Cloning, Expression, and Characterization of Babesia gibsoni Dihydrofolate Reductase-Thymidylate Synthase: Inhibitory Effect of Antifolates on Its Catalytic Activity and Parasite Proliferation

Aboge, Gabriel O; Terkawi, Mohamad A; Goo, Youn-Kyoung; Nishikawa, Yoshifumi; Sunaga, Fujiko; Namikawa, Kuzuhiko; Tsuji, Naotoshi; Igarashi, Ikuo; Suzuki, Hiroshi; Fujisaki, Kozo; Xuan, Xuenan
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Abstract

Dihydrofolate reductase-thymidylate synthase (DHFR-TS) is a well-validated antifolate drug target in certain pathogenic apicomplexans, but not in the genus Babesia, including Babesia gibsoni. Therefore, we isolated, cloned, and expressed the wild-type B. gibsoni dhfr-ts gene in Escherichia coli and evaluated the inhibitory effect of antifolatesonits enzymeactivity,aswellasoninvitroparasitegrowth.Thefull-lengthgene consists of a 1,548-bp open reading frame encoding a 58.8-kDa translated peptide containing DHFR and TS domains linked together in a single polypeptide chain. Each domain contained active-site amino acid residues responsible for the enzymatic activity. The expressed soluble recombinant DHFR-TS protein was approximately 57 kDa after glutathione S-transferase (GST) cleavage, similar to an approximately 58-kDa native enzyme identified from the parasite merozoite. The non-GST fusion recombinant DHFR enzyme revealed K m values of 4.70□ 0.059 (mean□ standard error of the mean) and 9.75□ 1.64□M for dihydrofolic acid (DHF) and NADPH, respectively. Methotrexate was a more-potent inhibitor of the enzymatic activity (50% inhibition concentration [IC50]□ 68.6□ 5.20 nM) than pyrimethamine (IC50□ 55.0□ 2.08□M) and trimethoprim (IC50□ 50□ 12.5□M). Moreover, the antifolates' inhibitory effects on DHFR enzyme activity paralleled their inhibition of the parasite growth in vitro, indicating that the B. gibsoni DHFR could be a model for studying antifolate compounds as potential drug candidates. Therefore, theB.gibsoni DHFR-TS is a molecular antifolate drug target.