

Ribokinase: A Possible Target for Chemotherapy of Protozoans

Ogbunude POJ^{1*}, Udeogaranya PO², Eze AA¹, Ikekpeazu JE¹ and Okoli UA¹

¹Department of Medical Biochemistry, College of Medicine, University of Nigeria, Nigeria

²Department of Clinical Pharmacy, University of Nigeria, Nsukka, Nigeria

Abstract

The development of anti-parasitic agent is quite challenging particularly when exploiting biochemical differences between the parasite and the host. However, with post-genome bioinformatics and experimental research, drug targets can be more easily identified. Ribose metabolism in protozoans is of interest because protozoa in general are auxotrophic for purines and acquire these nutrients from the hosts¹. Ribose is utilized in the production of ribose 5-phosphate required for the synthesis of 5-phosphoribosyl-1-pyrophosphate that is used with purine bases for the synthesis of nucleic acids. The enzyme responsible for the conversion of ribose to ribose 5-phosphate is ribokinase (an ATP-dependent phosphoribosyl kinase, EC 2.1.7.15). Four possible pathways exist for mobilization of free nucleobases for the synthesis of nucleic acids in protozoans, three of these use ribokinase and one uses transketolase / transaldolase pathway. Inhibition of these pathways, that is, the ribokinase pathway and transketolase / transaldolase pathway will deny the parasites ability to use the nucleobases to make nucleic acids.

Keywords: Combination therapy; ribokinase; transketolase-transaldolase; Protozoan

Summary

The development of anti-parasitic agent is quite challenging particularly when exploiting biochemical differences between the parasite and the host. However, with post-genome bioinformatics and experimental research, drug targets can be more easily identified.

Ribose metabolism in protozoans is of interest because protozoa in general are auxotrophic for purines and acquire these nutrients from the hosts [1]. Ribose is utilized in the production of ribose 5-phosphate required for the synthesis of 5-phosphoribosyl-1-pyrophosphate that is used with purine bases for the synthesis of nucleic acids. The enzyme responsible for the conversion of ribose to ribose 5-phosphate is ribokinase (an ATP-dependent phosphoribosyl kinase, EC 2.1.7.15). Four possible pathways exist for mobilization of free nucleobases for the synthesis of nucleic acids in protozoans, three of these use ribokinase and one uses transketolase/transaldolase pathway. Inhibition of these pathways, that is, the ribokinase pathway and transketolase/transaldolase pathway will deny the parasites ability to use the nucleobases to make nucleic acids.

Introduction

Protozoans are widely distributed in nature and are pathogens to humans as well as to plants with devastating health and economic consequences. In humans, the diseases include trypanosomiasis, leishmaniasis and chagasis. Half a billion people, primarily in tropical and subtropical areas of the world, are at risk of contracting these diseases, and it is estimated that more than 20 million individuals are infected with the pathogens that cause them, resulting in extensive suffering and more than 100,000 deaths per year. These forms of diseases have been classified by the World Health Organization (WHO) as major tropical diseases [2] (Table 1).

The cellular biology of kinetoplastids protozoa is essentially similar, for example, they are all motile protozoans with a single flagellum that originates close to their large single mitochondrion. They all have glycosomes, that is, micro-bodies that perform glycolysis. All typically grow asexually although sexual recombination has been shown or inferred but is not obligate in any one of them. They divide by binary fission during which their nucleus does not undergo membrane

dissolution or chromosome condensation. They are well adapted to their hosts and evade immune elimination by antigenic variation, and alteration of immune responsiveness. There is no effective immune response against human trypanosomiasis which invariably results in fatality. In the case of *T. cruzi* and *Leishmania* spp., the immune response tends to control rather than eliminate them.

Leishmaniasis is a treatable disease; however, the antileishmanial therapy is bewildering largely because of the complexities of the disease. The few effective agents available generally are potentially toxic and mostly are difficult to administer. Furthermore the treatment of cutaneous leishmaniasis, for example, is often complicated by rapid self-healing making it difficult to assess efficacy of trials. The pentavalent antimony compounds, sodium stibogluconate (Pentostam, Glaxo Wellcome, UK) and meglumine antimonite (Glucantime, Rhône-Poulenc Rorer, France) have been the mainstays of antileishmanial therapy since 1940s [4,5]. These drugs, although, effective suffer from disadvantages of long duration of therapy, parenteral mode of administration, almost always reversible toxic effects among other disadvantages.

In the case of human African trypanosomiasis, only four drugs are currently registered for its treatment, two of them suramine and pentamidine are useful only against early stage disease before parasites have entered and become manifest within cerebrospinal fluid. Metarsoprol and eflornithine are used for late stage disease. Melarsoprol is exceedingly toxic, killing 5% of those taking the drug. Eflornithine which is actually effective against only *T. gambiense* infection is prohibitively expensive in terms of large scale treatments. Treatment failures with melarsoprol is on the increase possibly because of emergence of drug resistance parasites in the field.

***Corresponding author:** Patrick Ogbunude, Department of Medical Biochemistry, University of Nigeria, Enugu Campus, Nigeria, Tel: +2348033101851; E-mail: ogbunudejim@yahoo.co.uk

Received December 31, 2015; **Accepted** February 22, 2016; **Published** February 29, 2016

Citation: Ogbunude POJ, Udeogaranya PO, Eze AA, Ikekpeazu JE, Okoli UA (2016) Ribokinase: A Possible Target for Chemotherapy of Protozoans. J Biotechnol Biomater 6: 219. doi:10.4172/2155-952X.1000219

Copyright: © 2016 Ogbunude POJ, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

	HAT	CHAGAS	LEISHMANIASES
Principal disease forms or stages	Early(hemolymphatic) stage, late (CNS) stage	Acute phase, indeterminant phase, chronic phase (cardiac and digestic forms)	Vicersl, Mucocutaneous and Cutaneous leishmaniasis
Causative organisms	<i>T. gambiense</i> , <i>T. rhodesiense</i>	<i>T. cruzi</i>	21 <i>Leishmania</i> spp. e.g. <i>L. donovani</i> (VL) <i>L. braziliensis</i> (MCL) <i>L. major</i> (CL)
Host cell/tissue	Extracellular in blood, lymph, cerebral spinal fluid, intercellular spaces	Intracellular, in cytoplasm of heart, smooth muscles, gut, CNS, adipose tissue cells	Intracellular, in phagolysosomes of macrophages
Vectors of medical impotence	Tsetse flies (\approx 20 <i>Glossina</i> spp)	Reduviid bugs (Triatominae spp)	<i>Phebotominae sandflies</i> (\approx 70 spp)
Transmission	Infected fly bite, congenital (rare), blood transfusion (rare)	Contamination by feces of infected bugs	Infected fly bite
Geographic distribution	Sub-Saharan Africa	South and Central America	South and Central America, Europe, Africa, Asia and Middle East
Population at risk	50 million	100 million	350 million
Infected	70,000-80,000	8-1 million	12 million
Deaths per annum	\approx 30,000	\approx 14,000	\approx 51000 (VL)
Health Burden (DALYs)	1.5 million	0.7 million	2-1 million

Table 1: Summary of the kinetoplastids that cause human diseases [3].

Currently only nitrofurantoin derivative, nifurtimox and the nitroimidazole benznidazole are available for treatment of Chagas disease. Both are administered orally but have long treatment course, approximately two months and they have side effects.

New effective, safe and affordable drugs are needed for all kinetoplastids. It will be better to have more than one new drug so that combination therapy can be employed whenever drug resistance arises and also to provide back-up when resistance emerges.

Rational development of anti-protozoal drugs that will exploit biochemical differences between host and parasite is most desirable. There are challenges to achieving this goal but with current available technologies, it is possible. With post-genomic bioinformatics and experimental research, it is possible to identify drug targets, vaccine candidates and pathogenic processes. It is also possible to identify candidate diagnostics which realistically should be non-invasive, inexpensive and deployable at the poor resource sites. The new diagnostics in addition should discriminate between types of diseases.

Ribose metabolism in protozoans is of interest because they are auxotrophic for purines and expresses multiple pathways for purine uptake that enable them to acquire and use these vital metabolites from the hosts [1,6,7]. A metabolic pathway of interest is that involved in production of ribose 5-phosphate (R5P) required for the synthesis of 5-phosphoribosyl-1-pyrophosphate used with nucleobases for the synthesis of nucleic acids. Ribose 5-phosphate is generated in cells by one or combination of the following pathways: ribokinase conversion of ribose; condensation of fructose 6-phosphate and glyceraldehyde 3-phosphate mediated by transaldolase and transketolase; conversion of glucose via oxidative pathway of pentose phosphate pathway and hydrolysis of nucleoside to nucleobase and ribose followed by the first reaction above.

Hydrolysis of nucleosides is unique to trypanosomatids in the sense that the reaction is catalysed by purine nucleoside hydrolase unlike in humans where it is catalysed by purine nucleoside phosphorylase. The nucleoside hydrolase activity results in the abundance of intracellular ribose available to trypanosomatids. The extracellular ribose has been shown previously to be efficiently incorporated into the nucleic acids of leishmania [8]. Ribose may also serve as a source of energy for organisms [9] hence there is every need to study the enzyme (ribokinase) that is involved in the mobilization of ribose.

Summary of Cloning of Ribokinase in *L. Major* [10]

Design of oligonucleotide primers

The gene coding a putative ribokinase was identified in the *L. major* genome using BLAST searches with ribokinase of diverse organisms. Oligonucleotide primers were designed against conserved regions of proteins, which represented an open reading frame. The forward primer (5'-AAACATATGCACCGTGTGCAGAACGTT-3') was designed from the first peptide of the protein while the reverse (5'-AAACTCGAGCTACGTGACACCAGCC-3') was designed from the second peptide. The underlined bases represent restriction sites (*Nde*I and *xho*I) that were inserted to facilitate cloning of the PCR amplified products. The oligonucleotides were synthesized by MWG-Biotech AG, UK.

PCR amplification

Amplification was carried out in a DNA thermal cycler (MJ Research Inc., Western Town, MA, USA). PCR was routinely performed in 100 μ l reaction containing 10 mM Tris-HCl, pH 8.3, 1.5 mM MgCl₂, 50 μ M KCl, 200 μ M each of dNTPs, 40 ng of each of the primer and 1 unit of Pfu DNA polymerase. The tube containing the reactants were placed in the thermo cycler programmed for 30 cycles, a single cycle at 94°C for 120 s; was followed by 30 cycles at 63°C for 15 s and 72°C for 120 s; with a final cycle at 72°C for 600 s.

Detection of PCR products

Five micro litres of the PCR product was electrophoresed in a 1% agarose gel containing 0.1 μ g of ethidium bromide per ml, and bands were visualized by UV trans illumination.

Preparation of cloning vector and insertion

The amplified DNA, after gel electrophoresis was isolated using the commercially available QIAquick Gel Extraction Kit (Qiagen, West Sussex). The gel-purified PCR product (1.2-kb amplicon) was cut with *Nde*I and *xho*I at the primer sequence sites underlined. This was ligated with the pGEM-T Easy plasmid (Novagen) cut with the same enzymes. The recombinant plasmid was introduced into *E. coli* strain DH5 α (Invitrogen) by heat transformation in Luria broth (LB) medium at 42°C for 50 sec. Agar plates with LB medium supplemented with ampicillin, isopropyl-1-thio- β -D-galactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl- β -galactoside (X-gal) were used to test the

recombinant strain. Positive strain was used for recombinant protein over-expression experiments.

Protein overproduction

For recombinant protein over expression, the Nde I and xho I digest of pGEM-T was inserted between the same sites of plasmid pET28a⁺ (Novagen). The resulting plasmid pET28a⁺ribo construct harbors the rbk gene under the control of a hybrid promoter-operator region, consisting of sequence of T7 promoter and a *lac* operator. The pET28a⁺ribo was introduced into the BL21 (strain DE3) (Statagene) for protein over expression. Expression of the His-tagged ribokinase was induced by 0.5 mM IPTG overnight at room temperature. Cells were harvested by centrifugation, washed once and stored at -20°C in 5 ml of 50 mM Hepes buffer, pH 7.0 containing 300 mM NaCl and 10 mM EDTA. The cells were lysed by sonication in buffer A (50 mM NaH₂PO₄ containing 300 mM NaCl, pH 8.0) and the soluble fraction recovered by centrifugation at 10,000 g for 30 min at 4°C. This was applied to nickel-nitrilotriacetic acid column (bioCAD) pre-equilibrated with the buffer A. The column was washed with 100 ml of the buffer A containing 0.5 mM imidazole and then with the same buffer containing 50 mM imidazole and finally the his-tagged recombinant protein was eluted with 500 mM imidazole in the buffer A. The eluant was dialysed overnight in 50 mM Tris-HCl, pH 7.0 at 4°C and stored at -70°C in glycerol (1:1).

Characteristic of Ribokinase

Ribokinase is an ATP-dependent phosphoribosyl kinase (EC 2.1.7.15), which catalyses the conversion of ribose to ribose 5-phosphate, a substrate of 5-phosphoribosyl 1-pyrophosphate synthetase, that uses nucleobases and ribose 5-phosphate to synthesize nucleic acids. X-ray crystallography of *Escherichia coli* ribokinase shows the protein to be a homodimer in solution with each subunit having two domains. Each subunit has a molecular weight of about 33 KDalton [11,12]. The turnover numbers for *Leishmania* ribokinase for the substrates D-ribose and ATP are respectively 10.8 s⁻¹ and 10.2 s⁻¹ and the catalytic activity is strongly dependent on the presence of monovalent cations [10,14].

Conclusion

Ribokinase, although, a possible target for chemotherapy is not yet proven to be essential in protozoa parasites. Because of the multiplicity of pathways for utilization of nucleobases for nucleic acids synthesis, the parasites can bypass the ribokinase pathway to satisfy its nucleic acids requirements. However, if the transketolase / transaldolase pathway

is blocked with specific inhibitors, then all other pathways become susceptible to ribokinase inhibitors. Hence a combination therapy with inhibitors of transketolase/transaldolase and ribose kinase pathways will effectively block all the salvage pathways for purine nucleotide synthesis in trypanosomatids.

References

1. Hammond DJ, Gutteridge WE (1984) Purine and pyrimidine metabolism in the trypanosotidae. *Molecular and Biochemical Parasitology* 13: 243-261.
2. WHO (2016) Neglected tropical diseases.
3. Stuart K, Brun R, Croft S, Fairlamb A, Gürtler RE, et al. (2008) Kinetoplastids: related protozoan pathogens, different diseases. *Clinical Investigation* 118: 1301-1310.
4. Berman JD (1997) Human leishmaniasis: clinical, diagnostic and chemotherapeutic developments in the last 10 years. *Clinical Infectious Disease* 24: 684-703.
5. Herwalt BL, Bermand (1992) Recommendations for treating leishmaniasis with sodium stibogluconate (Pentosam) and review of pertinent clinical studies. *American Journal of Tropical Medicine and Hygiene* 46: 296-306.
6. König E (1976) Comparative aspects of nucleotide biosynthesis in pathogenic protozoa. In: *Biochemistry of Parasites and Host-Parasite Relationships* (Van de Bossche, edn.), Elsevier/North-Holland Biomedical Press, Amsterdam.
7. Gutteridge WE, Davies MJ (1981) Enzymes of purine salvage in *Trypanosoma cruzi*. *Federation of European Biochemical Society Letter* 127: 211-214.
8. Maugeri DA, Cazzulo JJ, Burchmore JS, Barrett MP, Ogbunode POJ (2003) Pentose phosphate metabolism in *Leishmania mexicana*. *Molecular and Biochemical Parasitology* 130: 117-125.
9. Berens RL, Deutsch-King LC, Marr JJ (1980) *Leishmania donovani* and *Leishmania braziliensis*: hexokinase, glucose 6-phosphate dehydrogenase and pentose phosphate shunt activity. *Experimental Parasitology* 49: 1-8.
10. Ogbunode POJ, Ikekpeazu JE, Ugonabo J, Barrett M, Udeogaranya PO (2011) Cloning the Ribokinase of kinetoplastidae: *Leishmania major*. In: *Molecular Cloning, Selected Applications in Medicine and Biology* (Brown GG edn.), Intech Janeza Press, Croatia.
11. Sigrel JA, Cameron AD, Jones TA, Mowbray SL (1997) Purification, characterization and crystallization of *Escherichia coli* ribokinase. *Protein Science* 6: 2474-2476.
12. Sigrel JA, Cameron AD, Mowbray SL (1999) Induced fit on sugar binding activates ribokinase. *Journal of Molecular Biology* 290: 1009-1018.
13. Ogbunode POJ, Lamour N, Barret P (2007) Molecular cloning, expression and characterization of ribokinase of *Leishmania major*. *Acta Biochimica Biophysica (sinica)* 39: 462-466.
14. Chuvikovskiy DV, Esipov RS, Skoblov YS, Chupova LA, Muravyova TI, et al. (2006) Ribokinase from *E. Coli*: Expression, purification and substrate specificity. *Biorganic Medicinal Chemistry* 14: 6327-6332.