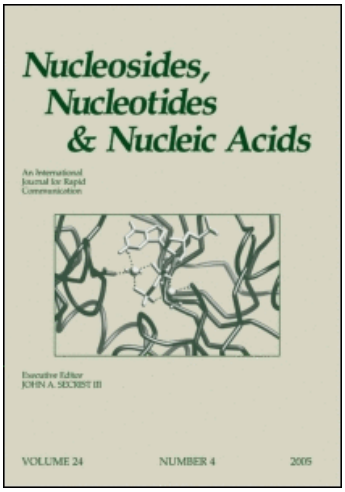

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L-PURINE NUCLEOSIDES AS SELECTIVE ANTIMALARIALS

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ABSTRACT: L-nucleosides selectively enter malaria infected erythrocytes and have the unique ability to be metabolised by the malarial adenosine deaminase. This has allowed us to design novel L-nucleosides as potential anti-malarials.

Malaria is arguably the most important infectious human parasitic disease in the world as 300 million people world wide are infected every year. Due to resistance to known drugs there is an increased need for novel antimalarials, particularly those which have no affect on the host. When the malarial parasite invades the red blood cell, it induces a unique transport pathway in the membrane of the infected red blood cell[1]. This transport pathway takes up both purine and pyrimidine nucleosides and bases, but in particular it takes up non-physiological L-purine nucleosides [2,3]. The concept of selective drug design is based on the fact that the malarial parasite must salvage purine nucleosides from the serum as it has no purine *de novo* pathway and thus it utilises preformed purines as nucleosides. As shown in Fig. 1, the nonphysiological L-nucleoside, L-adenosine, can be rapidly transported into *Plasmodium falciparum*-infected red blood cells. We have also shown that L-nucleosides can be transported not only into the parasite infected red blood cell but also into the parasite itself by crossing the parasitophorous vacuole membrane and the plasma membrane of the parasite[3]. One major advantage in the development of an L-nucleoside as a "magic bullet" chemotherapeutic agent is that L-nucleosides enter normal cells poorly since they are not recognised as substrates for purine transporters in mammalian cells such as human erythrocytes (Fig. 1), HeLa cells and Buffalo Green Monkey cells (BGM)[1].

L-Nucleosides are metabolised by the enzymes of the parasite but not those of the host erythrocyte or other mammalian cells. Many enzymes of the parasite are significantly different to the corresponding enzymes in the host, both quantitatively and qualitatively.

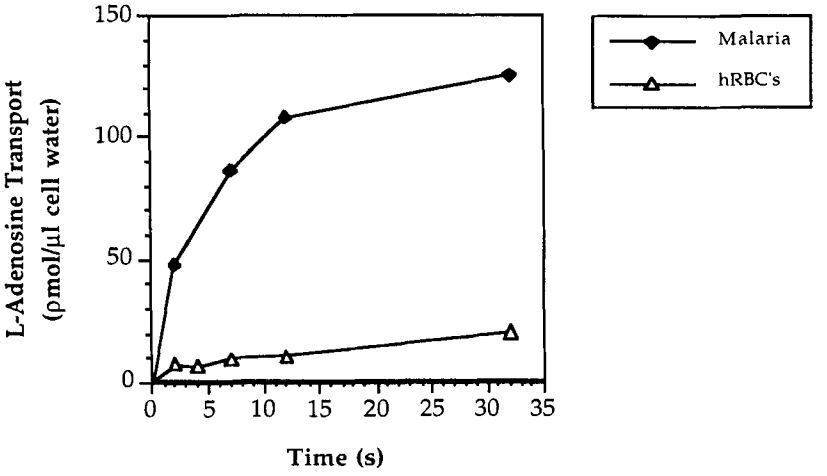


Fig. 1. L-Adenosine transport into uninfected and *P. falciparum*-infected human erythrocytes. Transport of 100 µM [^{3}H]L-adenosine into cell preparations (2 x 10^8 cells/ml) of trophozoite-stage *P. falciparum* -infected human erythrocytes (≈90% parasitemia, ♦) and uninfected human erythrocytes (Δ) was determined by a rapid oil-stop assay as previously described[4]. Data are means of triplicate determinations.

Previous reports have shown that *P. falciparum* adenosine deaminase (ADA) exhibits higher levels of activity in parasitised cells (28,500 nmol/mg/h compared) when compared to human erythrocytes (37 nmol/mg/h)[5]. We have confirmed these observations and shown that *P. falciparum* ADA demonstrates significant differences in substrate specificity when compared with the corresponding human enzyme. It was observed that L-adenosine (see Fig. 2) and many synthetic L-nucleoside analogs (e.g. L-ribofuranosyl-2-6-diamino-purine, L-ribofuranosyl-pyrazolo-[3,4-d]pyrimidine-4-amine) (data not shown) were metabolised by the parasite ADA. In contrast L-nucleosides were not metabolised in mammalian cell lysates such as human erythrocytes (data not shown), HeLa and BGM cells indicating that even if such compounds could enter normal host cells they would not be metabolised to their corresponding toxic compound[1]. As demonstrated in Fig. 2., L-adenosine is deaminated to L-inosine by the parasite ADA. No further metabolism of L-inosine wasobserved. We have also examined the transport and toxicity of a large number of L-nucleosides (Table I). Many of the L-nucleosides tested were either substrates or inhibitors of the parasite ADA. As demonstrated in Table I, 6-thio-L-inosine, L-coformycin and L-isocoformycin were inhibitors of malarial ADA. The differences between mammalian and *P. falciparum* ADA are further highlighted by the observation that L-isocoformycin, while being a potent inhibitor of parasite ADA, did not inhibit mammalian ADA and that D-

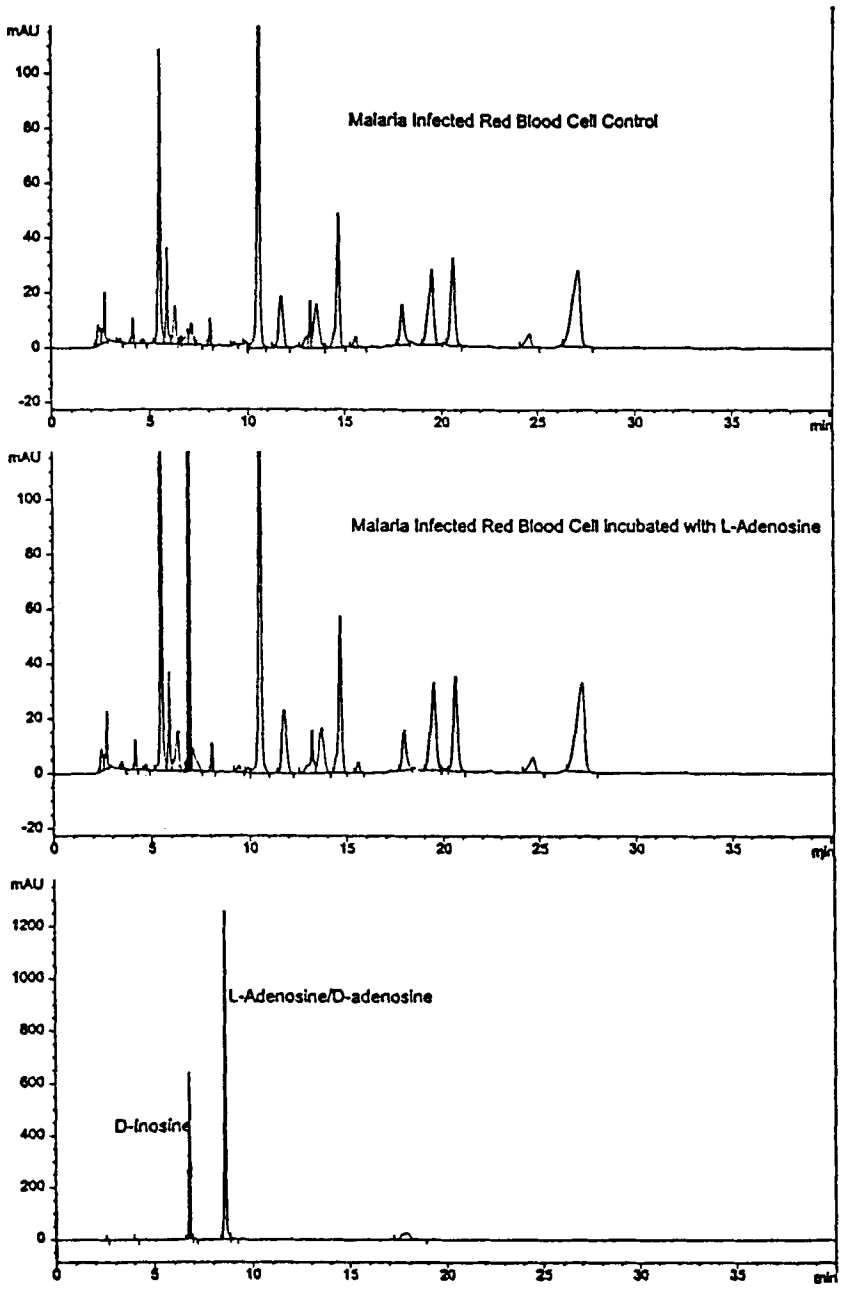
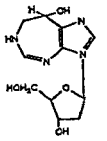
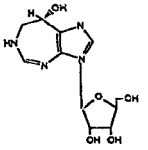
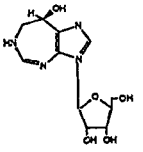
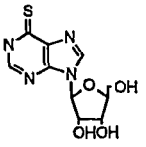


Fig. 2. Analysis of L-adenosine influx into *P. falciparum*-infected human erythroytes by HPLC. Cell preparations of 90% *P. falciparum*-infected human erythrocytes were incubated with L-adenosine (100 μM) at 37°C for 90 min, and the reaction terminated by centrifugation through silicon oil (16 000 x g, 15 s). The cell pellet was precipitated with 3 M perchloric acid and neutralised with 1.3 M K_2CO_3. Samples (25 μl) were analysed by UV detection (254 nm) on a adsorboshpere nucleotide column (Activon) using a Hewellet Packard HP1100 HPLC. The buffer system employed was 10 mM potassium phosphate, 2 mM tetrabutylammonium phosphate and a linear pH (5.25 - 7.25) and acetonitrile (3-20%) gradient over 10 min. Isocratic HPLC was performed from 10 - 40 min.

Table I. Comparison of Inhibitors on Malaria and mammalian ADA activity. ADA activity was assayed in malaria-infected human erythrocytes and bovine spleen. *P. falciparum*-infected erythrocytes (2 x 10^8 cells/ml at 90% parasitemia) were lysed by freeze-thawing and pure bovine spleen ADA was diluted in phosphate-buffered saline prior to assaying for ADA activity by a spectrophotometric assay[6]. Lysates were incubated with 50 μM D-adenosine and activity determined by measuring the change in UV absorbance at 265 nm. Results are means of duplicate experiments. nd, not determined.

Inhibitor (2 μM)	% Inhibition of ADA	
	Malaria	Mammalian
Deoxy-D-coformycin 	96.2 ± 6.5	96.1
L-Coformycin 	21 ± 1.2	nd
L-Isocoformycin 	90.6 ± 5.9	0
L-Thio-inosine 	20	nd

deoxycoformycin, which is used clinically in humans and eliminates parasitemias in *P. knowlesi* infected monkeys), inhibits both enzymes[5] (Table I).

Those compounds which were simply L-enantiomers of physiological D-nucleosides entered *P. falciparum*-infected cells and the parasite within but exhibited no toxicity. However, L-enantiomers of known toxic D-nucleosides, such as 6-thio-L-adenosine, 6-thio-L-guanosine and L-sangivamycin exhibited toxicity against *P. falciparum in vitro* in both wild type and resistant strains with ID_{50} values (concentration of drug required to

inhibit cell growth by 50%) of 160.2, 200 and 25 μM, respectively. The D-nucleoside analogs of these compounds have the disadvantage of being toxic to normal cells. In comparison, none of these L-compounds entered normal cells, thus increasing the therapeutic activity of L-nucleosides over the D-derivatives.

As none of the purine L-nucleosides tested entered normal cells, the increased therapeutic activity of L-nucleosides over the D-derivatives demonstrates the potential of L-nucleosides as selective antimalarials. Although the parasite is forced to salvage purine nucleosides, we have been able to show that, in addition, L-pyrimidine nucleosides were also transported into intact *P. falciparum*-infected erythrocytes and "free" parasites but not into uninfected human erythrocytes[3]. This provides an additional tool in that L-pyrimidine nucleosides could also act as carriers to take a prodrug into the infected cell. It is our aim to further develop L-nucleosides with properties which allow them to be selectively toxic to malaria and other parasites. The research area of L-nucleosides as chemotherapeutic agents has forged ahead in the field of cancer and viral chemotherapy over the last 20 years [7,8]. L-Nucleosides as potential antimalarials present us with several singular advantages over other proposed antimalarials due to their selective entry into parasite infected cells. It also raises possibilities for treatment of other parasitic diseases such as Babesia, in which we have shown al altered transporter similar to malaria[9].

Acknowledgements
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