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Editorial

An Aquaglyceroporin as a New Drug Target in *Leishmania*

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INTRODUCTION

Aquaporins belong to MIPs (Major Intrinsic Protein), a large super-family of integral membrane proteins. They are present throughout the five kingdoms, including bacteria, archaea, protista, fungi, plantae, animalia and even viruses [1]. The ubiquitous nature of this channel underlines its importance in life processes. However, most of these aquaporins are redundant and null mutants for any one of them from bacteria to mammals do not cause lethality [2,3] with one exception from the parasite protozoan *Leishmania* (Mukhopadhyay and Ouellette, unpublished observation). The aquaporin family can be functionally divided into two sub-groups, the orthodox aquaporins, which are water-specific channels, and aquaglyceroporins, which allow the transport of water, glycerol, metalloids and other small, uncharged solutes. These channels are all tetrameric complexes with each monomer functioning independently; unlike ion channels the center of the quaternary structure is impermeable. Each monomer contains six transmembrane helices and two short half-helices which meet in the middle of the membrane to form a pseudo-transmembrane span. The comparison between the crystal structures of *E. coli* aquaglyceroporin GlpF and human aquaporin AQP1 showed a larger pore for aquaglyceroporins accommodating the larger glycerol molecule [4]. The constriction at the pore mouth is termed as aromatic arginine (ar/R) constriction. An additional constriction in the middle of the pore is the Asn-Ala-Pro (NPA) region. These two NPA motifs form the central of the pore and act as a proton filter acting as the capping amino acids at the positive ends of helices B and E and act as hydrogen donors to the oxygen atoms of passing permeants [5]. The aquaglyceroporins facilitate the transport of wide variety of uncharged solutes in addition to water. These include glycerol, urea, dihydroxyacetone (DHA), methylglyoxal (MG), polyols and metalloids such as trivalent arsenic (AsIII) and antimony (SbIII) [6]. In mammals, thirteen AQPs (0-12) have been identified so far. Among them four are classical aquaglyceroporins AQP3, 7, 9 and 10 [7]. A number of aquaporins have been identified in parasitic protozoa from a single aquaglyceroporin in *Plasmodium*, to three in *Trypanosoma brucei*, five in *Leishmania*, two in *Toxoplasma* and one in *Cryptosporifium* [8-11]. A common feature of the parasite AQPs is that they are generally better water transporters when compared to *E. coli* (GlpF) and mammalian ones. It appears that protozoal aquaglyceroporins are bifunctional and conduct both water and glycerol at reasonable rates [12]. Among five members of the AQP family in *Leishmania*, we identified and characterized the first aquaglyceroporin AQP1. AQP1 is involved in the accumulation of metalloids in *Leishmania* promastigotes and amastigotes along with water, glycerol, methylglyoxal, glyceraldehyde to name a few [9]. The other four AQPs are not involved in metalloid transport (Mukhopadhyay, unpublished data) and are closer to classical aquaporins. Channels such as AQPs are at the interphase of host parasite interactions and could be attractive drug targets and/or mediator of specific drugs such as arsenic and antimony. Protozoan parasites cause deadliest of diseases throughout the world. The situation is worse in developing countries because of poor hygiene and infrastructure.

Leishmaniasis is a protozoan parasitic infection ranging from self healing cutaneous lesions to non-healing mucocutaneous and visceral ailments caused by *Leishmania* spp. The disease is endemic in parts of 88 countries across five continents - the majority of the affected countries are in the tropics and subtropics. Approximately 12 million people worldwide are affected by leishmaniasis and 2 million new cases are considered to occur annually. The *Leishmania* parasite exists in two morphologically distinct forms: promastigotes and amastigotes. The promastigotes reside in the intestinal tract of the sandfly vector and have a slipper like body with an anterior flagellum. Inside the mammalian host, the promastigote forms of the parasites are transformed into amastigotes that appear as small, oval-shaped, aflagelleted structures, and reside in macrophages and other mononuclear phagocytes. The female phlebotomine sandflies are solely responsible for the transmission of *Leishmania* parasites amongst vertebrate hosts. Transmission of leishmaniasis could be anthroponotic that is transmission from human to human through the sandfly vector, where humans are the sole reservoir host. The disease can also spread from animals to humans (zoonosis), in this case, domestic animals (dogs) and wild animals (foxes, jackals, rodents, hyraxes) serve as the reservoir hosts. The disease in humans has been classified in three different forms, each having a broad range of clinical manifestations. Visceral leishmaniasis (VL) is the most severe form of the disease and is fatal if left untreated. VL is caused by

Leishmania donovani, *Leishmania infantum* or *Leishmania chagasi* and is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and anemia. Approximately 90% of the 500,000 new cases of VL reported annually occur in Bangladesh, Brazil, India, Nepal and Sudan. Cutaneous leishmaniasis (CL) is caused by a variety of species including *Leishmania major*, *Leishmania tropica, Leishmania mexicana*, and *Leishmania panamensis*. CL is characterized by skin lesions on the exposed parts of the body, such as the face, arms and legs, causing serious disability and leaving the patient permanently scarred. It is the most common form of the disease with 1-1.5 million new cases reported annually worldwide, and 90% of all CL cases are reported from Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria. Mucocutaneous leishmaniasis (MCL) due to *Leishmania braziliensis* infection produces lesions which can lead to extensive and disfiguring destruction of mucous membranes of the nose, mouth and throat cavities. 90% of MCL cases occur in Bolivia, Brazil and Peru [13]. The US military is not immune to *Leishmania* infection. During operations Desert Storm and Desert Shield in the 90s, many soldiers contacted cutaneous leishmaniasis and some viscerotropic leishmaniasis, a visceral and deadlier form of the disease caused by the cutaneous species *L. tropica*. Visceral infection due to *L. tropica* in a veteran of Operation Desert Storm was presented two years after leaving Saudi Arabia [14]. Incubation periods of up to ten years have been reported for *L. donovani,* the causative agent of the classical visceral form of the disease (VL) (the predominant species in Pakistan, and the Afghanistan-Pakistan border). Additionally, the parasite can masquerade as inflammatory diseases such as Wegener granulomatosis for up to 6-7 years (Brahn et al 2010, [15]. *L. tropica* is also endemic to the Iraq and Afghanistan region where US military is currently deployed. A burst of cutaneous infection cases took place in the US military between 2003 and 2005, so much so that Lt. Col. Peter Weina, director of *Leishmania* diagnostics at Walter Reed Army Medical Center commented in Nature Medicine, *"This is probably the largest outbreak of leishmaniasis that the US military has ever seen*" (February, 2004, Vol:10, page: 110). Additionally, *Leishmania*/HIV co-infection is currently emerging as an extremely serious, new disease and is being considered a real threat in various parts of the world. VL has been widely recognized as an opportunistic infection among persons who are immunosuppressed, particularly in patients infected with human immunodeficiency virus [16].

The first line compounds against all forms of leishmaniasis are the two pentavalent antimonials, sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime). However, clinical resistance to this treatment is becoming prevalent [17,18]. In fact, more than 50% of VL cases in North-East India are resistant to pentostam [19]. *Leishmania* resistant to trivalent antimony has also been reported [20]. The second line of anti-leishmanial drugs includes amphotericin B and pentamidine. Recently, alkyl-lysophospholipids (ALP) such as miltefosine and edelfosine, originally developed as anticancer drugs, have shown significant antiproliferative activity against *Leishmania* [21]. Miltefosine is the first oral drug that has been used against VL in India, including antimony resistant cases [22]. Other drugs in various stages of clinical trials include allopurinol, atovaquone, fluconazole, paromomycin, and sitamaquine.

The *L. major* genome encodes for five aquaporins: LmAQP1, LmAQPα, LmAQPβ, LmAQPγ, and LmAQPδ. While LmAQP1 shows strong similarity to bacterial aquaporins the other *L. major* aquaporins (LmAQP α-δ) are closer to plant aquaporins [12]. This is a unique peculiarity of *L. major* aquaporins, since other parasitic aquaporins known to date are either bacteria- or plant-like, and not a mixed population [12]. The roles of the other *L. major* aquaporins have not yet been determined although they are not involved in metalloid transport (Mukhopadhyay, unpublished data). The *Leishmania major* aquaglyceroporin (LmAQP1) is adventitiously permeable to antimonite [Sb(III)], an activated form of Pentostam or Glucantime [23]. Besides the two metalloids arsenite [As(III)] and Sb(III), LmAQP1 is also permeable to water; its water conduction capacity is 65% of that of the classical water channel, human AQP1 [9]. In contrast to *Plasmodium* and *Trypanosome* aquaglyceroporins (AQPs) that are inhibited by mercurials, water movement through LmAQP1 is not inhibited by mercuric chloride, and it is therefore a mercurial independent water channel. LmAQP1 also conducts glycerol, glyceraldehyde, dihydroxyacetone, and sugar alcohols. Expression of LmAQP1 is limited exclusively to the flagellum of promastigotes, while in amastigotes it is found in the flagellar pocket, rudimentary flagellum, and contractile vacuoles. LmAQP1 plays an important physiological role in water and solute transport, volume regulation and osmotaxis [9]. Disruption of one of the two *LmAQP1* alleles in *L. major* conferred a 10-fold increase in resistance to Sb(III) [23]. LmAQP1 mRNA levels are significantly less in either the Sb(III) or As(III) resistant *L. major* and *Leishmania tarentolae* cells, indicating that downregulation of LmAQP1 leads to drug resistance [24]. Later these findings were corroborated in field isolates from India [25] and Nepal [26]. Therefore, LmAQP1 plays a major role in *Leishmania* cellular physiology and drug resistance. However, down-regulation of LmAQP1 is not the sole cause of drug resistance in *Leishmania*. Several other factors include trypanothione (TSH), antimonate reductase, MRP homologue PGPA and an antimony-TSH conjugate exporter in the plasma membrane. In absence of a crystal structure, our structure-function studies have defined the pore mouth residues of LmAQP1. We have shown that an extracellular loop C residue glutamate 152 helps the channel to differentiate between metalloids and glycerol [27]. In a recent paper, we have also shown that alanine 163 in loop C is localized near the pore mouth, critical for channel function and alter drug sensitivity of the parasite [28]. Despite several attempts we have not been able to generate the null mutant, which supports the idea of *LmAQP1* being an essential gene for *Leishmania* survival and growth (R. Mukhopadhyay and M. Ouellette, unpublished observation).

Hence, *Leishmania* AQP1 probably becomes the best target to control the parasite growth. On one hand, overexpression of this channel will make the parasite hypersensitive to the traditional antimony containing drugs (Figure 1) may be even at lower concentrations combating the toxicity. On the other, just shutting

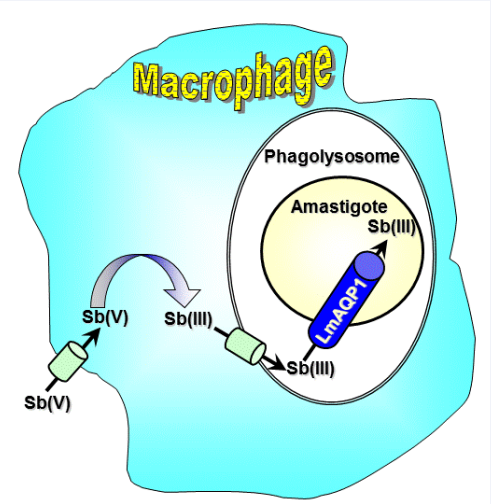

Figure 1

the channel down may cause adverse effect to the general well-being of the parasite. Identification of pharmacological agents that induce AQP1 expression by manipulating the regulatory pathways is a feasible goal. Our present data show that induction of AQP1 makes the wild type and drug resistant field isolates susceptible to lower concentrations of antimonials. Thus co-administration of inducing agents of AQP1 along with pentavalent antimony will overcome drug resistance and avoid toxicity. However, control of gene expression is complicated in *Leishmania*. Genes of this parasite are transcribed as a large polycystronic group and regulated at the post transcriptional level largely by the 3'UTR, although controlling cis-elements and trans acting factors are largely unknown. In a recent paper we have shown that Mitogen Activated Protein Kinase 2 (MAPK2 regulated AQP1 expression positively [29]. The positive regulation of *Leishmania* AQP1 by an MAP kinase might provide a new approach for drug targeting. Since protozoan MAP kinases are distantly related to human MAP kinases, it might be possible to design compounds that specifically target the protozoan enzymes. One of the approaches might be the development of small molecule inhibitors of *Leishmania* MPK2 activity. Such compounds would prevent phosphorylation of the protozoan AQP1 channel, resulting in its increased turnover, and consequently, exposing the parasites to osmotic stress. Another approach would be to look for small molecule inducers of MPK2, which will stabilize AQP1, and thereby either resensitize drug resistant isolates to Sb(III) and/or reduce the effective dose, eliminating drug toxicity.

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