol* 1994b; 80 : 158-60.

Yeo AET, Rieckmann KH. Increased antimalarial activity of azithromycin during prolonged exposure of *Plasmodium falciparum in vitro. Int J Parasitol* 1995; 25 : 531-2.

Yeo AET, Seymour KK, Rieckmann KH, Christopherson RI. Effects of dual combinations of antifolates and atovaquone or dapsone on nucleotide levels in *Plasmodium falciparum. Biochem Pharmacol* 1997; 53: 943-50.