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Review

Parasite aquaporins: Current developments in drug facilitation and resistance☆

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ABSTRACT

Background: Although being situated in a niche, research on parasite aquaporins is a lively field that has provided new insight into basic aquaporin structure–function relationships and physiological roles of water and solute transport. Moreover, it bears the potential to find novel approaches to antiparasitic chemotherapy.
Scope of review: Here, we summarize the current knowledge about the structure and substrate selectivity of aquaporins from protozoan and helminth parasites, review the current views on their physiological roles, and discuss their potency for chemotherapy.
Major conclusions: Parasite aquaporins fulfill highly diverse tasks in the physiology of the various organisms, yet their general protein structure is well conserved. Aquaporins are directly (antimonials) and indirectly (melarsoprol, pentamidine) linked to the uptake of antiparasitic drugs. Unfortunately, drug-like aquaporin inhibitors are still missing.
General significance: Aquaporins expression levels determine the degree of parasite resistance against certain drugs. Further studies on parasite aquaporins may provide data about overcoming drug resistance mechanisms or even spark novel treatments. This article is part of a Special Issue entitled Aquaporins.

1. Introduction

A parasite is an organism that lives in and draws nutrition from a host organism. Parasites are classified into two main categories: ecto-parasites (living in contact with the host but outside of the host organism) and endo-parasites (living inside the host's body, e.g. in the digestive tract, in the blood, or in certain tissues). Endo-parasites can be further divided into protozoa (microscopic, one-celled organisms) and helminths (large, multicelluar organisms). Protozoan parasites are the infectious agents of devastating diseases worldwide, such as malaria, African trypanosomiasis, Chagas' disease, leishmaniasis, and toxoplasmosis. Helminth parasites cause severe tissue infections, such as schistosomiasis and fasciolosis.

The arsenal of chemotherapeutic drugs against parasite infections remains quite limited. This is in part due to the parasite's diverse and complex life styles and their cycling between an invertebrate vector and one or more vertebrate hosts, connected with changing metabolic profiles [1]. Further, parasites develop rapidly with short generation times and, hence, propagate the generation and spreading of drug-resistant strains [2].

The increasing number of parasite genome projects has identified and highlighted various genes coding for proteins of potential value as drug targets, with a special focus on integral membrane proteins at the host–parasite interface [3]. The interface fulfills essential physiological functions for the parasite, including adhesion to a host cell during invasion [4], coping with osmotic stress by rapid water transport [5], uptake of nutrients [6], release of metabolic end-products [7], and secretion of toxic proteins [8]. For antiparasitic drugs, whose targets are located within the parasite cytosol, the parasite plasma membrane forms the final barrier and also can be regarded as a drug delivery system. The selective pressure during parasite–host co-evolution has optimized the interface proteins with regard to their structure and function [9].

Aquaporins (AQPs) constitute an ancient family of transmembrane channel proteins. They are present in virtually all organisms [10] and have been thoroughly characterized with respect to their structure–function relationships since their discovery 20 years ago. However, questions regarding the physiological roles of AQPs or design and discovery of potent and selective inhibitors, remain challenging. The AQP family comprises water-specific channels (orthodox aquaporins), and channels that additionally facilitate transport of glycerol, urea, ammonia or other small, uncharged molecules (e.g. aquaglyceroporins). Water and solute flux through AQPs depends on the presence of osmotic or chemical gradients, which concomitantly define the direction of transport [11].

The elucidation of the physiological roles of mammalian as well as plant AQPs is a thriving topic of research and the work has been

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Table 1
Parasite AQPs discussed in this review.

	Phylum	Species	AQP
Protozoa	*Apicomplexa*	*Plasmodium falciparum*	PfAQP
		Toxoplasma gondii	TgAQP
	Kinetoplastida	*Trypanosoma brucei*	TbAQP1–3
		Trypanosoma cruzi	TcAQP, TcAQPβ–δ
		Leishmania major	LmAQP1, LmAQPα–δ
Helminthes	*Platyhelminthes*	*Fasciola gigantica*	FgAQP1–2
		Schistosoma mansoni	SmAQP

intensively reviewed (see for instance [12]). The functions of parasite AQPs are less well understood. Here, we summarize the current views and knowledge about parasite AQPs (Table 1) mainly with respect to the structural and functional context and discuss their proven and putative relations to chemotherapeutic treatments, which can be either direct as a drug target or indirect as a facilitator for the uptake or release of drugs.

2. Structural peculiarities of parasite AQPs

AQPs form homotetrameric complexes with each monomer functioning independently as a water/solute channel (see Fig. 1 for visualization of structural details). Each monomer is made of six transmembrane helices and two short half-helices, which dip into the membrane from either side to form a seventh pseudo-transmembrane span. Two highly conserved constrictions in the pore region determine the selectivity of aquaporins (Fig. 1) [13,14]. The constriction at the pore mouth is termed aromatic arginine (ar/R) constriction or selectivity filter [15]. It selects permeants by size and by their electrostatic nature. The ar/R constriction of water specific AQPs is narrow and consists of polar amino acid residues offering optimal hydrogen bond acceptor and donor sites for isolating a water molecule from the bulk. Aquaglyceroporins contain a wider and amphipathic ar/R constriction providing hydrogen bonds only on the hydroxyl side of a glycerol molecule and lipophilic interactions on the alkyl back [16]. The ar/R constriction further co-constitutes the exquisite AQP proton filter together with a second filter region, i.e. the Asn-Pro-Ala (NPA) region [14,16–19]. The NPA motifs are located at the positive ends of two highly conserved half-helices, which act as macro-dipols, and reside in the center of the channel [20]. The Asn residues act as hydrogen donors to the oxygen atoms of passing permeants and also form the filter against inorganic cations [14,17,21].

The aquaglyceroporin from *Plasmodium falciparum* (PfAQP) is the best-characterized parasite AQP. Unlike its close structural homolog from *E. coli* (GlpF), PfAQP not only conducts glycerol but also water at high rates [22–25]. To elucidate the structural peculiarities of PfAQP that are responsible for this functional difference a series of in vivo, in vitro, and *in silico* experiments has been conducted.

Sequence comparison shows almost identical residues in both filter regions of PfAQP and GlpF. While the ar/R constriction of PfAQP and GlpF is composed of the same set of residues (Arg, Trp, and Phe), the NPA motifs of PfAQP are slightly changed to Asn-Leu-Ala (NLA) and Asn-Pro-Ser (NPS). Yet, mutation of the two varying amino acids back to canonical NPA motifs did not alter the water or glycerol permeability of PfAQP [22].

Later, a so-far neglected structural element of the AQPs was linked to the special permeability properties of PfAQP, i.e. the extracellular connecting loop C, which folds into the outer pore vestibule and lies just above the ar/R region. Specifically, a Glu (E125) was identified to be responsible for the high water permeability of PfAQP [23]. The mutation of E125 to Ser, i.e. the residue present at the corresponding position of GlpF, largely abolished water permeability with only little effect on glycerol permeability [23]. The mutation further resulted in an increase of the activation energy by about 3 kcal mol^{-1} and it was suggested that a change in the hydrogen bond network around the pore Arg might cause stronger binding of a passing water molecule and, thus, slower passage. Further evidence for this interpretation came from the 2.05-Å resolution crystal structure of PfAQP (Fig. 1) [25], which shows exactly this: the pore Arg of PfAQP is linked to neighboring residues by four hydrogen bonds, whereas GlpF exhibits only three hydrogen bonds. This free valence in GlpF is thought to enhance binding of a passing water molecule and to considerably increase its residence time in the ar/R filter resulting in the observed low water permeability rate.

The crystal structure also indicated a higher degree of decoration of PfAQP with glycerol molecules, especially within the extracellular vestibule where two glycerol molecules are located in direct vicinity to each other (Fig. 1) [25]. This finding was used to explain discrepant data on water permeability of PfAQP, which was obtained by different research groups and which had confused discussions of the matter. It was found that contrary to GlpF, water permeability of PfAQP strongly depends on the nature of the external osmolyte. PfAQP exhibited high water permeability using salt [22] or saccharose gradients [25], whereas no water permeability above background was seen when sorbitol buffers were used [26]. A screening of various solutes led to the conclusion that sorbitol (effectively representing two linked glycerol molecules) could take the place of the two glycerol molecules bound to the extracellular vestibule that are visible in the crystal structure and, hence, interfere with channel water permeability.

In the in vivo situation, PfAQP is exposed to a mixture of different physiological solutes. A simple functional assay was created to test the permeability of mixtures of glycerol and urea [27]. Under isotonic conditions, any ratio of glycerol and urea passed PfAQP equally well, whereas in a hypertonic buffer where the solute needs to diffuse in a countercurrent against water, glycerol was clearly preferred over urea [27]. Simulation of molecular dynamics and energy calculations of solute permeation through PfAQP equally indicate that PfAQP should conduct glycerol at higher rates than urea [28]. Other theoretical models showed that already micromolar concentrations of glycerol binding to PfAQP should inhibit water permeation [29]. Together, the experimental and theoretical analyses strongly suggest that the structure of PfAQP is particularly optimized for glycerol facilitation and, accordingly, a main task of PfAQP should be in the uptake of glycerol as a precursor for glycerolipid biosynthesis in the malaria parasite [30]. Deletion of the AQP encoding gene in the rodent malaria parasite *Plasmodium berghei* eventually confirmed this hypothesis because this strain has lost its capability of transmembrane glycerol transport [31]. These results further show that individual physiological functions for a parasite cell can be deduced from in-depth in vitro analyses of AQP permeation properties in combination with in vivo studies on the protein localization and expression profiles during development.

Unfortunately, there is no crystal data available of other parasite AQPs. Accordingly, any structural peculiarities must be inferred from sequence comparison. Considering the large effect on PfAQP water permeability of slight changes in the hydrogen bond network around the pore Arg residue, such an attempt can only be crude. In this regard it is notable that the *T. gondii* AQP and one AQP from *T. brucei*, TbAQP2, do not contain an arginine in the ar/R region at all but lipophilic alkyl residues instead, i.e. a valine and a leucine, respectively. Indeed, both showed considerable water permeability. Effects on their proton and cation filter capability are not reported.

3. Physiological functions of parasite aquaporins

Here, we can focus only on certain aspects of parasite physiology. For more detailed information about the biology (morphology, life cycles, metabolism etc.) of the parasites described below, the reader is kindly asked to consult respective literature.

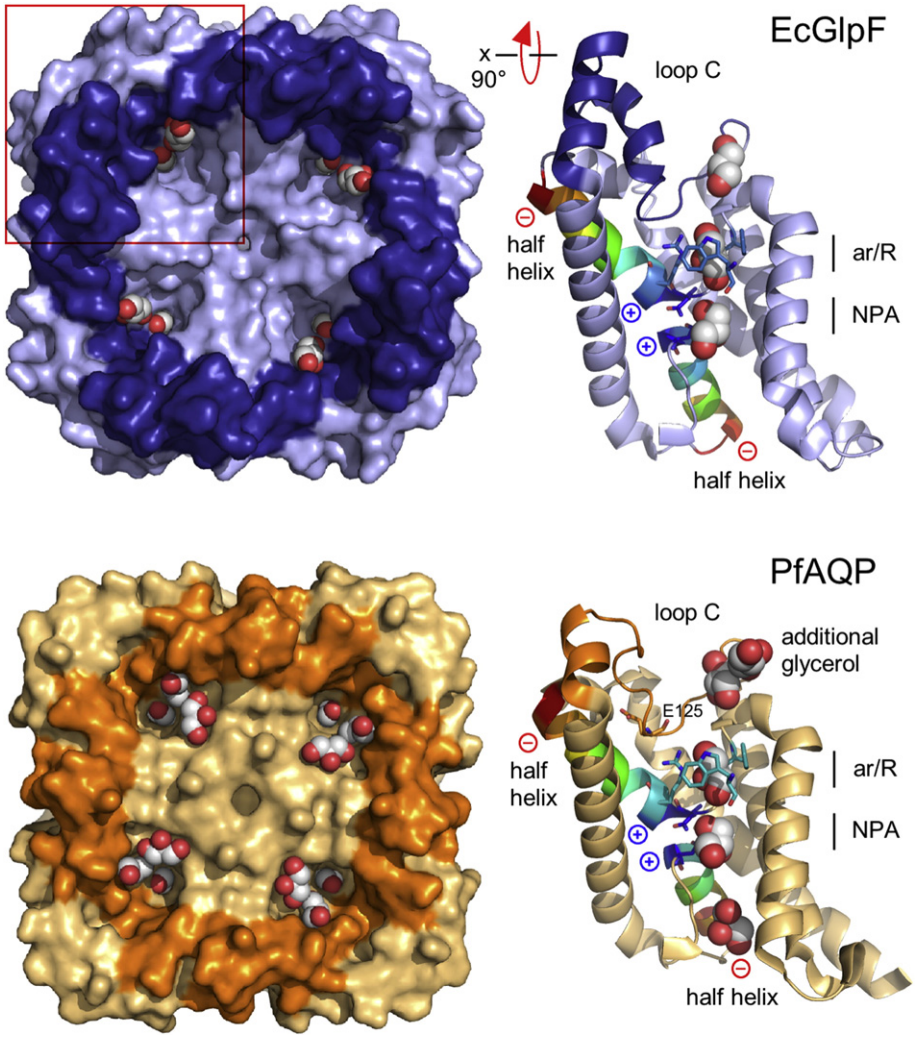

Fig. 1. Protein structures of the aquaglyceroporins from *E. coli*, EcGlpF (top; PDB ID: 1FX8), and *Plasmodium falciparum*, PfAQP (bottom; PDB ID: 3C02). Shown are surfaces of functional tetramers (left) with the extracellular connecting loops C labeled in a darker color and bound glycerol molecules as spheres. The cartoons of EcGlpF and PfAQP monomers on the right indicate structural details; the transmembrane domains 1 and 2 were removed to open up the view on the channel interior. The AQP-typical half helices are shaded in rainbow colors to visualize the macro-dipolmoment, respective partial charges at the helix ends are indicated. Residues of the constriction sites (aromatic arginine constriction, ar/R; Asn-Pro-Ala region, NPA) are drawn as sticks. Compared to EcGlpF, the PfAQP structure contains two additionally bound glycerol molecules, one at the extracellular and one at the intracellular opening of the vestibules.

3.1. Plasmodium aquaporins

Genome analyses of various malaria parasite species indicate a reduced set of membrane proteins [2]. It fits into the scheme that the *Plasmodium* genome carries a single aquaporin gene [5]. PfAQP is constitutively expressed during all stages of the parasite's asexual developmental cycle as well as in the sexual gametocyte form and is localized to the plasma membrane [22]. PfAQP is a bifunctional channel facilitating water and glycerol [22] with the caveat, as pointed out above, that under physiological conditions, i.e. in the presence of glycerol, water permeability may be low. Besides glycerol, several other physiological solutes, such as sugar alcohols, urea, methylglyoxal, and ammonia were found to be substrates of PfAQP [22,32,33]. Plasmodia proliferate rapidly in the blood stage and require a high rate of lipid biosynthesis for cell membrane biogenesis. For this purpose, the parasites efficiently use glycerol from the host serum for the production of glycerolipids [31]. PfAQP exhibits the necessarily high glycerol permeability to fulfill this task [22]. Additionally, blood-stage plasmodia massively consume energy; metabolism in the main growth phase peaks at a 100-fold increased rate compared to that of uninfected red blood cells [30]. During this process, a large amount of toxic and waste metabolites must be eliminated probably via PfAQP, which exhibits high permeability for methylglyoxal (derived as a side-product from glycolysis) [33], urea (from arginine degradation via ornithine) [22], and ammonia (conversion of amino acids to α-ketocarbonic acids) [32].

3.2. Toxoplasma aquaporins

Toxoplasmosis is a disease caused by the obligate intracellular parasite *Toxoplasma gondii*. Similar to plasmodia, which constitutively express a single AQP, the *T. gondii* genome encodes a single classical AQP, TgAQP [34]. Further, a second *T. gondii* gene with some resemblance of AQPs has been annotated as TgAQP2. The curious point about this gene is its obvious duplication resulting in a tandem AQP sequence. The deduced protein sequence contains four NPA motifs of which only the second and third are canonical, whereas the first (NPI) and fourth (NVQ) deviate (www.uniprot.org/uniprot/Q6WN50). Accordingly, the tandem TgAQP2 protein would be expected to form homodimers rather than tetramers in order to assume the AQP-typical intra-membrane structure. The literature does not provide any information on the TgAQP2 expression or functionality. TgAQP1 is a

bifunctional aquaporin with intermediate water and high glycerol permeability [34]. Further, polyols of up to five carbon atoms in length and urea [34] as well as other nitrogen metabolites [32] pass TgAQP1. Phylogenetic analysis shows a surprisingly high similarity of TgAQP1 with plant AQPs of the tonoplast intrinsic protein family, TIPs [34], which are found in lytic vacuoles [35]. Apicomplexans in general express various plant-like proteins, which are thought to derive from an endosymbiosis event in which a predecessor cell ingested a green algae. Recently, a plant-like vacuole in *T. gondii* has been described [36] and TgAQP1 has been shown to be located in the membrane of this organelle, which seems to be part of the endosomal/lysosomal pathway of the parasite [36].

3.3. Trypanosome aquaporins

The genus *Trypanosoma* comprises two major parasitic species, namely *T. cruzi* and *T. brucei*. *T. cruzi* is the causative agent of Chagas' disease in South America whereas *T. brucei* causes human African trypanosomiasis (HAT).

Three AQPs have been reported in *T. brucei*, TbAQP1, TbAQP2, and TbAQP3 [37]. All belong to the aquaglyceroporin subfamily and show high water and glycerol permeability [37]. The higher number in AQPs expressed in these and other extracellular parasites may be due to their direct exposure to the environment and the need for higher differentiation in morphology and functionality of the interface membrane of the parasite with the surroundings. TbAQP1 is exclusively localized in the membrane of the parasite's flagellum [38], TbAQP2 is restricted to the flagellar pocket [39], and TbAQP3 depicts a more general distribution around the plasma membrane [38]. It is generally assumed that *T. brucei* live under constant environmental conditions in the blood and are typically not exposed to osmotic stress. Abrupt extracellular osmotic changes occur, however, when they pass the renal medulla [40]. Further, osmoregulation may become pivotal for survival of trypanosomes during the course of transmission between the insect vector and the human host or during development within the fly. Compared to other protozoa, *T. brucei* has developed a unique pathway of glucose metabolism making use of a parasite-specific organelle, i.e. the glycosome. Under anaerobic conditions, glucose is converted into equimolar amounts of pyruvate and glycerol phosphate. Glycerol kinase can then, in the sense of a backward-directed reaction, transfer the phosphate moiety from glycerol phosphate to ADP forming ATP [41]. To maintain this energetically unfavorable reaction, glycerol as a product has to be readily removed from the equilibrium [42]. It is not farfetched to conclude, that the glycerol-facilitating TbAQPs could fulfill a vital role in this physiological situation.

The *T. cruzi* genome contains four genes encoding AQP-like proteins, TcAQP and TcAQP β–δ, of which only TcAQP has been functionally characterized so far. Its water permeability was found to be very low, and solute permeability was not found. TcAQP has been localized to the acidocalcisomes and contractile vacuole complex of *T. cruzi* [43]. Acidocalcisomes, i.e. acidic, calcium-containing organelles, are rich in phosphate in the form of pyrophosphate and polyphosphate. Several functions are attributed to these organelles including storage of calcium and phosphate, pH regulation, as well as osmo-regulation [44,45]. It is tempting to connect the presence of the apparently water-specific TcAQP to the latter function of cellular osmotic regulation. Another single-celled organism, *Amoeba proteus*, relies on contractile vacuoles for osmo-regulation and a water-specific AQP was localized to this organelle system [46].

3.4. Leishmania AQPs

The genus *Leishmania* comprises more than 20 different species that cause leishmaniasis. Most studies deal with the AQPs from *L. major*. Five AQP genes have been identified in the *L. major* genome, i.e. LmAQP1 and LmAQPα-δ. LmAQP1 shows highest similarity to bacterial aquaglyceroporins, while the deduced amino acid sequences of LmAQP α-δ are closer to plant AQPs. LmAQP1 has been studied in some detail, while functionality of the other LmAQPs has not been shown, yet. LmAQP1 exhibits different localizations in the promastigote insect form, where it was found in the flagellum, and in the intra-phagocytic amastigote form of the human host, where is appears to reside in the flagellar pocket membrane and the contractile vacuole complex [47]. In vitro functional analysis showed good permeability of LmAQP1 for water and various physiological solutes, such as glycerol and methylglyoxal [47]. LmAQP was reported to be regulated by a mitogen-activated protein kinase [48] leading to developmental stage-specific and environmental stress-dependent phosphorylation. Particularly in the metacyclic parasite stage, phosphorylation of LmAQP may trigger redistribution of the protein to the pellicular membrane [48] where regulated transport of water and solutes may be beneficial during the transmission between the insect and mammalian hosts [47,48].

L. donovani similarly codes for five aquaporins termed LdAQP1, LdAQP9, LdAQP2860, LdAQP2870 and LdAQP *putative* [49]. Again, one protein sequence, LdAQP1, exhibits similarity to bacterial aquaglyceroporins, whereas the remaining four are related to plant AQPs. Expression in *L. donovani* promastigotes of GFP-fusion constructs hints at an intracellular localization of the plant-like AQPs [49]. Yet, at this stage, it is too early to speculate about functions of these AQPs.

3.5. Helminth aquaporins

Flukes of the genus *Fasciola* cause a local tissue infection of the liver, called fascioliasis [50]. The complex life-cycle of the parasites involves freshwater snails as intermediate hosts and definitive hosts, such as cattle, sheep and humans. In the mammalian host, the parasites actively penetrate different tissue barriers to reach the liver where they form capsules. Two AQPs, FgAQP1 and FgAQP2, from *F. gigantica* have been identified and characterized. The AQPs are genetically closely related and exhibit a rare amino acid exchange in the first NPA motif, i.e. Thr-Ala-Ala (TAA). As critical members of the AQP cation filter, the Asn residues in both NPA motifs are the most conserved positions in the AQP protein family. Only one other AQP from the bacterium *Burkholderia coenocepacia* [17] carrying a similar exchange, Ser-Pro-Ala (SPA), has been functionally characterized so far. Both FgAQPs show low water permeability when expressed in *Xenopus laevis* oocytes, permeability for glycerol, urea, or methylamine could not be detected [51]. Interestingly, mutation of the Thr of the TAA motif to Asn increased water permeability 3–4 fold. Immunolocalization identified FgAQP in the tegument, i.e. the interface between the parasite and its environment, as well as in the epithelial lining of the testes and ovary. The importance of regulated tegument water permeability has been shown by knock-down studies using another helminth parasite, *Schistosoma mansoni*, briefly described below.

Schistosomiasis is a chronic disease caused by parasitic platyhelminths of the *Schistosoma* genus. Parasites coming from a freshwater environment and invading a mammalian host undergo a complex series of adaptive morphological and biochemical changes, which are further accompanied by changes in the energy metabolism. A proteomics approach using *Schistosoma mansoni* proteolytic peptides led to the identification of a water/solute channel, SmAQP [52]. SmAQP was localized to the tegument and showed highest expression levels in the intravascular life stages. Knock-down by siRNA rendered the parasite less reactive to osmotic changes and viability was compromised. Several solutes were reported to be permeants of SmAQP, i.e. mannitol, fructose, alanine and lactate, whereas glucose was excluded [53]. Considering the size (sugar alcohols) and charge (zwitterionic alanine, negatively charged lactate) of these compounds the permeability profile is quite surprising [53]. Based on the data of the in vitro determination of the permeability profile, it was proposed that, besides osmotic regulation,

functions of SmAQP could also be in nutrient uptake, e.g. amino acids, and metabolic wastes excretion, e.g. lactate from glycolysis [53]. Yet, further more quantitative studies including in vivo experiments seem to be required to conclude safely on the physiological roles of helminth AQPs and their potential as drug targets.

4. Parasite AQPs — new drug targets for the treatment of infections?

The idea that AQPs could serve as direct therapeutic targets has been brought up soon after their discovery [54]. A variety of human disease states has been shown to be related to a malfunction or dysregulation of AQPs [54]. Today, efforts are undertaken towards the inhibition of the human water channels AQP1 for treating glaucoma, brain edema, inflammatory pain and certain types of cancer [55], and AQP4 to prevent acute ischemic cerebral edema [56]. Inhibitors of the human water/glycerol channel AQP9 may be used to reduce the uptake of glycerol into the liver as a prevention of steatosis and for lowering glycemic levels in type-2 diabetes [57]. However, potent drug-like AQP inhibitors are yet to be found. Interestingly, aquaglyceroporins can facilitate the passage of arsenious acid, $As(OH)_3$, and antimonious acid, $Sb(OH)_3$, into cells due to some resemblance of these compounds with the glycerol molecule [58]. Arsenic trioxid, As_2O_3, which dissolves in water into arsenious acid, is a treatment of promyelocytic leukemia and its accumulation in leukocytes depends on the presence of AQP9 as the uptake route [59].

Parasite aquaporins have been cloned and studied also with regard to fathom their potential as targets for chemotherapy [5]. The permeability profiles derived from this work suggest crucial functions in the osmotic volume regulation of the parasite cells, in energy metabolism by release of end or side products, e.g. ammonia or methylglyoxal, and in the biosynthesis of cellular components, e.g. glycerolipids via glycerol uptake. However, as with the human AQPs, besides sulfhydryl-modifying cations, such as Hg^{2+} or Ag^{+} that non-specifically inhibit some AQPs via cysteine binding in the pore region, no potent drug-like blockers are available at this time.

Are there peculiar difficulties linked to AQP proteins, which hamper the design of adequate small-molecule inhibitors?

One challenge lies in the rigid, narrow, funnel-like internal protein structure of AQPs. For the typical orthodox aquaporins and aquaglyceroporins the narrowest pore region is located close to the extracellular site and its diameter is a mere 2.8 Å or 3.4 Å, respectively. The former perfectly fits a water molecule and the latter accommodates the passage of a carbon hydroxyl group of polyols, such as glycerol [60]. Putative inhibitor molecules are, thus, strongly limited in their dimensions [16]. A major portion of an inhibiting compound must therefore interact with domains outside of the actual channel [61]. The outer vestibule of water-specific aquaporins is polar, small, and exposed to the solvent; accordingly, is it heavily decorated with water molecules from the extracellular space. An efficient inhibitor would need to bind at least six orders of magnitude stronger to the AQP vestibule than a water molecule to overcome the 55 molar concentration of water in the aqueous solvent [61]. This would probably require lipophilic shielding of the interacting groups, which is hard to achieve due to the shallow nature of the vestibule. The outer vestibule of aquaglyceroporins typically appears wider and deeper enhancing the chance of success in finding well-binding organic molecules. We noted inhibition of PfAQP water permeability due to binding of sorbitol to the outer vestibule while saccharose did not show this effect. However, concentrations equal to the osmotic gradient were required [27]. Even though it would be advantageous to block AQPs from the extracellular side, because this will prevent resistance mechanisms of the parasite cell, such as metabolism of the compound or expulsion by transporters, the intracellular AQP vestibule and channel appear wider and reasonably sized inhibitor compounds may be able to travel even up to the central NPA region for better binding.

A second challenge lies in the intracellular life style of many parasites, e.g. of *T. gondii* or apicomplexan parasites of the *Plasmodium* species, whereas extracellular parasites, e.g. helminths or trypanosomes, can be targeted directly. The central position of the *Plasmodium* aquaglyceroporin in transmembrane metabolite flow seems to render it a promising drug target. Yet, blood-stage plasmodia are well shielded from the environment by three consecutive lipid bilayers, i.e. its own plasma membrane, the parasitophorous vacuole membrane, and, finally, the red cell membrane [30]. To reach the parasite any drug compound needs to penetrate these barriers and withstand metabolizing erythrocytic and plasmodial enzymes in order to reach its target [62]. The membrane permeability properties of infected erythrocytes further differ largely from those of uninfected red cells by still unknown processes induced by the parasite [9]. Hence, the physicochemical properties of a suitable chemotherapeutic drug must comply with several requirements: high-affinity binding to the target AQP, sufficient membrane penetration capability either by passive diffusion or active transport by carrier proteins, and chemical resistance against metabolic conversion.

Finally, it is debatable, whether parasite AQPs are indeed essential for their viability. It is quite surprising that the gene deletion of the rodent malaria parasite *Plasmodium berghei* AQP results in a comparably mild phenotype. The PbAQP-null parasites are highly deficient in glycerol transport; yet, they are viable in vitro as well as in infected mice. The proliferation rate is reduced by half and the infected mice survive twice as long as their mates infected with the wild-type parasites [31]. It seems that metabolite transport is indeed hampered but the parasites can cope with the situation by using alternative biochemical pathways, e.g. for glycerol biosynthesis, or other routes of transmembrane transport, e.g. passive diffusion of ammonia across lipid bilayers. The genomes of the *Kinetoplastida* (*Trypanosoma, Leishmania*), encode up to five individual AQP genes [5]. Knockdown experiments of the *Trypanosoma brucei* AQP1 and AQP3 well survived hypo-osmotic stress conditions [38]. A double-deletion AQP2/AQP3-null strain did not show significant differences to the wild-type mother strain under normal growth conditions [63]. Similarly, the deletion of AQP1 in the *Leishmania major* genome showed unaltered viability [64]. Parasite AQPs are, thus, probably not suitable as a single novel target for chemotherapy. The possibility remains, however, that inhibition of parasite AQPs increases metabolic stress and respective drugs may be usable in combination therapy regimes. A reduced proliferation rate of the parasites may also provide that amount of additional time required by the immune system to generate the appropriate response [65].

5. Aquaporins as facilitators of drug uptake

Aquaglyceroporins are permeable for aliphatic organic molecules as long as they are small, i.e. with a molecular weight below approx. 300 Da, of linear shape, and uncharged [60]. From a pharmaceutical point of view, such structural restrictions do not leave much room for the design of potent and specific drug compounds. There are, however, three examples of antiparasitic molecules within this category, i.e. hydroxyurea [34], dihydroxyacetone [33,66], and methylglyoxal (Fig. 2) [33]. The compounds lack specificity and are generally cytotoxic or antineoplastic. But if such molecules are more toxic for the parasite than for the host cells, they bear a certain potential as antiparasitic drugs and the facilitating aquaglyceroporins provide the direct entry pathway. The inorganic metalloids, $As(OH)_3$ and $Sb(OH)_3$ [64], must be added to this group of drugs, whose uptake is facilitated by AQPs.

Hydroxyurea (Fig. 2), an anticancer drug, is toxic to replicating eukaryotic cells. In vitro experiments showed its repressive effect on the proliferation of *T. gondii* [67], *Leishmania mexicana* [68] and *P. falciparum* [69]. The AQPs of *T. gondii* and *P. falciparum* have been tested for hydroxyurea permeability and flux rates in the range of the original substrate glycerol were observed. This indicates that parasite AQP provide a pathway for the uptake of hydroxyurea [34].

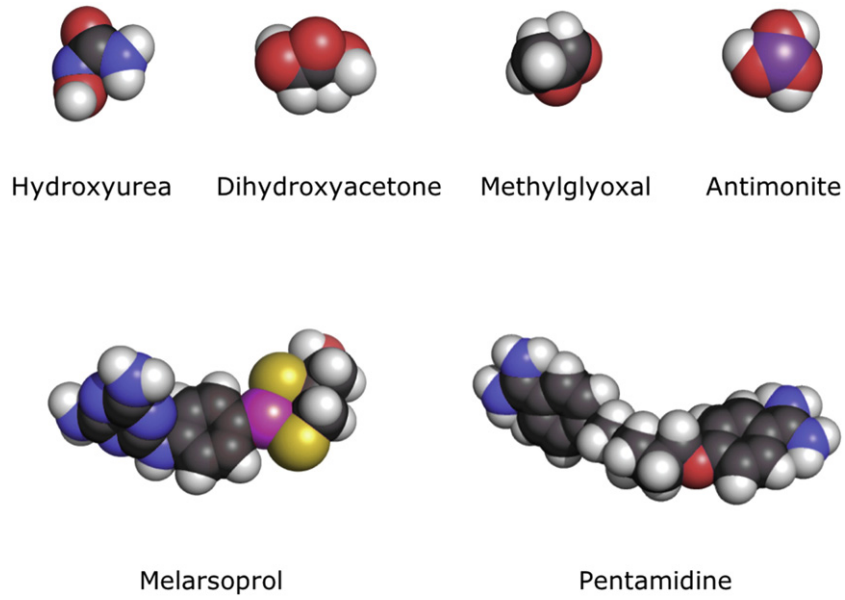

Fig. 2. Structures of drugs that are related to parasite aquaporins either by direct transport through the channels (upper row) or by as yet unresolved mechanisms (lower row). Carbon — black, hydrogen — white, oxygen — red, nitrogen — blue, sulfur — yellow, arsenic — pink, antimony — purple.

Dihydroxyacetone is a simple three-carbon sugar molecule, a triose, highly similar to glycerol (Fig. 2). For many cell types dihydroxyacetone is not toxic at all because it will be phosphorylated by dihydroxyacetone kinase to yield dihydroxyacetone phosphate, which enters the glycolytic metabolic pathway. However, some parasites cannot convert dihydroxyacetone because they lack dihydroxyacetone kinase so that the chemical reactivity of accumulating dihydroxyacetone results in DNA or protein modification and cell damage. The antiproliferative effect of dihydroxyacetone has been shown in vitro for *T. brucei* [66,70], and *P. falciparum* [33]. Aquaglyceroporins are highly permeable for dihydroxyacetone, e.g. PfAQP [33], TbAQP1–3 [38], and LmAQP1 [47].

Methylglyoxal (Fig. 2) is a non-enzymatic glycolytic side-product of toxic and mutagenic potency due to the chemical reactivity of its vicinal di-carbonyl moieties [71]. Protozoan parasites express a detoxifying glyoxalase enzyme system [71], nevertheless, growth of various parasites is inhibited by micromolar concentrations of methylglyoxal: *P. falciparum* (IC_{50} of 223 μM for the Binh1 strain, 128 μM for 3D7) [33], *T. brucei* (IC_{50} of 70 μM) [72], *T. cruzi* (IC_{50} of 171 μM) [72], and *L. major* (IC_{50} of 397 μM) [72]. High permeability of PfAQP [33] and LmAQP [47] for methylglyoxal has been shown by direct assays.

A clinically probably more relevant example for the AQP facilitated transport of compounds with antiparasitic activity is the permeability for $Sb(OH)_3$ (Fig. 2). Pentavalent antimonials, i.e. sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucanitime), are still the first line treatment of leishmaniasis [64]. *Leishmania* reside in macrophages or other phagocytic cells. Within the macrophage cytosol, the pentavalent antimonials are reduced to the active trivalent antimony form, $Sb(OH)_3$, which is then transported into the amastigote by the aquaglyceroporin LmAQP1 [64,73]. Accordingly, a direct correlation was found between the LmAQP expression levels and the degree of resistance of *Leishmania* against antimonials [74].

Recently, a truly unexpected link between drug resistance and AQP expression was identified in *T. brucei* [63]. The genome-wide screening for gene products responsible for cross-resistance against African trypanosomiasis drugs revealed that down-regulation of TbAQP2 leads to pentamidine and melarsoprol resistance (Fig. 2) [63]. Generation of a TbAQP2 gene knockout strain confirmed the data, and episomal expression of TbAQP2 restored drug sensitivity [39].

Melarsoprol is thought to bind and inactivate trypanothione, a trypanosome-specific thiol compound produced by the parasite as a defense against oxidative damage [75]. Pentamidine has been shown to interfere with the kinetoplast DNA [76], and an effect of pentamidine on the mitochondrial membrane potential is discussed [77]. Whether the mentioned mechanisms of action are the only ones or the most relevant ones is still under debate; it is highly unlikely, however, that inhibition of water or solute transport of TbAQP2 is the mode of action of the compounds, especially considering the viability of the TbAQP2 knockout strain.

It is similarly questionable, that melarsoprol (mol. weight 398 Da) and pentamidine (mol. weight 346 Da plus two positive charges) can directly pass the TbAQP2 water/solute channel. Nevertheless, both compounds obviously transit the parasite membrane in their intact form [78,79]. One attempt to explain the uptake mechanism proposes that TbAQP2 may regulate the expression or function of the so-far unidentified high-affinity pentamidine transporter (HAPT1) and, thus, controls pentamidine uptake in an indirect way [80]. Two additional uptake pathways for pentamidine are the known P2 aminopurine transporter and the non-identified low-affinity pentamidine transporter (LAPT1) [81]. In any case, the newly established connection of a *T. brucei* AQP with the action of two drugs that are in use since the 1940s offers a fresh view on the treatment of African trypanosomiasis and spurs further research.

6. Conclusion

The roles of the individual AQPs in the physiology of the various human-pathogenic parasites are probably as diverse as their ways of transmission, intra-/extracellular life style, developmental cycles, and energy metabolism. In contrast, AQPs form a highly conserved group of proteins throughout all species. Variations in the structure are mainly found outside of the actual channel domain. Most promising for the development of compounds of sufficient affinity are the vestibules at the channel openings. Binders to the extracellular vestibule may be preferable because in this case the compounds do not need to enter the parasite's cytosol, which should render respective drugs less susceptible to metabolic inactivation. However, the intracellular vestibule may allow inhibitory compounds to penetrate deeper into the channel domain, thus, enhancing binding, because the NPA region is wider than the ar/R constriction (Fig. 1). Anyway, the pressingly anticipated discovery of AQP inhibitors of reasonable affinity and specificity will provide highly useful pharmacological tools for the

investigation of the true function of parasite AQPs and will eventually reveal their real potential for novel chemotherapeutic approaches.

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