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Mechanism of growth inhibition of intraerythrocytic stages of *Plasmodium falciparum* by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR)

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ABSTRACT

Purine nucleotide synthesis in *Plasmodium falciparum* takes place solely by the purine salvage pathway in which preformed purine base(s) are salvaged from the host and acted upon by a battery of enzymes to generate AMP and GMP. Inhibitors of this pathway have a potent effect on the *in vitro* growth of *P. falciparum* and are hence, implicated as promising leads for the development of new generation anti-malarials. Here, we describe the mechanism of inhibition of the intraerythrocytic growth of *P. falciparum* by the purine nucleoside precursor, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR). Our results show that AICAR toxicity is mediated through the erythrocyte in which AICAR is phosphorylated to its nucleotide, ZMP. Further, purine metabolite labeling of the parasitized erythrocytes by [^{3}H]-hypoxanthine, in the presence of AICAR, showed a significant decrease in radioactive counts in adenylate fractions but not in guanylate fractions. The most dramatic effect on parasite growth was observed when erythrocytes pretreated with AICAR were used in culture. Pretreatment of erythrocytes with AICAR led to significant intracellular accumulation of ZMP and these erythrocytes were incapable of supporting parasite growth. These results implicate that in addition to the purine salvage pathway in *P. falciparum*, AICAR alters the metabolic status of the erythrocytes, which inhibits parasite growth. As AICAR and ZMP are metabolites in the human serum and erythrocytes, our studies reported here throw light on their possible role in disease susceptibility, and also suggests the possibility of AICAR being a potential prophylactic or chemotherapeutic anti-malarial compound.

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1. Introduction

Malaria, one of the major tropical diseases is caused by protozoa of the genus *Plasmodium*. The disease is responsible for approximately 1 million deaths annually [1], with 90% of these being caused by *Plasmodium falciparum*. Due to the emergence of drug resistance in this parasite to most of the first line antimalarials [2], development of new drugs that could specifically inhibit the parasite's metabolism and, at the same time, cause minimal toxicity to the host is required.

P. falciparum, unlike its human host, lacks the *de novo* purine biosynthetic pathway [3] and hence, is a purine auxotroph whose survival is dependent on the salvage of either purines or purine nucleosides from the host. It has been shown that hypoxanthine, guanine, adenosine, guanosine and inosine are transported by PfNT1 transporter [4,5], which is localized predominantly on the parasite plasma membrane [6]. Of the different purines and purine nucleosides, PfNT1 has the highest affinity for hypoxanthine, which gets transported rapidly into the parasite cytosol. Hypoxanthine is phosphoribosylated to IMP by hypoxanthine/guanine phosphoribosyltransferase (HGPRT). Alternatively, salvage of adenosine is followed by its deamination to inosine by adenosine deaminase (ADA) and subsequent conversion to hypoxanthine by purine nucleoside phosphorylase (PNP). IMP is converted to GMP by the concerted action of IMP dehydrogenase (IMPDH) and GMP synthetase (GMPS) and to AMP by adenylosuccinate synthetase (ADSS) and adenylosuccinate lyase (ASL) (Fig. 1A). The complete dependence of the parasite on the salvage pathway for its purine nucleotide requirements makes the constituent purine transporters and/or enzymes putative drug targets [7] and thereby, necessitating their detailed biochemical characterization. Recombinant ADA [8–10], PNP [11], HGPRT [12–14], ADSS [15,16], GMPS [17] and ASL [18] from *P. falciparum* have been cloned, expressed, purified and extensively characterized. These studies highlight differences in the biochemical and/or kinetic properties between the

Abbreviations: ASL, adenylosuccinate lyase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; ZMP, 5-aminoimidazole-4-carboxamide ribonucleotide.

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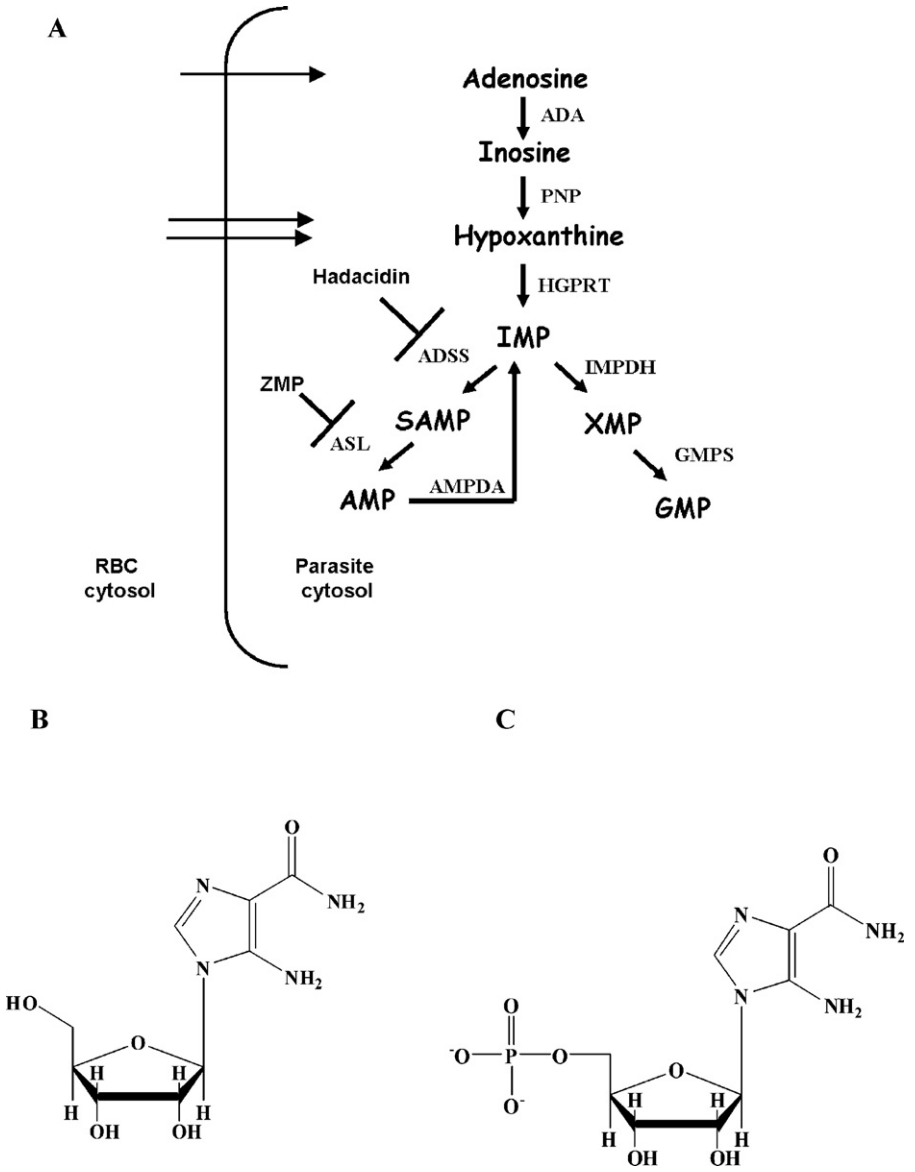

Fig. 1. Schematic representation of the purine salvage pathway in *P. falciparum* and chemical structures of AICAR and ZMP. (A) Arrows across RBC and parasite compartments indicate uptake of metabolites from the infected erythrocyte cytosol into the parasite. ADA, adenosine deaminase (EC 3.5.4.4); PNP, purine nucleoside phosphorylase (EC 2.4.2.1); HGPRT, hypoxanthine guanine phosphoribosyltransferase (EC 2.4.2.8); ADSS, adenylosuccinate synthetase (EC 6.3.4.4); ASL, adenylosuccinate lyase (EC 4.3.2.2); AMPDA, AMP deaminase (EC 3.5.4.6); IMPDH, IMP dehydrogenase (EC 1.1.1.205); GMPS, GMP synthetase (EC 6.3.4.1). These enzymes are annotated in the *P. falciparum* genome database, PlasmoDB [53]. Hadacidin and ZMP are inhibitors of ADSS and ASL, respectively. (B) Chemical structure of 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR). (C) Chemical structure of 5-aminoimidazole-4-carboxamide ribonucleotide (ZMP).

parasite and human enzymes that could be exploited for the development of chemotherapeutic interventions.

5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) (Fig. 1B) is the nucleoside component of AICARibotide (ZMP) (Fig. 1C), a metabolic intermediate of the *de novo* purine biosynthetic pathway. We have earlier shown that AICAR has anti-parasitic activity against the intraerythrocytic stages of *P. falciparum* with an IC_{50} value of $167 \pm 5\,\mu M$ [18]. AICAR has been used extensively at very high concentrations not only in rodent models [19–22], but also in humans as a potential drug for use during myocardial ischemia and cardiac bypass surgery [23]. Thus AICAR, which is non-toxic to the human host but toxic to *P. falciparum* could be a promising candidate as an antimalarial. Therefore, deciphering the mechanism of inhibition of *P. falciparum* growth by AICAR through alternate routes becomes an interesting study. We have shown that ASL from *P. falciparum* (PfASL) catalyzes, with equal efficiency, the cleavage of 5-aminoimidazole-4-(N-succinylcarboxamide) ribonucleotide (SAICAR) to 5-aminoimidazole-4-carboxamide ribonucleotide (ZMP) and fumarate, which forms the eighth step of the *de novo* purine biosynthetic pathway, in addition to the cleavage of succinyl-adenosine monophosphate (SAMP) to adenosine monophosphate (AMP) and fumarate, the final step in AMP synthesis [18]. This led us to hypothesize that *in vivo*, in the intraerythrocytic parasite, AICAR probably gets phosphorylated to ZMP, which might then compete with SAMP for PfASL active site thereby, bringing about its inhibition leading to death of the parasite.

Our studies reported here delineate the mechanism of toxicity of AICAR. Interestingly, we find that AICAR exhibits toxicity only on parasitized erythrocytes and not on isolated parasites implicating a pivotal role for the erythrocyte. This is explained by the

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