Review articles

Transporter protein and drug resistance of *Trypanosoma*

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ABSTRACT. *Trypanosoma* infection is one of the most important infections in livestock and humans. One of the main problems of its therapeutic control and treatment is the resurgence of drug resistance. One of the most studied causes of such resistance is the function of its adenosine transporter gene. A trypanosomal gene TbAT1 from *Trypanosoma brucei* has been cloned in yeast to demonstrate its function in the transport of adenosine and trypanocidal agents. Drug resistant trypanosomes showed a defective TbAT1 variant; furthermore, deletion of the gene and set point mutations in the transporter gene has been demonstrated from isolates from relapse patients. The molecular understanding of the mechanism of action trypanocidal agents and function of transporter gene can lead to control of drug resistance of Trypanosomes.

Key words: *Trypanosoma*, TbAT1 gene, adenosine transporter

Introduction

One of the major problems in livestock production is the recurrence of infection primarily due to treatment failure. Development of resistance to broad range of therapeutic drugs leads to lesser therapeutic options and economic sabotage. *Trypanosoma* infection for instance has been studied vastly as there is increasing trend of treatment resistance. This organism cause a range of infectious diseases that includes sleeping sickness (human African trypanosomiasis), Nagana in cattle, Dourine in equines and Surra in buffaloes [1–3]. Several species infecting domestic and wild animals (*Trypanosoma congolense*, *Trypanosoma vivax* (*T. vivax*) and *Trypanosoma brucei bruce* (*T. b. brucei*)) are transmitted by tse-tse flies, while *T. vivax*, closely related to *Trypanosoma evansi* (*T. evansi*) and *Trypanosoma equiperdum* (*T. equiperdum*) can also be transmitted sexually or by biting insect [4].

Trypanosomiasis is controlled either by controlling the vector or by controlling the parasite or the combination of both. Over the years the control of animal trypanosomiasis has been dependent heavily on the use of trypanocidal drugs. There are few chemoprophylactic and chemotherapeutic compounds use against *Trypanosoma* such as phenanthridines (isomethamidium chloride), ethidium bromide, melaminophenyl arsenical (melarsoprol and melarsen oxide) and diamidines (diminazene aceturate and pentamidine) [5,6]. The uses of the trypanocidal drugs are unsatisfactory for various reasons, including unacceptable toxicity, poor efficacy, undesirable route of administration, and drug resistance [5].

There have been an increasing number of reports on drug resistance in animal trypanosomes, and it is unclear whether this increase is due to a higher incidence of drug-resistant strains. Growing scientific interest on these areas has revealed possible causes of multidrug resistance. Multidrug resistance may develop in response to specific drugs or drug combinations. One of the investigated

causes is the lack of transport of drug into the microorganism. Transport proteins play a major role in the absorption, distribution and elimination of wide variety of drugs in clinical use [7].

This review will focus on the properties of nucleoside transporter with particular emphasis on its role on the *Trypanosoma* drug resistance.

Transporter Proteins

Drug resistance in African trypanosomes has been well studied for the past years. The reduction of net drug uptake has emerged as the most frequent cause of resistance [12]. Drug uptake is a function of transport proteins that play a role in the absorption, distribution, and elimination of wide variety of drugs [7,8].

Transporter protein is responsible for the influx or efflux molecules through the membrane of a cell or organelle [7,9]. Nucleoside transporter is an example of transporter protein that transports nucleoside substrates like adenosine across the membranes of cells and/or vesicles [10]. Nucleoside consists of nitrogenous base covalently attached to a sugar ribose or deoxyribose that upon phosphorylation by specific kinases produces nucleotides (purines and pyrimidines). Nucleotides are very important in mammalian and parasitic protozoan cells as they are involved in biologic production of energy (ATP), phospholipids for the cell membrane and importantly as building blocks for nucleic acids [19,20].

Unlike mammalian cells, protozoans lack the ability to synthesize purines de novo, instead they depend exclusively on purines salvaged from the blood of their mammalian host. Protozoans have elaborate variety of salvage pathways that enable them to acquire preformed purines from their hosts. These salvage pathways entails the uptake of purine nucleosides or nucleobases from the host milieu and is mediated by various nucleoside or nucleobases transporters, located in the cell membrane of the parasitic hosts. Nucleoside transporters are therefore required for parasite viability in all life cycle stages [19].

Molecular genetic work over the past years has been devoted to identifying and characterizing the genes that encode various nucleoside and nucleobase transporters of protozoans such that of *Trypanosoma* spp. Maser et al. [6] revealed that *T. b. brucei* has a gene that encodes adenosine transporter designated as TbAT1. When *T. brucei* bloodstream form cDNA was transformed with *Saccharomycetes cerevisiae*, a yeast that does not take up exogenous adenosine, the yeast was able to grow on a media containing adenosine as sole purine source. This proves that when expressed in yeast, TbAt1 gene enabled expression of transporter proteins that is responsible for cellular uptake of adenosine.

Mechanism involve in Trypanosomal resistance

T. evansi was found to have two classes of adenosine transporters, P1 and P2 [13]. P1 is specific for adenosine and inosine transport while
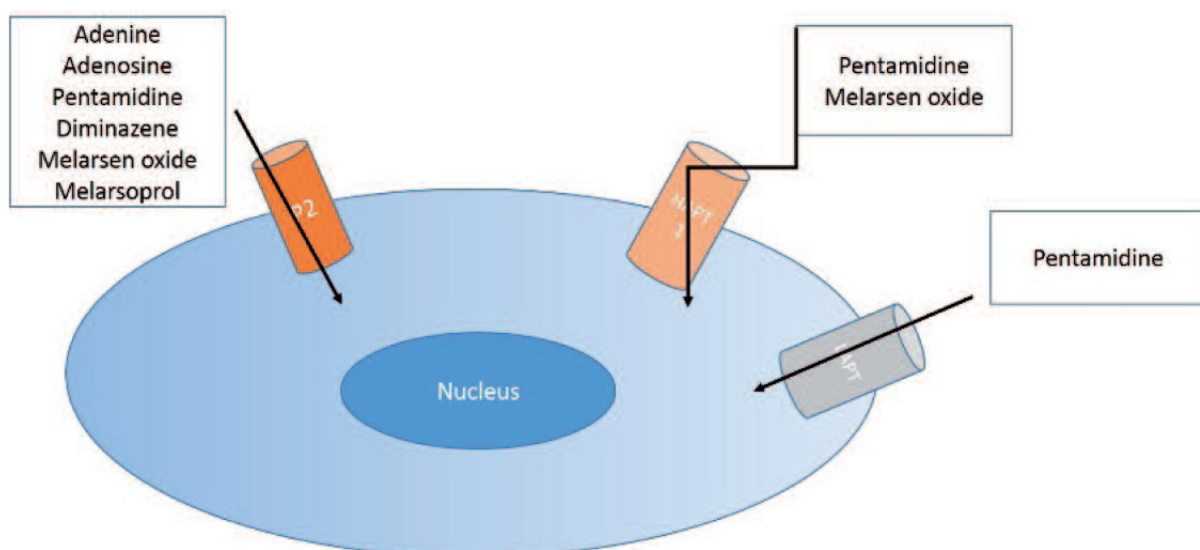

Fig.1. Model for the uptake of most commonly available trypanocides in Trypanosomes. Main tranporter proteins: P2 (Purine transporter protein); HAPT (High affinity pentamide transporter); LAPT (Low affinity pentamide transporter)

P2 transports adenosine, and adenine [11]. These transporters mediate the uptake of various antiparasitic drugs, both purine analogs and non-analogs, hence alteration or loss of this transport function can induce partial or pronounced resistance to these therapeutic agents [13,19].

There are two main classes of drugs against trypanosoma, arsenical-based trypanocides (melarsoprol) and diamidines (pentamidine). Researchers found that purine permease P2 mediates influx of trypanosomal drugs melarsoprol and diamines [3,12–14]. Structural similarities between aminopurines adenosine and adenine and melarsoprol and pentamidine may be responsible for the common recognition by the P2 transporters. Increase concentration of these trypanocides causes lethal effects on the parasites [15,19]. Prolonged used of these trypanocides, however, have resulted in the resistant strains of trypanosoma. This heightened the interest to study transport of drugs into trypanosomes to elucidate the cause of drug resistance (Fig. 1).

With the use of genetic approaches, mechanism of resistance can be elucidated. One is the identification of the gene encoding the trypanosome P2 adenosine transporter protein. P2 adenosine transporter is encoded by TbAT1 gene by *T. b. brucei*, the same gene in *T. equiperdium* referred to as TeAT1. *Trypanosoma brucei gambiense* (*T. b. gambiense*) *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) as well as *T. evansi* and *T equiperdium* are very close phylogenetic relatives of *T. brucei* [3].

Studies using melarsamine hydrochloride-resistant *T. b. gambiense* strain, diamidine furamidine -resistant *T. b. brucei* strain and arsenical-resistant *T. b. rhodesiense* strain have shown deletion of the TbAT1 gene as shown by PCR analysis of genomic DNA. Consequently, fluorescence-based assay showed lack of functional P2 or TbAT1 transporter protein. Hence, deletion of TbAT1 gene can lead to loss of functional TbAT1 expression of transporter proteins leading to resistance [3].

Additionally, studies on inhibition, knocking down or silencing of the gene responsible for the encoding of P2 aminopurine transporter has been described and reviewed as one of the causes of resistance [15]. Studies using the RNA interference (RNAi) produced a knock-out of TevAT1 gene resulted to 10-fold depletion of TevAT1 mRNA resulting to concomitant resistance to diminazene aceturate [17].

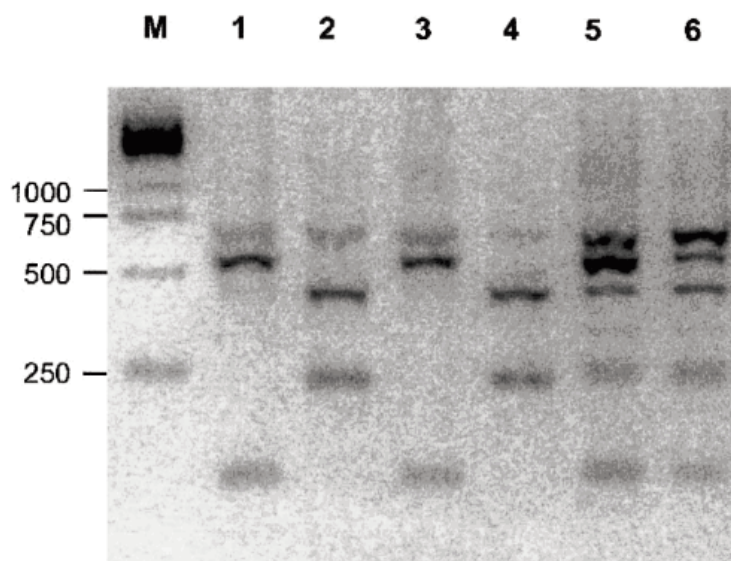However, trypanosomes exhibited varied

Fig. 2. SfaN1 RFLP of TbAT1 reveals three different banding patterns. A fragment of TbAT1 (nucleotides 430–1108) from each patient (totally 65) was subjected to digestion with SfaN1 and analyzed on a 2% agarose gel. Lanes 1 and 2: DNA from cultured melarsoprol sensitive (777S) and resistant stocks (777R), respectively; lanes 3–6: representative samples for the analysis of PCR fragments amplified from patient CSF (patients F015, R027, R015 and R026). Lanes 1 and 3 represent the RFLP of wild-type TbAT1; lanes 2 and 4 represent the RFLP of the mutated TbAT1 present in stock 777R; lanes 5 and 6 represent a mixed RFLP containing all bands from both prototype patterns [14]

response to trypanocides. The production of *T. equiperdum* diminazene-resistant strain has been shown to retain TeAT1 gene [3]. Hence, deletion is not the mechanism of resistance. Using Reverse-Transcription-PCR analysis, the *T. equiperdum* resistant line showed loss of stable expression or no mRNA transcript was detected, hence P2 transport activity was lacking.

Another mechanism for occurrence of resistance is explained by the presence of genetic variants of TbAT1 from *T. b. gambiense* as shown in relapse infections following melarsoprol therapy [14,16]. A rapid screening for the potential presence of mutants using single-stranded DNA conformation polymorphism (SSCP) analysis showed a wild-type, mutant and mixed genotypes of TbAT1. Using *Sfa*N1 restriction fragment length polymorphism (RFLP) and allele-specific PCR (AS-PCR), mutant TbAT1 and wild-type TbAT1 genes were isolated (Fig. 2). Mutant genes can lead to amino acid

substitutions or lead to deletion of an entire codon and others are silent mutations.

In some experiments, TbAT1 gene alone does not confer more than marginal pentamidines resistance [4]. Pentamidines are also taken up by a non-P2 transport mechanism as shown by the TbAT1 negative trypanosomes that remain sensitive to high concentrations of the drugs in vitro. Studies show two adenosine-insensitive transporters for pentamide: the high affinity pentamide transporter HAPT1 and low-affinity pentamide transporters LAPT1 that contributes to the uptake of these drugs. Though diminazene (diamidines) is structurally related to pentamidine, it is not an effective substrate for HAPT1 or LAPT1 [11]. Recent studies also showed that HAPT1 also contributed to the melarsen oxide (arsenicals) uptake by TbAT1 negative trypanosome in vitro despite the low affinity of the drug to the transporter. Physiological function of HAPT is unknown yet and the gene encoding the transporter has not been positively identified [18].

Other theory being investigated to explain this occurrence of resistance is the expression of catabolic enzymes or pathway for diminazene, introduction of an efflux pump or other sequestration/extrusion mechanism or additional non-transport-related adaptations that alter drug sensitivity of the parasite at the target level (mitochondria) [18].

The efficient operation of important element sequestration and extrusion mechanisms within the cell is crucial for the maintenance of normal homeostasis. Different buffering processes involved in the maintenance of normal cellular homeostasis. This gradient is maintained by the effective operation of pumps located in mitochondria, endoplasmic reticulum, and plasma membrane. All of these processes are energy-dependent and require the hydrolysis of adenosine triphosphate (ATP) [21].

Another drug investigated is Isometamidium (ISM) have additional mechanism of resistance. ISM transport within the trypanosome is energy-dependent. From the cytoplasmic compartment, ISM enters the mitochondrion by the same process. Development of resistance could be then due to the following: decrease in diffusion through mitochondrial membranes, modification of a possible transporter located in the inner mitochondrial membrane, or increased extrusion of the drug by a transporter located in the cytoplasmic membrane, or combination of these processes [15].

Conclusions

Getting a better understanding on the mechanism that leads to resistance of trypanosome to most trypanocide is very important for the success of chemotherapy against Trypanosoma. Researches showed that TbAt1 encodes adenosine transporter mediating uptake and susceptibility of trypanocidal drugs. Defects in TbAT1 contribute to defects in transporter proteins leading to resistance to these agents. Cloning of TbAt1 opens prospects for therapy of trypanosomiasis. Development and validation of molecular markers that identify polymorphism in the gene can contribute to faster detection of trypanocidal drug resistance. The exploitation of alternative pathways for drug uptake will be crucial for the development of new-generation trypanocides and can be used to develop strategies to stop or delay the development of drug resistance in trypanosome.

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