

ADENOSINE DEAMINASE IN MALARIA INFECTION :  
EFFECT OF 2'-DEOXYCOFORMYCIN *In vivo*

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OBJECTIVE : To determine whether 2'-deoxycoformycin, a specific inhibitor of adenosine deaminase, has antimalarial activity *in vivo*.

BACKGROUND : We have observed a dramatic increase in erythrocyte (RBC) adenosine deaminase (ADA: E.C 3.5.4.4) activity during malaria (*P. knowlesi*) infection of the rhesus monkey. Previous studies in our laboratory using *P. knowlesi* infected rhesus monkeys showed cyclic changes in RBC purine metabolites - particularly ATP and GTP - that were associated with the stage of parasite schizogony (1).

Purine nucleotides are required by the rapidly proliferating malaria parasite primarily for nucleic acid synthesis and energy metabolism (Figure 1). The malaria parasite cannot synthesize purines *de novo* and depends for its intraerythrocytic (IE) growth and development on salvage of purine bases from the host RBC and extracellular environment (2). We have shown with *P. falciparum*, *in vitro*, that hypoxanthine is an essential purine base precursor for parasite synthesis of adenosine and guanosine nucleotide since specific inhibition of adenylosuccinate synthetase or IMP dehydrogenase disrupts parasite nucleic acid synthesis (3). Whether hypoxanthine is the malaria parasites preferred substrate *in vivo* is not known.

An increase in ADA activity, however, is an obvious means for production of IE hypoxanthine due to the action of purine nucleoside phosphorylase (PNP) on the inosine produced from deamination of adenosine. Increased availability of hypoxanthine would be a natural consequence of adenosine catabolism in the mature erythrocyte (viz: adenosine  $\xrightarrow{ADA}$  inosine  $\xrightarrow{PNP}$  hypoxanthine) since this cell lacks the enzyme xanthine oxidase (4). Conversely, inhibition of ADA activity could act to deprive the rapidly growing IE malaria parasite of a readily accessible hypoxanthine pool for purine nucleotide synthesis.

It was, therefore, of interest to determine whether specific inhibition of adenosine deaminase activity *in vivo* using the tight binding inhibitor, 2' deoxycoformycin, interfered with the malaria parasites' IE growth and development.

**METHODS :** Adult male rhesus monkeys (*Macaca mulatta*) were experimentally infected with the simian malaria parasite, *Plasmodium knowlesi*. *Plasmodium knowlesi* is an intrinsically synchronous, quotidian malaria that is characteristically fulminant in rhesus. Parasitemias were expressed as percent of parasitized erythrocytes (% PRBC).

Samples of heparinized whole blood were collected at selected times during the IE infection cycle and at various levels of parasitemia. Each animal served as its own uninfected control. Lysates were prepared from washed RBC and stored frozen (-70°C). Perchloric acid extracts were prepared with whole blood by described methods (1).

The ADA assay was done by a radiochemical method using an automated HPLC system (5). ADA activity was measured by the conversion of (<sup>14</sup>C) adenosine to (<sup>14</sup>C) inosine (any labelled hypoxanthine formed was included as part of the inosine product. The standard reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), (<sup>14</sup>C) adenosine 1.8 mM, 1.6mCi/mmol) and erythrocyte lysate (200 µl) in a total volume of 700 µl. The range of protein concentration was 162 to 308 mg/ml. The reaction was linear with time and protein concentration. Specific activity was expressed as nanomoles/min/mg of protein.

Purine nucleotides were determined by an anion-exchange gradient HPLC method (6). This method separates all major purine nucleotides as well as the ribonucleotide from the deoxyribonucleotide form.

Administration of 2'-deoxycoformycin (Warner-Lambert Pharmaceuticals) to malaria infected rhesus monkeys was by a single 250 µg/kg (i.v.) dose. Evaluation of the drugs effect on parasitemia and parasite morphology was by microscopic examination of Giemsa stained thin blood smears.

**RESULTS AND DISCUSSION :** Table 1 shows the change in erythrocyte ADA level of *P. knowlesi* infected rhesus monkeys with moderate parasitemias. There was a 3.6 fold increase in RBC ADA activity of infected rhesus with a mean parasitemia of 6.2% PRBC. Several animals were serially sampled at increasing parasitemias and ADA levels were observed to increase in direct proportion to the number of RBC parasitized. A similar observation has been made for *P. falciparum* in culture (7). Malaria infected RBC have been shown by starch gel electrophoresis to contain a distinct parasite ADA (7,8). At high parasitemia (> 15% PRBC) considerable amounts of ADA activity were observed in the infected animals circulation (Monkey L901 showed a 17 fold increase in erythrocyte ADA activity with a 23% parasitemia).

It is apparent, therefore, that IE malaria parasite growth and proliferation is associated with an increase in PRBC ADA activity.

Table 2 shows that a single i.v. dose of 2'-deoxycoformycin effectively inhibited erythrocyte ADA activity in malaria infected monkeys at 6 and 24 hours. The parasitemia was decreased at 6 hours and continued to fall over the ensuing 24 hours. Microscopic examination of 6 hour 2'-deoxycoformycin treated PRBC revealed parasite nuclear and cytoplasmic deterioration. By 24 hours the majority of PRBC contained degenerate parasite forms.

2'-Deoxyformycin thus appears to produce a potent antimalarial effect *in vivo*.

2'-Deoxycoformycin has been shown to be toxic for malaria parasites *in vivo* only when exogenous adenosine or deoxyadenosine was added to the culture (7). Malaria erythrocyte cultures do not normally contain detectable levels of adenosine or deoxyadenosine; and, hypoxanthine is present in component serum and from RBC catabolism (9).

There are three plausible mechanisms to explain malaria parasite killing following *in vivo* 2'-deoxyformycin inhibition of ADA. First, there could be an IE deficiency of the catabolite hypoxanthine which is needed by the parasite for nucleotide synthesis. Second, there may be direct toxicity to critical parasite enzymes from accumulated adenosine or deoxyadenosine. Increased levels of 2'-deoxyadenosine, for example, have been shown to irreversibly inactivate S-adenosylhomocysteine hydrolase due to accumulation of S-adenosylhomocysteine which inhibits methyltransferase reactions (10).

Third, there could be an accumulation of deoxyadenosine triphosphate, dATP, such as has been observed in ADA associated severe combined immunodeficiency disease (SCIC) (11). DeoxyATP could act to inhibit ribonucleotide reductase and thereby interfere with DNA synthesis (12). In line with this third mechanism we observed an accumulation of both dADP and dATP in nucleotide profiles (24 hr) of PRBC following *in vivo* 2'-deoxycoformycin treatment of the *P. knowlesi* infected rhesus monkeys (Figure 2).

Finally, the mechanism for 2'-deoxycoformycin's antimalarial activity *in vivo* could be a combination of all three stated mechanism (Figure 3 summarizes the alternative metabolic fates of adenosine (deoxyadenosine) in normal and malarious erythrocytes). 2'-Deoxycoformycin is not suggested as a candidate antimalarial drug per se. Its value rather, has been to provide a specific molecular tool through which ADA has been identified as a potential metabolic target for the design of new antimalarial chemotherapy. Obviously, however, an analogue of 2'-deoxycoformycin specific for the parasite's adenosine deaminase would be an excellent candidate antimalarial agent.

Work is currently under way in our laboratory to more fully understand the action of 2'-deoxycoformycin on the malaria parasite and the possible role of ADA in cellular development and proliferation.

Table 1. Adenosine deaminase activity in malaria (*P. knowlesi*) infected erythrocytes of rhesus monkeys.

Condition	Adenosine deaminase activity (nanomoles/min/mg. protein)
Uninfected (control) Erythrocytes	1.91 ± 0.18 <sup>1</sup>
Infected <sup>2</sup> Erythrocytes	6.95 ± 0.70 (p < .005) <sup>3</sup>

<sup>1</sup> Mean ± SEM (n = 12)

<sup>2</sup> Parasitemia (6.2% ± 0.9; n = 12)

<sup>3</sup> Student's T-test for paired values

Table 2. Adenosine deaminase activity in malaria (*P. knowlesi*) infected rhesus monkeys treated with deoxycoformycin.

Monkey	Adenosine deaminase (nanomoles/min/mg. protein)		Deoxycoformycin treated <sup>1</sup>	
	Uninfected	Infected	(6 h)	(24 h)
M 032	1.47	6.26 <sub>2</sub> (8.2) <sup>2</sup>	0.12 (4.3)	0.69 <sub>3</sub> (1.8) <sup>3</sup>
M 025	1.07	6.36 (6.8)	0.65 (3.0)	0.62 <sub>3</sub> (2.0) <sup>3</sup>
M 189	2.13	8.19 (6.1)	0.37 (1.6)	0.23 <sub>3</sub> (0.1) <sup>3</sup>
Mean SEM (n = 3)	1.56 ± 0.31	6.94 ± 0.63 (6.7 ± 0.89)	0.38 ± 0.15 <sup>4</sup> (3.0 ± 0.78)	0.51 ± 0.14 <sup>4</sup> (1.3 ± 0.60)

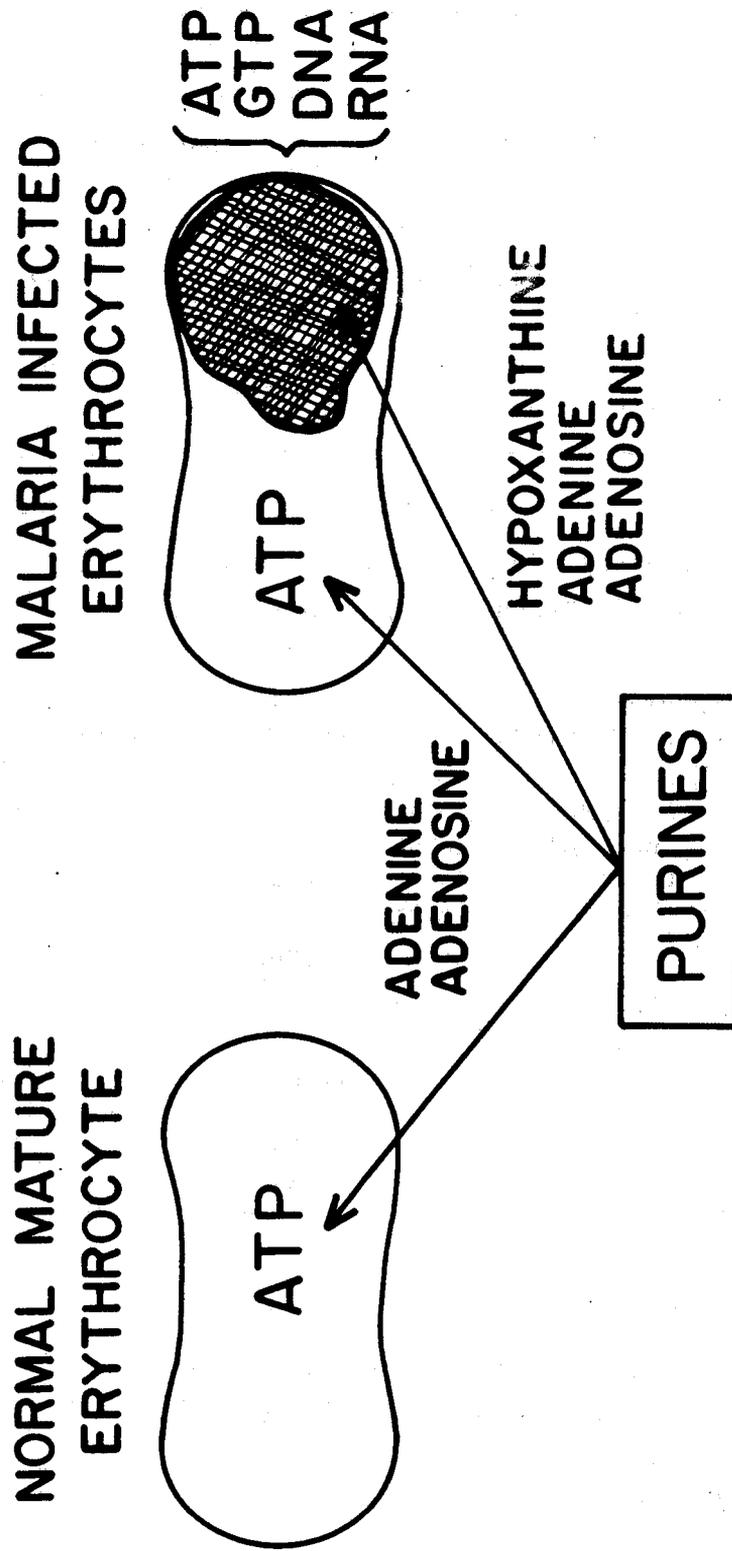
<sup>1</sup> Single i.v. dose of deoxycoformycin (250 µg/kg)

<sup>2</sup> Value in parenthesis is parasitemia (% PRBC)

<sup>3</sup> Remaining malaria infected erythrocytes showed parasite nuclear and cytoplasmic deterioration

<sup>4</sup> (p < .01)

# FIGURE I. PURINES IN MALARIA INFECTION



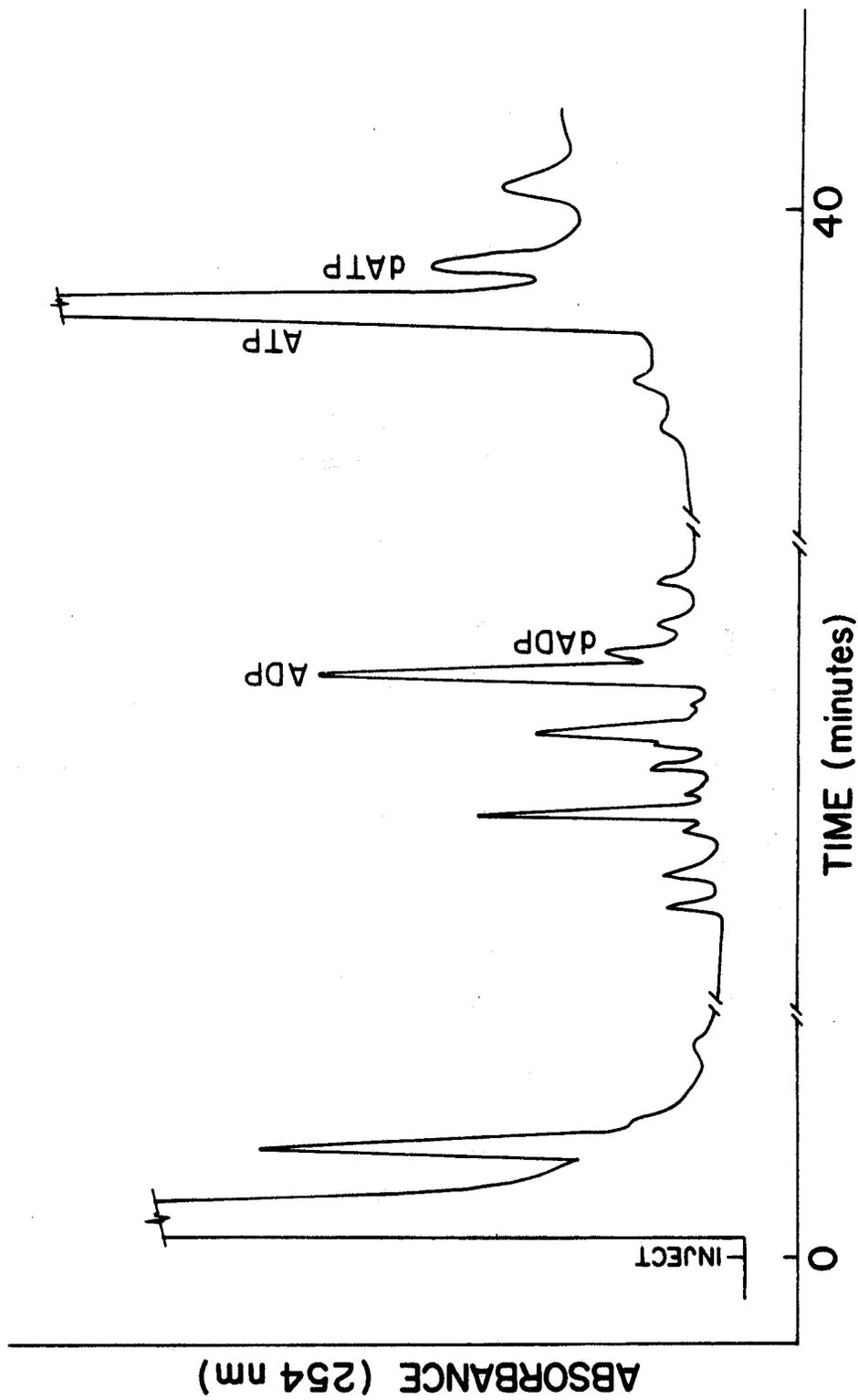
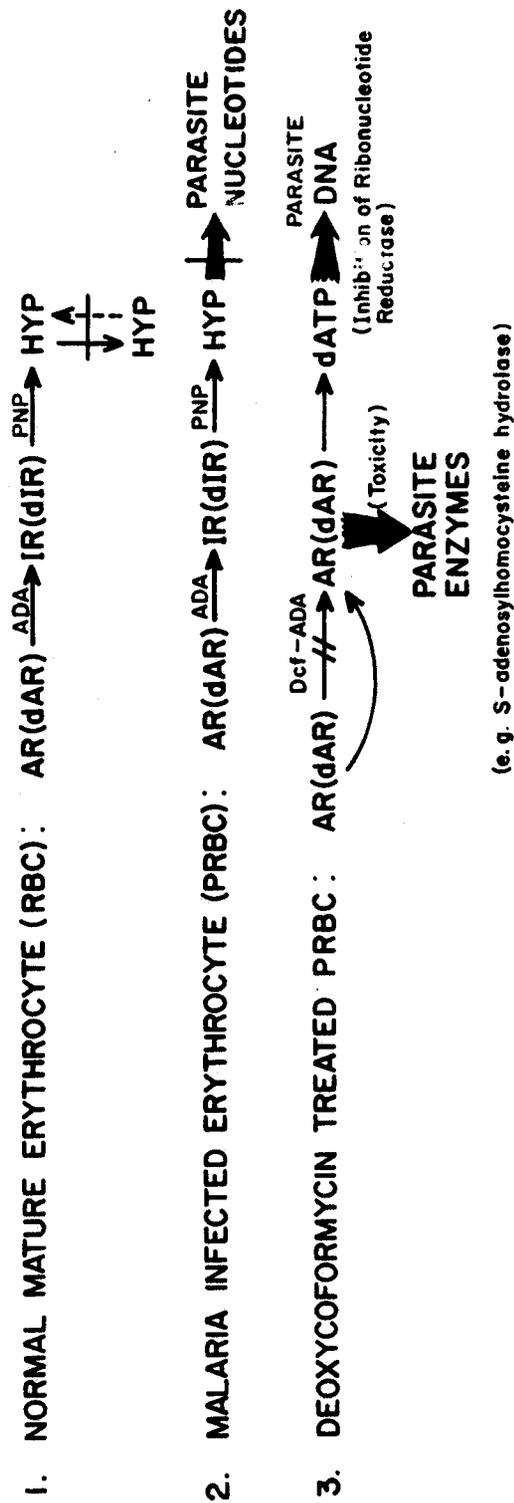


Figure 2. Purine nucleotide profile of malaria infected erythrocytes from deoxycoformycin treated monkey (24 hours).

**Figure 3. Schematic showing fate of adenosine (deoxyadenosine) in vivo in normal and Malaria infected erythrocytes.**



**ABBREVIATIONS:** AR = adenosine, dAR = deoxyadenosine, IR = inosine, dIR = deoxyinosine,  
 HYP = hypoxanthine, ADA = adenosine deaminase, PNP = purine nucleoside phosphorylase,  
 Dcf = deoxycoformycin.

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