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Cloning, characterization and validation of inosine 5′-monophosphate dehydrogenase of *Babesia gibsoni* as molecular drug target

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ABSTRACT

The inosine monophosphate dehydrogenase (IMPDH) enzyme has been characterized and validated as a molecular drug target in other apicomplexans but not in the genus *Babesia*. Subsequently, we cloned and expressed a *Babesia gibsoni* IMPDH (BgIMPDH) cDNA in *Escherichia coli*. We also determined the inhibitory effect of mycophenolic acid (MPA) on recombinant BgIMPDH (rBgIMPDH) activity and the *Babesia*-growths in vitro. The translated BgIMPDH peptide contained thirteen amino acid residues responsible for substrate and cofactor binding in its catalytic domain with Gly374 in BgIMPDH being replaced by Ser388 in mammalian IMPDH. The native BgIMPDH enzyme in the parasite was approximately 54-kDa a mass similar to His-tag rBgIMPDH protein. The K_m values of the rBgIMPDH were 8.18 ± 0.878 (mean ± standard error of the mean) μM and 360.80 ± 43.41 μM for IMP and NAD^+, respectively. MPA inhibited the rBgIMPDH activity yielding a K_i value of 20.93 ± 1.83 μM with respect to NAD^+. For *Babesia* growths, the IC_{50}s were 0.95 ± 0.21 and 2.88 ± 0.49 μM for *B. gibsoni* and *B. bovis*, respectively. Therefore, our results suggest that MPA may inhibit the replication of *Babesia* parasites by targeting IMPDH enzyme of the purine pathway.

1. Introduction

The *Babesia* parasites are protozoan pathogens transmitted by ticks and cause babesiosis in mammals including human [10,15,22]. *Babesia bovis* infects cattle causing massive economic losses in livestock industry worldwide [4] while *Babesia gibsoni* causes canine babesiosis worldwide, sometimes leading to deaths [3]. Babesiosis is one of the most widely distributed, serious and poorly controlled infection globally. Chemotherapy is the only practical way of saving infected animals, and thus reducing economic losses as well as safeguarding animal welfare [13,23]. However, only few drugs are available for babesiosis treatment. Therefore, there is a need to develop novel antibabesia compounds to treat babesiosis.

The purine pathway of parasitic protozoa is an attractive chemotherapeutic target because these parasites rely entirely on the pathway to meet their purine demands for nucleic acid synthesis [18,24]. In this pathway, inosine 5′-monophosphate dehydrogenase (IMPDH; EC 1.1.1.205) is a rate limiting step catalyzing the conversion of inosine monophosphate (IMP) to xanthosine monophosphate (XMP) with concomitant reduction of NAD^+ to NADH [9]. The XMP, which is formed, is a precursor of guanine nucleotides required for biosynthesis of DNA, crucial for the survival of parasites [9]. IMPDH enzyme is susceptible to inhibition by mycophenolic acid (MPA), which is a structural analog of the cofactor NAD^+. MPA is an immunosuppressive drug [14] and has anticryptosporidial activity in cell cultures [18]. The IMPDHs have been studied as molecular drug target in other apicomplexans, especially *Plasmodium*, *Cryptosporidium* and *Toxoplasma* spp. [9,19,21], but not in *Babesia* spp. We think that MPA targets *Babesia* IMPDH enzyme, thereby disrupting the enzyme in *Babesia* cell and thus inhibit the growth of the parasite in vitro.

Therefore, we have cloned and expressed a *B. gibsoni* (BgIMPDH) cDNA in *Escherichia coli* BL21 (DE3) and validated the BgIMPDH enzyme as a molecular drug target. We then use the recombinant BgIMPDH (rBgIMPDH) to generate antibodies in mouse for characterization of a corresponding native enzyme in the parasite. The kinetic parameters of the rBgIMPDH enzyme and the enzyme inhibition constant of MPA are also reported. Finally, we have demonstrated that MPA inhibits the growths of both *B. gibsoni* and *B. bovis* in vitro. Therefore, our data suggest that MPA targets IMPDH of *Babesia* parasites.

2. Materials and methods

2.1. Reagents and experimental animals

Dimethyl sulfoxide (DMSO), isopropyl-β-D-thiogalactopyranoside (IPTG), sodium pyruvate and sodium bicarbonate were purchased

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