Evolution, function and roles in drug sensitivity of trypanosome aquaglyceroporins

Juan F. Quintana1 and Mark C. Field2,3

1Wellcome Centre for Integrative Parasitology (WCIP), Institute of Biodiversity, Animal Health and Comparative Medicine (IBAHCM), University of Glasgow, Glasgow G61 1QH, UK; 2School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK and 3Institute of Parasitology, Biology Centre, Czech Academy of Sciences, 37005 Ceske Budejovice, Czech Republic

Abstract

Aquaglyceroporins (AQPs) are membrane proteins that function in osmoregulation and the uptake of low molecular weight solutes, in particular glycerol and urea. The AQP family is highly conserved, with two major subfamilies having arisen very early in prokaryote evolution and retained by eukaryotes. A complex evolutionary history indicates multiple lineage-specific expansions, losses and not uncommonly a complete loss. Consequently, the AQP family is highly evolvable and has been associated with significant events in life on Earth. In the African trypanosomes, a role for the AQP2 paralogue, in sensitivity to two chemotherapeutic agents, pentamidine and melarsoprol, is well established, albeit with the mechanisms for cell entry and resistance unclear until very recently. Here, we discuss AQP evolution, structure and mechanisms by which AQPs impact drug sensitivity, suggesting that AQP2 stability is highly sensitive to mutation while serving as the major uptake pathway for pentamidine.

Introduction

Aquaglyceroporins (AQPs) were first identified in the 1990s as membrane proteins with functions in osmoregulation and the translocation of low molecular weight solutes, in particular glycerol and urea (Preston et al., 1992). In humans, dysfunction is associated with multiple cancers, kidney disease, oedema and other pathologies (King et al., 2004; Yool et al., 2010; Shi et al., 2012). AQPs have an evolutionarily broad representation, being found in most pro- and eukaryotic taxa and they retain a conserved architecture encompassing six hydrophobic domains. This structure is in turn derived through an internal duplication from a primordial protein with three membrane-spanning helices, reflected in the presence of two NPA (Asn-Pro-Ala) boxes that are involved in channel functions. Both the N- and C-termini face the cytoplasm (Fig. 1) and sequence and architectural conservation indicates vertical descent. Consequently, at least one mechanism for the control of water (and solute) passage across biological membranes arose very early in the history of life (Ishibashi et al., 2020). However, AQPs are not present in all taxa, for example the bacterial phyla Fibrobacteres and Lentisphaerae, as well as some parasites and extremophiles. As AQPs can also be deleted in some eukaryotes, for example immortalized mammalian cells and trypanosomatids (Jescock et al., 2017; Calvanese et al., 2018), it is clear that AQPs are non-essential, at least under some circumstances. Control of osmolarity therefore likely utilizes additional mechanisms in both pro- and eukaryotes. Below we will consider initially the evolution and origins of AQP paralogues in protists and then the uncovering of drug-related functions in trypanosomes.

Evolution, functions and roles in protists

The evolution of the AQP family is surprisingly complex and at least three subfamilies with apparently distinct functions are recognized. These include AQPs able to translocate glycerol, others that only uptake water and a final third group, the superAQPs, that arose late in evolution. This latter subfamily is frequently intracellular, indicating a distinct function from the other members of the AQP family, which are usually located at the surface in most cells (Ishibashi et al., 2011), and are only found in metazoan. Significantly, the two ancestral forms are clearly differentiated in all prokaryotes, indicating an origin dating back to an early period of cellular life (Tong et al., 2019). The number of AQP paralogues in different species is highly variable, with land plants and vertebrates having the largest repertoires, as is the case for many other protein families.

There has been a considerable degree of expansion and contraction within specific lineages, or ‘churning’, with the result that functional differentiation between paralogues is difficult to predict (Ishibashi et al., 2020). Interestingly, in mussels (molluscs) there is evidence that expansion of AQP paralogues correlates with freshwater colonization events and hence facilitating adaptation to decreased environmental salinity (Calcino et al., 2019). Similar events may have facilitated tetrapod colonization of land habitats where desiccation is a considerable challenge (Finn et al., 2014) and underscores the importance of AQP evolution to life history.
Fig. 1. Structure and copy number of AQP paralogues. (A) Left panel: Depiction of the Trypanosoma brucei AQP2 monomer. The trans-membrane domains are highlighted in magenta. Right panel: Details of the unique NPS/NSA TbAQP2 selectivity pore. (B) Left panel: Top view of the proposed tetrameric structure of T. brucei AQP2 model. The lysine residues in position K147 and K234 are shown as spheres. Right panel: Expanded view of the conformational change observed during TMD simulations on TMD1 and TMD3 as a result of the K147R mutation. Wild type TbAQP2 is shown in green. TbAQP2 displaying the K147R and K234R mutations is shown in light orange. Other residues important for intramolecular interactions between trans-membrane domains (N70, D73, K142 and Y151) are also highlighted. Mutations on these residues profoundly impair protein stability, rendering the parasites resistant to pentamidine and melarsoprol. (Quintana et al., 2020). (C) Number of clear AQP paralogues detected in representative taxa. Note that for the protists these are all represented by the more permissive glycerol-capable class.

In unicellular eukaryotes the number of AQP paralogues is comparatively small when compared with multicellular organisms and it has been proposed that the numbers of AQP paralogues are correlated somewhat with environmental complexity (von Bülow and Beitz, 2015). Most protist AQPs appear to be the more peripherally correlated somewhat with environmental complexity (von Bülow et al., 2012). In the parasites the number of AQP genes in the American trypanosome, Trypanosoma cruzi also has four TIP-like AQPs, representing the entire repertoire in that organism and these are associated with the contractile vacuole and acidocalcisomes (Montalvietti et al., 2004). Trypanosoma brucei has three AQPs; AQP1 is shared with other kinetoplastida, while AQP2 and AQP3 arose from a recent gene duplication in the African trypanosome lineage and remain contiguous.

In addition to interactions between trans-membrane domains, two major selectivity filters restrict the molecular weights and properties of the solutes being translocated by AQPs and that can effectivly pass through the central pore; these are the ar/R and NPA/NPA motifs (Fig. 1) (Beitz, 2005; Baker et al., 2013; Verkman et al., 2014; Munday et al., 2015; Fairlamb and Horn, 2018). Trypanosoma brucei AQP1 and AQP3 display the internal arrangements in the protein pore observed in canonical AQPs, including the canonical ‘NPA’ within two half α-helices and a narrower ‘aromatic/arginine’ (ar/R) motif (Beitz, 2005).

Interestingly, TbAQP2 does not retain this canonical configuration, displaying an unconventional ‘N’PS/NSA’ filter motif and rearrangement in the ar/R motif that is replaced by a neutral leucine at position 264 (L264), followed by alphatic, rather than aromatic, residues (A88, 1110, V249 and L258), which are equivalent to the ‘TVL’ motif observed in the selectivity pore of canonical AQPs (de Groot and Grubmuller, 2001; Baker et al., 2013; Quintana et al., 2020). These structural features indicate that TbAQP2 can accommodate larger solutes through the selectivity pore (Uzcategui et al., 2004).

These examples demonstrated that AQP evolution is highly plastic, with the creation of additional paralogues, facilitating altered specificity. Hence, the AQP family contributes to surviving environmental complexity and exploitation of new ecological niches, with a considerable impact on the life history of the earth. However, the absence of AQPs from many lineages or a genetic demonstration of essentially in many organisms serves to underscore the challenges remaining for the full understanding of AQP function.

TbAQP2 and multidrug resistance

The treatment of sleeping sickness relies on drugs to clear first- or second-stage infections, and the choice of drug depends on the capacity to penetrate the blood–brain barrier (BBB) (Denise and Barrett, 2001; Steverding, 2010; Fairlamb and Horn, 2018). Of these, pentamidine and melarsoprol represent two of the most potent drugs currently used to treat first- and second-stage
Endocytosis or membrane uptake: competing models for drug entry

Suggesting that the role of a channel protein is not the primary mechanism for pentamidine to access the trypanosome cytoplasm may seem to be a straw man, but this possibility has been proposed. Specifically, as AQP2 binds pentamidine with high affinity, the hypothesis that pentamidine enters the cell at the cell surface (see text) and is then translocated into the mitochondrion to interact with the kinetoplast is perhaps not surprising that resistance to these compounds has been frequently observed in endemic countries. Indeed, pentamidine is unable to reach the central nervous system (CNS), in part due to its high affinity interactions with serum proteins, charge and relatively high retention in tissues and is therefore ineffective for the treatment of second-stage meningoencephalic HAT (Barrett et al., 2007; Maclean et al., 2012). Melarsoprol, on the contrary, is an arsenical compound used for the treatment of second-stage HAT, including T. rhodesiense HAT (Fairlamb et al., 1989; Keiser et al., 2000; Field et al., 2017). This compound is thought to be metabolized to melarsen oxide prior to uptake by African trypanosomes, leading to the formation of a stable adduct with trypanothione known as Mel T (Burri et al., 1993, 1994; Fairlamb and Horn, 2018). Melarsoprol penetrates the BBB comparatively more effectively than pentamidine, reaching the minimum concentration required for parasite clearance in the CNS (Mäser et al., 1999; Stewart et al., 2010). Melasoprol also displays reactive encephalopathy in ∼10% of patients, which is frequently fatal (Fairlamb and Horn, 2018).

Given the limited repertoire of drugs available for treatment it is perhaps not surprising that resistance to these compounds has been frequently observed in endemic countries. Indeed, diamidine-arsenical cross-resistance was initially reported in the 1940s, suggesting that mechanisms of uptake and/or action were common to these otherwise divergent chemical compounds, but with the molecular details poorly understood. The identification of the pentamidine/melarsoprol transporter has been a serendipitous process. Initial studies in cross-resistance in laboratory strains (Bernhard et al., 2007; Bridges et al., 2007; Graf et al., 2015a) and field isolates (Shahi et al., 2002; Alsford et al., 2012) from relapsed patients identified the gene encoding for the purine transporter responsible for drug uptake as T. brucei adenosine transporter 1 (TbAT1). In addition to TbAT1, the high-affinity pentamidine transporter (HAPT1) (Bernhard et al., 2007) as well as the ATP-binding cassette transporter MRPA (Baker et al., 2012) were also proposed to mediate drug resistance by various mechanisms, but neither explained the drug resistance levels observed in field isolates (Baker et al., 2013).

Using genome-wide RNAi-mediated genetic screening and functional assays, the locus encoding the closely related AQP2 and AQP3 was identified as a bona fide hit for pentamidine/melarsoprol cross-resistance (Graf et al., 2015b). Further biochemical and genetic manipulation studies demonstrated that deletion of AQP2, but not AQP3, led to a significant increase in the EC$_{50}$ of both compounds, mirroring the behaviour observed in previously generated laboratory strains and field isolates (Munday et al., 2014; Graf et al., 2015b; Song et al., 2016). Other observations such as localization to the flagellar pocket in the bloodstream form (Munday et al., 2014; Graf et al., 2015b; Song et al., 2016; Quintana et al., 2020), as well as the unusual pore structure discussed above, led to the hypothesis that pentamidine and melarsoprol are likely to interact with high affinity to AQP2 located in the flagellar pocket (Alghamdi et al., 2020), posing the question of how these compounds are internalized and also the mechanisms for resistance.

Endocytosis or membrane uptake: competing models for drug entry

Suggesting that the role of a channel protein is not the primary mechanism for pentamidine to access the trypanosome cytoplasm may seem to be a straw man, but this possibility has been proposed. Specifically, as AQP2 binds pentamidine with high affinity at the first selectivity pore, the possibility that AQP2 is a receptor for uptake by endocytosis is not unreasonable (Fig. 2) and could act as a parallel to ISG75-mediated uptake of suramin (Graf et al., 2015b). This model was further supported by reports demonstrating that pentamidine binds AQP2 with nanomolar affinity, thus
potentially acting as a highly selective inhibitor of AQP2 (Fig. 2) (Alghamdi et al., 2020). However, consideration of structural features of the pore do support TbAQ2 acting as a channel for larger and more structurally flexible solutes including pentamidine (Petersen and Beitz, 2020). In the endocytosis model, ubiquitination of TbAQ2 at the flagellar pocket is central for subsequent ubiquitination-mediated intracellular trafficking and delivery to intracellular organelles such as the lysosome. Indeed, TbAQ2 forms a stable homomultimeric complex in the flagellar pocket where ubiquitination is likely to take place on individual monomers (Quintana et al., 2020).

The opposing membrane uptake model proposes that pentamidine, and potentially melarsoprol, are taken up via the intrinsic channel properties of TbAQ2. Indeed, a recent report demonstrates that drug permeation is possible due to a highly conserved amino acid motif in the central pore architecture of TbAQ2, facilitating the passage of high molecular weight solutes (Alghamdi et al., 2020). This was demonstrated by TbAQ3 mutants containing the amino acids of the selectivity pore from TbAQ2 possessing increased capacity for pentamidine uptake (Alghamdi et al., 2020). Moreover, pentamidine permeation through TbAQ2 appears to be further aided by the intrinsic membrane potential and is not abrogated by partially blocking endocytic uptake (Alghamdi et al., 2020; Quintana et al., 2020), albeit at a rate that is considerably slower than for lower molecular weight solutes, which in essence implies a leak in the AQ2 permeability barrier.

Concerning the likely site for pentamidine uptake, there is no evidence that endocytosis or post-translational modification of AQP2 is required. Specifically, additional genes identified from the genome-wide RNAi screen identified a kinase and phosphatase for melarsoprol and pentamidine respectively, as well as one unique hypothetical each (Alford et al., 2012). None of these genes have evidence for roles in ubiquitylation, endocytosis or trafficking in general, suggesting that translocation of drugs from the surface is sufficient for toxicity and that blocking ubiquitylation or endocytosis does not offer resistance. However, it needs to be acknowledged that a role for endocytosis that is overshadowed by the channel-mediated mechanism, remains a possibility.

**Stability and folding of TbAQ2 contribute to pentamidine resistance**

In common with most membrane proteins, AQPs undergoing translation are inserted into the endoplasmic reticulum through the Sec61 translocon and assisted in folding via a cohort of chaperones (Pitonzo and Skach, 2006). Given that most AQPs are also glycoproteins it is likely that the calnexin/calreticulin quality control system is involved in monitoring quality and rapidity of folding. Importantly, formation of homotetrameric complexes is important for AQP stability and the formation of heterotetrameric complexes has not been observed (Duchesne et al., 2002; Furman et al., 2003). The residues responsible for this specificity are not clear, but AQP tetramers can assemble into higher order quasi-crystalline arrays (Kitchen et al., 2016). Furthermore, there are clear differences in the stabilities of the water and solute permeable AQP tetramers with the former exhibiting greater stability than the latter and likely due to features within the final two trans-membrane domains and loops D and E (Lagreé et al., 1998; Duchesne et al., 2002; Buck et al., 2007; Kitchen et al., 2016), albeit with the functional consequences, if any, unclear. Significantly the folding pathway is not identical for all AQPs, being controlled at least partly by sequences within the second trans-membrane domain (Carrington et al., 2010). Finally, mammalian AQPs are both phosphorylated and ubiquitylated, with at least the latter contributing to protein turnover, endocytosis and quality control (Kamsteeg et al., 2006; Mandal et al., 2012; Sharma et al., 2015; Quintana et al., 2020). Although it is most likely that similar pathways operate in trypanosomes, with direct evidence for ubiquitylation and most of the relevant folding chaperones present, the precise mechanisms of AQP maturation, at least in African trypanosomes, remain to be investigated in detail (Field et al., 2010; Tiengwe et al., 2016a, 2016b).

To understand folding, stability and trafficking of AQP2 in *T. brucei* we examined sequence-dependence and trans-membrane domain exchange designed to mimic natural AQ2/3 chimeras expressed in a triple null background (Peacock et al., 2017; Quintana et al., 2020). TbAQ2 forms both tetramers and tetramers of tetramers and is degraded in the lysosome by a ubiquitin-dependent process (Fig. 2) (Quintana et al., 2020). Attempts to influence ubiquitylation by mutating cytoplasmic lysine residues unexpectedly reduce stability rather than preventing lysosomal targeting (Quintana et al., 2020). This is due to reduced folding and tetramerization efficiency, which triggers ER-associated degradation, indicating a failure to complete quality control (Quintana et al., 2020). Perhaps the most significant finding is that chimerical TbAQ2/3 proteins also lead to impaired folding and reduced stability (Quintana et al., 2020). This was also the case for constructs mimicking chimeras found in trypanosomes from patients where pentamidine treatment had failed.
Clearly rigorous quality control mechanisms operate within the ER of *T. brucei*, but with a consequence that mutations in the non-essential AQPs can render parasites refractory to treatment. Moreover, the instability of AQP2 is likely an underlying cause of pentamidine treatment failure while the production of chimeric forms is potentially a high frequency event and stems directly from generation of contiguous paralogues initially derived by gene duplication; presumably the chimeras have poor folding capability due to mismatch between the N- and C-terminal regions.

**Concluding remarks**

Remarkable advances to understanding mechanisms for classical therapies against African trypanosomiases, as well as development of new drugs and the successes of public health programmes, auger well for the control of both human and animal African trypanosomiasis. Remarkably, we now have considerable understanding of pentamidine and melarsoprol uptake as well as mechanisms for resistance. The evolutionary history of trypanosome AQPs reveals both how pentamidine sensitivity arose, with a specifically broad-spectrum AQP2, and resistance arising from recombination. Placed in context (Fig. 3) the millennia-old relationship between trypanosomes and humans has been complex, with periods where one organism had the upper hand and then the other. Recently, humans have been in the ascendant, with case numbers having dropped precipitously and even exceeding the WHO roadmap predictions. Indeed, several countries previously considered endemic have reported no cases for several years. It can only be hoped that the advances made in the last decade are not eroded by the COVID-19 pandemic, which threatens to undermine global progress on many fronts. The successes of public health programmes, of new drugs and the relationships of leishmania donovani aquisopors shows presence of subcellular aquaporins similar to tonoplast intrinsic proteins of plants. *PLoS* ONE 6, e24820.

Barrett MP, Boykin DW, Brun R and Tidwell RR (2007) Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *British Journal of Pharmacology* 152, 1155–1171.

Beit E (2005) Aquaporins from pathogenic protozoan parasites: structure, function and potential for chemotherapy. *Biological of the Cell* 97, 373–383.

Bernhardt SC, Nerima B, Mäser P and Brun R (2007) Melarsoprol- and pentamidine-resistant *Trypanosoma brucei* rhodesiense populations and their cross-resistance. *International Journal for Parasitology* 37, 1443–1448.

Biyani N, Mandal S, Seth C, Saint M, Natarajan K, Ghosh I and Madhubala R (2011) Characterization of leishmania donovani aquisopors shows presence of subcellular aquaporins similar to tonoplast intrinsic proteins of plants. *PLoS* ONE 6, e24820.

Bray PG, Barrett MP, Ward SA and De Koning HP (2003) Pentamidine uptake and resistance in pathogenic protozoa: past, present and future. *Trends in Parasitology* 19, 232–239.

Bridges DJ, Gould MK, Nerima B, Mäser P, Burchmore RJ and de KH (2007) Loss of the high-affinity pentamidine transporter is responsible for high levels of cross-resistance between arsical and diamidines in African trypanosomiasis. *Molecular Pharmacology* 71, 1098–1108.

Buck TM, Wagner J, Grund S and Skach WR (2007) A novel tripartite motif involved in aquaporin topogenesis, monomer folding and tetramerization. *Nature Structural & Molecular Biology* 14, 762–769.

Burri C, Balz T, Giroud C, Doua F, Welker HA and Brun R (1993) Pharmacokinetic properties of the trypanocidal drug melarsoprol. *Chemotherapy* 39, 225–234.

Burri C, Onyango JD, Auma JE, Burudi EM and Brun R (1994) Pharmacokinetics of melarsoprol in uninfected vetex monkeys. *Acta Tropica* 58, 35–49.

Calcino AD, De Oliveira AL, Simakov O, Schwaala T, Zieger E, Wollesen T and Wanninger A (2019) The quagga mussel genome and the evolution of freshwater tolerance. *DNA Research* 26, 411–422.

Calvanese L, D'Auria G, Vangone A, Falcigno L and Oliva R (2018) Structural basis for mutations of human aquaporins associated to genetic diseases. *International Journal of Molecular Sciences* 19, 1577.

Carrington M, Field MC, Sergeanton T, Wang Y and Bo S (2010) Chaperone requirements for biosynthesis of the trypanosome variant surface glycoprotein. *PLoS* ONE 5, e8648.

de Groot BL and Grubmuller H (2001) Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and OLP. *Science* (New York, N.Y.) 294, 2353–2357.

Denise H and Barrett MP (2001) Uptake and mode of action of drugs used against sleeping sickness. *Biochemical Pharmacology* 61, 1–5.

Duchesne L, Pellerin I, Delamarche C, Deschamps S, Lagrée V, Froger A, Bonnek G, Thomas D and Hubert JF (2002) Role of C-terminal domain and transmembrane helices 5 and 6 in function and quaternary structure of major intrinsic proteins: analysis of aquaporin/glycerol facilitator chimeric proteins. *Journal of Biological Chemistry* 277, 20598–20604.

Fairlamb AH and Horn D (2018) Melarsoprol resistance in African trypanosomiasis. *Trends in Parasitology* 34, 481–492.

Fairlamb AH, Henderson GB and Cerami A (1989) Trypanothione is the primary target for arsenical drugs against African trypanosomes. *PNAS* 86, 2607–2611.

Field MC, Sergeanton T, Wang YN, Böhm S, Carrington M, Field MC, Sergeanton T, Wang YN and Bo S (2010) Chaperone requirements for biosynthesis of the trypanosome variant surface glycoprotein. *PLoS* ONE 5, e8648.

Field MC, Horn D, Fairlamb AH, Ferguson MAJ, Gray DW, Read KD, De Ruyck M, Torrie LS, Wyatt PG, Wylie S and Gilbert IH (2017) Anti-trypanosomatid drug discovery: an ongoing challenge and a continuing need. *Nature Reviews Microbiology* 15, 217–231.

Figarella K, Uzcátegui NL, Zhou Y, LeFurgey A, Ouellette M, Bhattacharjee H and Mukhopadhyay R (2007) Biochemical characterization of *Leishmania major* aquaglyceroporin LmAQP1: possible role in volume function and potential for chemotherapy. *Biology of the Cell* 97, 383–397.

Finn RN, Chauvigné F, Hildberg JB, Cutler CP and Cerda J (2014) The lineage-specific evolution of aquaporin gene clusters facilitated tetrapod terrestrial adaptation. *PLoS* ONE 9, 1–38.

Furman CS, Gorelick-feldman DA, Davidson KGV, Yasumura T, Neely JD, Aquarelli T, Koenig F, Belcher J, Mili MG and Hogg S (2009) Helix-loop-helix DNA motif and site-specific DNA methylation in trypanosome regulatory sequences. *Proc Natl Acad Sci U S A* 106, 10064–10069.

Gould MK and Schnaufer A (2014) Independence from kinetoplast DNA actions of M1 and M23 isoforms. *Molecular Microbiology* 93, 71–81.

Hancock AW, Etherington M, Hulme D, Lockman G, Underwood R, Cooper T and Beales PL (2011) Chaperone requirements for biosynthesis of the trypanosome variant surface glycoprotein. *PLoS* ONE 6, e25055.
Trypanosoma brucei in vitro. Antimicrobial Agents and Chemotherapy 58, 2925–2928.

Graf FE, Baker N, Munday JC, de Koning HP, Horn D and Mäser P (2015a) Chimerization at the AQP2-AQP3 locus is the genetic basis of melarsoprol-pentamidine cross-resistance in clinical Trypanosoma brucei gambiense isolates. International Journal for Parasitology: Drugs and Drug Resistance 5, 65–68.

Graf FE, Baker N, Munday JC, De Koning HP, Horn D and Mäser P (2015b) Chimerization at the AQP2 – AQP3 locus is the genetic basis of melarsoprol – pentamidine cross-resistance in clinical Trypanosoma brucei gambiense isolates. International Journal for Parasitology: Drugs and Drug Resistance 5, 65–68.

Ishibashi K, Kondo S, Harra S and Morishita Y (2011) The evolutionary aspects of aquaporin family. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 300, 566–577.

Ishibashi K, Tanaka Y and Morishita Y (2020) Perspectives on the evolution of aquaporin superfamily. 112, 1-27.

Jeacock L, Baker N, Wiedemar N, Mäser P and Horn D (2017) Aquaglyceroporin-null trypanosomes display glycerol transport defects and respiratory inhibitor sensitivity. PLoS Pathogens 13, 1–16.

Kamsteeg EE-JEJ, Hendriks G, Boone M, Konings IBM, Oorschot V, van der Suijs P, Klumperman J, Deen PMT, Van Der Suijs P, Klumperman J and Deen PMT (2006) Short-circuit ubiquitination mediates the regulated endocytosis of the aquaporin-2 water channel. Proceedings of the National Academy of Sciences 103, 18344–18349.

Keiser J, Ericsson O and Burri C (2000) Investigations of the metabolites of the trypanocidal drug melarsoprol. Clinical Pharmacology & Therapeutics 67, 478–488.

Kennedy PGE and Rodgers J (2019) Clinical and neuropathogenetic aspects of human African trypanosomiasis. Frontiers in Immunology 10, 1–11.

King LS, Kozono D and Agre P (2014) From structure to disease: the evolving tale of aquaporin biology. Nature Reviews Molecular Cell Biology 5, 679–687.

Kitchen P, Conner MT, Bill RM and Conner AC (2016) Structural determinants of oligomerization of the aquaporin-4 channel. The Journal of Biological Chemistry 291, 6858–6871.

Lagréve V, Froger A, Deschamps S, Pellerin I, Delamarche C, Bonnet G, Gouranton J, Thomas D and Hubert JF (1998) Oligomerization state of water channels and glycerol facilitators: involvement of loop E. Journal of Biological Chemistry 273, 33949–33953.

Lin YC, Hsu JY, Shu JH, Chi Y, Chiang SC and Lee ST (2008) Two distinct arsenic-resistant variants of Leishmania amazonesis take different routes to achieve resistance as revealed by comparative transcriptomics. Molecular and Biochemical Parasitology 162, 16–31.

Maclean I, Reiber H, Kennedy PGE and Sternberg JM (2012) Stage progression and neurological symptoms in Trypanosoma brucei rhodesiense sleeping sickness: role of the CNS inflammatory response. PLoS Neglected Tropical Diseases 6, e1857.

Mandal G, Sharma M, Kruse M, Sander-Juelch C, Munro LA, Wang Y, Vilg JV, Tamás MJ, Bhattacharjee H, Wiese M and Mukhopadhyay R (2012) Modulation of Leishmania major aquaglyceroporin activity by a mitogen-activated protein kinase. Molecular Microbiology 85, 1204–1218.

Marquis N, Gourbal B, Rosen BP, Mukhopadhyay R and Ouellette M (2005) Modulation in aquaglyceroporin AQP1 gene transcript levels in drug-resistant Leishmania. Molecular Microbiology 57, 1690–1699.

Mäser P, Sütterlin C, Kraji A and Kaminsky R (1999) A nucleoside transporter from Trypanosoma brucei involved in drug resistance. Science (New York, N.Y.) 285, 242–244.

Mathis AM, Holman JL, Sturk LM, Ismail MA, Boykin DW, Tidwell RR and Hall JE (2006) Accumulation and intracellular distribution of antitypanosomal diamidine compounds DB75 and DB820 in African trypanosomes. Antimicrobial Agents and Chemotherapy 50, 2185–2191.

Montalvetti A, Rohloff P and Docampo R (2004) A functional aquaporin co-localizes with the vacuolar proton pyrophosphatase to acidocalcisomes and the contractile vacuole complex of Trypanosoma cruzi. Journal of Biological Chemistry 279, 38673–38682.

Munday JC, Ece AA, Baker N, Glover I, Lucass C, Andrés DA, Natto MJ, Teka IA, Maldonad J, Lee RS, Graf FE, Ludin P, Burchmore RJ, Turner CMR, Tait A, Macleod A, Mäser P, Barrett MP, Horn D and De Koning HP (2014) Trypanosoma brucei aquaglyceroporin 2 is a high-affinity transporter for pentamidine and melaminophenyl arsenic drugs and the main genetic determinant of resistance to these drugs. Journal of Antimicrobial Chemotherapy 69, 651–663.

Munday JC, Settimo I and de Koning HP (2015) Transport proteins determine drug sensitivity and resistance in a protozoan parasite, Trypanosoma brucei. Frontiers in Pharmacology 6, 1–10.

Nishihara E, Yokota E, Tazaki A, Orih H, Katsuchara M, Katoaka K, Igarashi H, Moriyama Y and Seiji TS (2012) Presence of aquaporin and V-ATPase on the contractile vacuole of Amoeba proteus. Biology of the Cell 100, 179–188.

Petersen LM and Beitz E (2020) The ionophores CCCP and gramicidin but not nigericin inhibit Trypanosoma brucei aquaglyceroporins at neutral pH. Cells 9, 2335.

Pitonzo D and Skach WR (2006) Molecular mechanisms of aquaporin bio-genesis by the endoplasmic reticulum Sec61 translocon. Biochimica et Biophysica Acta – Molecular Cell Research 1758, 976–988.

Preston GM, Carroll TP, Guggin WB and Agre P (1992) Appearance of water channels in xenopus oocytes expressing red cell CHIP28 protein. Science (New York, N.Y.) 256, 26–28.

Quintana JF, Bueren-Calabuig J, Zuccotto F, de Koning HP, Horn D and Field MC (2020) Instability of aquaglyceroporin (AQP) 2 contributes to drug resistance in Trypanosoma brucei. PLoS Neglected Tropical Diseases 14, 1–26.

Quintana JF, Bueren-Calabuig J, Zuccotto F, De Koning HP, Horn D and Field M C (2020) Instability of aquaglyceroporin (AQP) 2 contributes to drug resistance in Trypanosoma brucei. PLoS Neglected Tropical Diseases 14, 1–26.

Shahi SK, Krauth-Siegel RL and Clayton CE (2002) Overexpression of the putative thiol conjugate transporter TmMRPA causes melarsoprol resistance in Trypanosoma brucei. Molecular Microbiology 43, 1129–1138.

Sharma M, Mandal G, Mandal S and Bhattacharjee H (2015) Functional role of Y2see in Leishmania major AQPI. Molecular and Biochemical Parasitology 201, 139–145.

Shi Z, Zhang L, Luo I, Zhao H, Cheng J, Xiang J and Zhao C (2012) Aquaporins in human breast cancer: identification and involvement in carcinogenesis of breast cancer. Journal of Surgical Oncology 106, 267–272.

Song J, Baker N, Rother M, Henke B, Jeacock L, Horn D and Beitz E (2016) Pentamidine is not a permeant but a nanomolar inhibitor of the Trypanosoma brucei aquaglyceroporin-2. PLoS Pathogens 12, 1–14.

Steverding D (2010) The development of drugs for treatment of sleeping sickness: a historical review. Parasit Vectors, 3, 15.

Stewart ML, Burchmore RJS, Clucas C, Hertz-Fowler C, Brooks K, Tait A, MacLeod A, Turner CMR, de Koning HP, Wong PE and Barrett MP (2010) Multiple genetic mechanisms lead to loss of functional TbAT1 expression in drug-resistant trypanosomes. Eukaryotic Cell 9, 336–343.

Tiengwe C, Muratore KA and Bangs JD (2016a) Variants surface glycoprotein, transferrin receptor, and ERAD in Trypanosoma brucei. Cell Microbiology 18, 1673–1688.

Tiengwe C, Muratore KA and Bangs JD (2016b) Surface proteins, ERAD and antigenic variation in Trypanosoma brucei. Cellular Microbiology 18, 1673–1688.

Tong H, Hu Q, Zhu L and Dong X (2019) Prokaryotic aquaporins. Cells 8, 1–18.

Uzcategui NL, Szallies A, Pavlovic-Djurancic S, Palomada M, Figarella K, Boehmer C, Lang F, Beitz E and Duszenko M (2004) Cloning, heterologous expression, and characterization of three aquaglyceroporins from Trypanosoma brucei. Journal of Biological Chemistry 279, 42669–42676.

Verkman AS, Anderson MO and Papadopoulos MC (2014) Aquaporins: important but elusive drug targets. Nature Reviews Drug Discovery 13, 259–277.

von Bülow J and Beitz E (2015) Number and regulation of protozoal aquaporins reflect environmental complexity. Biological Bulletin 229, 38–46.

von Bülow J, Müller-Lucks A, Kais L, Bernhards F and Beitz E (2012) Functional characterization of a novel aquaporin from Dictyostelium discoideum amoebae implies a unique gating mechanism. Journal of Biological Chemistry 287, 7474–7479.

Yool AJ, Brown EA and Flynn GA (2010) Roles for novel pharmacological blockers of aquaporins in the treatment of brain oedema and cancer. Clinical and Experimental Pharmacology and Physiology 37, 403–409.