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MINI-REVIEW

Schistosoma mansoni sarco/endoplasmic reticulum Ca^{2+} ATPases (SERCA): role in reduced sensitivity to praziquantel

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Abstract

Praziquantel leads to increase Ca^{2+} influx and disrupts Ca^{2+} homeostasis in adult *Schistosoma*. However, calcium influx is only one component in a series of molecular events leading to the drug effect and some downstream constituents of the cascade that is initiated by this interaction differ between worms with different degrees of susceptibility to praziquantel. Extensive use of the drug raises the concern regarding the selection of drug resistant parasites. SERCA participates in maintenance of Ca^{2+} homeostasis. Up-regulation of SERCA has been found in *Schistosoma mansoni* worms with reduced sensitivity to praziquantel. This could be due to increase cytosolic Ca^{2+}, activation of calmodulin kinase II or may be due to SR/ER stress generated from oxidative stress that leads to impaired protein degradation. The significance of SERCA up-regulation is related to counter action of the drug effect by increasing the worm capacity to restore Ca^{2+} homeostasis, reducing cytosolic Ca^{2+} followed by lowering mitochondria Ca^{2+} and consequently inhibition of apoptosis beside its relation to P-glycoprotein. In schistosomes with reduced sensitivity to praziquantel, the agitations produced by Ca^{2+} influx and the downstream component of the cascade that is initiated by this interaction may be opposed by up-regulation of SERCA and possibly by certain elements of Ca^{2+} signaling which modulate the process determining cells entrance in the apoptotic state. Revealing the principal mechanisms of up-regulation of SERCA and its significance in reducing the effect of the drug could lead to possible strategies to reverse drug resistance or develop alternative therapies.

Keywords SERCA · Ca^{2+} ATPases · *Schistosoma mansoni* · PZQ · Resistance · Calcium

Introduction

Schistosomiasis is an important waterborne parasitic disease caused by helminth worms of genus *Schistosoma*, with three main species (*S. mansoni, S. haematobium* and *S. japonicum*) accounting for the majority of human infections. It is considered one of the most prevalent neglected tropical diseases, constituting a major public health problem and leading to chronic and even debilitating disease that impairs development and productivity. The disease is endemic in 78 countries located in Africa, Asia and the Americas and is responsible for 3.3 million disability adjusted life years (Hotez et al. 2014). Inadequate water supply, poor sanitary conditions as well as low socio-economic development are involved in the prevalence of the disease (Rollinson et al. 2013; Abou-El-Naga 2015).

Praziquantel (PZQ) is the drug of choice against schistosomiasis. Extensive use of the drug raises the concern regarding the selection of drug resistant parasites (Fallon and Doenhoff 1994). The main mode of action of the drug is disruption of calcium homeostasis, however, the exact mechanisms associated with this effect are not clear (Cioli and Pica-Mattoccia 2003).The constituents of calcium homeostasis and calcium signaling pathways in *Schistosoma* worms have been payed considerable attention and will be discussed in this review, focusing on the sarco/endoplasmic reticulum Ca^{2+} ATPase pump (SERCA) and the suggested mechanisms regarding its over expression and its significance in reduced sensitivity of the worms to PZQ.

Calcium homeostasis and calcium signaling process (Fig. 1)

Calcium ion plays a key role in signal transduction in eukaryotic cells and is an important second messenger that regulates

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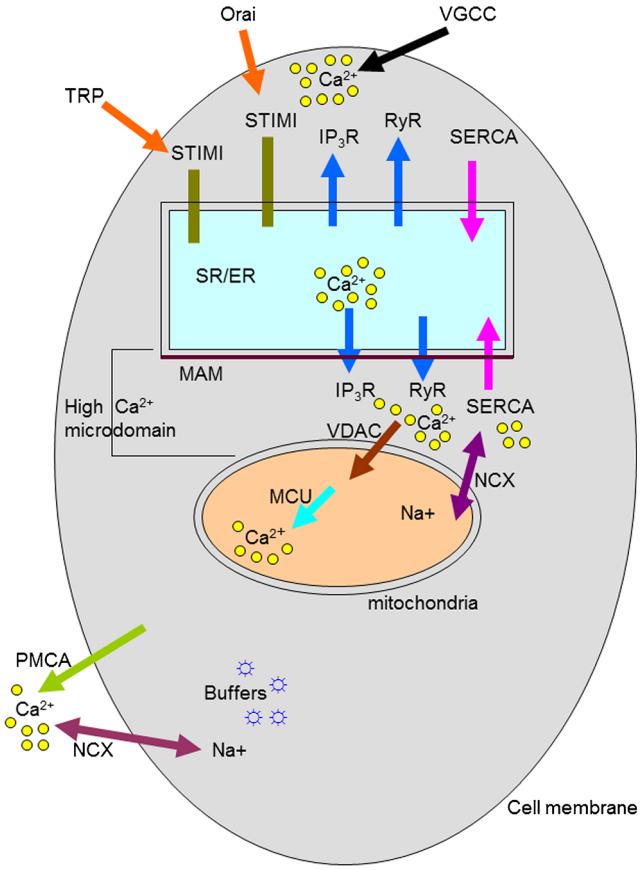

Fig. 1 Diagram illustrating components of calcium signaling process and calcium homeostasis in the cell. Ca^{2+} release from the SR/ER via the IP3 receptors (IP3R) and the ryanodine receptor (RyR). Voltage-gated Ca^{2+} channel (VGCC) and the Store-operated calcium entry (SOCE) through the plasma membrane also increase cytosolic Ca^{2+} levels. Major components of SOCE are the transient receptor potential (TRP) channel and Ca^{2+} pore forming channel Orai and Ca^{2+} sensor Stromal interaction molecule 1 (STIM1). Ca^{2+} clearance from the cytosol occurs through the plasma membrane Ca^{2+} ATPase (PMCA) as well as the $Na+/Ca^{2+}$ exchanger (NCX) which extrude Ca^{2+} to the extracellular compartment and also via sarco/endolasmic reticulum Ca^{2+} ATPase (SERCA) which sequester Ca^{2+} into the SR/ER. Ca^{2+} buffers (Buffers) bind a large proportion of cytosolic Ca^{2+}, removing it from the activating pool. Mitochondria also play an important role in regulating levels of Ca^{2+} in the cytoplasm. The ER and the mitochondria interact through a domain of the ER called mitochondria-associated membrane (MAM). Ca^{2+} moves between SR/ER and mitochondria through IP3R and RyR on the SR/ER side, and the voltage-dependent anion channel (VDAC) on the mitochondrial membrane, in addition the mitochondrial Ca^{2+} uniporter (MCU) moves Ca^{2+} from the mitochondrial intermembrane space to the mitochondrial matrix. Ca^{2+} is released from the mitochondria through the NCX

diverse cellular functions including muscular contraction, fertilization, cell proliferation and differentiation (Lee 2004). Like in other eukaryotes, $Ca^{2+}$is a key element affecting crucial aspects in different life cycle stages of *Schistosoma*.

Calcium signaling is initiated by various cell stimuli such as membrane depolarization, extracellular signaling molecules or intracellular messengers. These stimuli lead to transient and local increase in concentration of cytosolic calcium ions ten to hundreds of times above the basal level. Once Ca^{2+} signal is generated, the Ca^{2+}-sensitive processes translate this signal into an adequate cellular response. As maintenance of intracellular Ca^{2+} concentration is critical for cellular signaling, therefore cells have developed fine tune mechanisms to maintain Ca^{2+} homeostasis. These mechanisms comprise a vast collection of signaling units including channels, pumps receptors, exchangers, Ca^{2+}-sensitive enzymes and buffers as well as Ca^{2+}- binding proteins (Berridge et al. 2003).

The transient increases in cytosolic Ca^{2+} is brought through entry of extracellular Ca^{2+} or by release of intracellular Ca^{2+} stores. Entry of extracellular Ca^{2+} can be mediated by several components of plasma membrane ion channels such as voltage-gated Ca^{2+}-channels (VGCCs), store-operated calcium entry (SOCE) and transient receptors potential (TRP) channels. VGCCs couple membrane depolarization to rapid influxes of Ca^{2+} and contribute to impulse propagation. Store-operated calcium entry (SOCE) through the plasma membrane is another regulator of intracellular Ca^{2+} and has an impact in refilling of sarcoplasmic/endoplasmic reticulum (SR/ER) with Ca^{2+}. Ca^{2+} pore forming channel Orai and SR/ER Ca^{2+} sensor stromal interaction molecule 1 (STIM1) are major components of SOCE which is activated in response to a depletion of SR/ER Ca^{2+} stores leading to Ca^{2+} influx (Ambudkar et al. 2017). TRP channels are involved in regulating intracellular calcium and ions homeostasis and are critical for transducing sensory signals and responses to a wide range of external stimuli (Bais and Greenberg 2016).

Cytoplasmic Ca^{2+} concentration can also be abruptly and severely increased by the release from the Ca^{2+} storage organelles; chief amongst them is the SR/ER. Ca^{2+} can be released from SR/ER through activation of the inositol-1,4,5-triphosphate receptors (IP_3R) and by ryanodine receptors (RyR) calcium release channels localized on the SR/ER membrane (Clapham 2007). Ca^{2+}-binding proteins such as calreticulin and Ca^{2+}-dependent calnexin buffer the Ca^{2+} in the SR/ER lumen. Calcium signal is terminated when cytosolic free Ca^{2+} concentration is reduced to basal levels. This is achieved by sequestering Ca^{2+} in the calcium storage organelles mainly the SR/ER by means of SERCA or by extrusion of Ca^{2+} to the extracellular compartment through the plasma membrane Ca^{2+} ATPase (PMCA) as well as the $Na+/Ca^{2+}$ exchanger (Bagur and Hajnóczky 2017). Notably, there is an efficient coupling of the extracellular Ca^{2+} entry and the intracellular Ca^{2+} release mediated by interactions between components of the plasma membrane and the SR/ER (Ambudkar et al. 2017). Thus, the activity of SERCA lowers the concentration of Ca^{2+} in the cytoplasm by sequestering it to the SR/ER. The content of Ca^{2+} within the SR/ER controls important processes including the sensitivity of Ca^{2+} release and the activity of plasma membrane entry channels. Consequently, SERCA activity has a significant impact on patterns of Ca^{2+} homeostasis and

signaling as well as the cellular events these signals control (Bagur and Hajnóczky 2017).

Calcium homeostasis and calcium signaling in *Schistosoma mansoni* (Fig. 1)

Like in other eukaryotes, Ca^{2+} is a key element affecting crucial aspects in different life cycle stages of *Schistosoma*. $Ca2^{+}$ homeostasis is a vital process for *S. mansoni* as keeping low cytosolic Ca^{2+} concentration is important to the worm physiology, particularly in muscular contractility. Treatment of schistosomiasis relay only on PZQ which acts mainly by disrupting Ca^{2+} homeostasis of the worm. Schistosomes have different developmental stages living in different environmental conditions. Eggs, miracidia and cercariae are free living stages displaying geotropism and are sensitive to light. Schistosomula and adult worms are present inside the definitive host (mammals) while the sporocyst stage is present in the snail intermediate host and is exposed to the snail hemolymph. Signals from these different media stimulate physiological, morphological and biochemical adaptations of the different developmental stages of the parasite. Moreover, signals from adult male schistosomes are necessary for female development (Walker 2011).

Several cellular elements that are essential for maintenance of Ca^{2+} homeostasis have been described by many researchers in *S. mansoni*. As regards intracellular Ca^{2+} release channels, genes encoding homologues of mammalian intracellular Ca^{2+} release channels; inositol 1,4,5-trisphosphate receptors (IP3R), ryanodine receptors (RyR) have been shown in *S. mansoni* (Prole and Taylor 2011). The genomes of *S. mansoni* also encode homologues of mammalian Ca^{2+} influx channels which are important for muscle contraction and viability of the parasite including VGCC channels, TRP channels and Orai Ca^{2+}channel in addition to STIM Ca^{2+} sensor homologues, suggesting that store-operated Ca^{2+} entry may occur in *S. mansoni* (Greenberg 2005; Wolstenholme et al. 2011; Prole and Taylor 2011).

Schistosomes VGCCs are large multi-domain complexes, consist of a central pore-forming α_1 subunit associated with auxiliary subunits that modulate the properties of the channel (β, δ and). They are activated by depolarization and alteration of the membrane potential leading to passive influx of Ca^{2+} across the cell membrane (Hofmann et al. 1999). Schistosomes express at least two variant novel and exclusive β subunits (Sm Ca_vβ) among invertebrates (Kohn et al. 2001). Sm Ca_vβ lack the two conserved serines within their β interaction domains which are replaced by cysteine and arginine. These serines are consensus protein kinase C phosphorylation sites. Phosphorylation of VGCCs by protein kinase C and other protein kinases plays a critical role in regulating channel properties. Hence, the absence of the conserved consensus protein kinase C phosphorylation sites in Sm Ca_vβ leads to the unusual modulatory properties and pharmacological sensitivities of the VGCCs. These two Sm Ca_vβ subunits modulate α_1 subunits in an atypical manner, instead of increasing the current amplitude as the conventional β subunits do, these dramatically decrease the amplitude of the currents. The molecular target of PZQ thought to be the extraordinary Sm Ca_vβ subunits of VGCCs (Greenberg 2005).

TRP channels are non-selective Ca^{2+} permeable channels. They have critical roles in transduction of sensory signals, response to a wide range of external stimuli and modulate intracellular Ca^{2+} concentrations (Gees et al. 2010; Bais and Greenberg 2020). Different members of the TRP channels have been predicted in *S. mansoni* genome but there is no evidence about representatives of the *S. mansoni* TRPV channel subfamily (Wolstenholme et al. 2011; Bais and Greenberg 2016). PZQ activates a schistosome Ca^{2+}-permeable TRP channel, a member of the TRP melastatin (TRPM) subfamily, christened (*Sm*.$TRPM_{PZQ}$). The target effect of the drug on this channel is found to be mediated by its (R)-enantiomer (Park et al. 2019; Park and Marchant 2020). Mutation can alter the *Sm*.$TRPM_{PZQ}$ sensitivity to PZQ (Park et al. 2019). However, the implications for drug resistance highlight further analyses of *Sm*.$TRPM_{PZQ}$ homologs in other flatworms as well as all other schistosome TRPM channels (Park and Marchant 2020).

Sm.TRPA channel apparently differs from that of the mammalian channels, as they exhibit atypical mixed TRPA1/TRPV1-like pharmacology. Despite the absence of TRPV-like genes in schistosomes, the selective TRPV1 activators leads to hyperactivity and rapid separation of *S. mansoni* coupled worms. The TRPA1 channels could be a drug target as they transduce endogenous host signals that are required or exploited by the parasite leading to disruption of schistosome development, reproduction or survival within the host (Bais et al. 2018). However, the sensitivity of these channels to PZQ has not been demonstrated yet.

Schistosome genes involved in the cell signaling and the calcium ion binding including putative sodium/calcium exchanger and those of the voltage dependent anion channel are also expressed in the different developmental stages of the parasite (Parker-Manuel et al. 2011; Phuphisut et al. 2018). The main function of SERCA pumps is to sequester Ca^{2+} into the SR/ER. SERCA have been identified in schistosomes and this review will concentrate mainly on these pumps.

Ca^{2+} signaling in *Schistosoma* has many different Ca^{2+}-binding proteins which are either Ca^{2+} buffers or Ca^{2+} sensors. Signal transduction involves integrated networks that interact mostly by switching activity status via phosphorylation (protein kinases) and dephosphorylation (protein phosphatases) of amino acid residues. The buffer proteins have relatively high affinity behavior and modulate the shape and/or duration of Ca^{2+} signals and help maintain Ca^{2+} homeostasis. In contrast,

Ca^{2+} sensors have constant affinity thus can detect and respond to a physiologically relevant change in intracellular Ca^{2+} (Bagur and Hajnóczky 2017). Calmodulin is a small, calcium-sensing protein implicated in egg hatching and miracidia transformation and is essential for sporocyst development (Taft and Yoshino 2011). *S. mansoni* expresses two highly similar calmodulin isotypes which are shown to interact in a same affinity with the VGCC (Thomas and Timson 2018a). Calcineurin (CN) is a Ca^{2+}/CaM dependent serine-threonine protein phosphatase. It is regulated by Ca^{2+} both directly and via CaM and is activated during SR/ER stress (Cunningham and Fink 1996). It is present in different developmental stages in *S. mansoni* and has been found at high concentrations in the excretory systems of the schistosomula and adult worms, perhaps suggesting a role for CN in the regulation of ion fluxes (Mecozzi et al. 2000). Calmodulin kinase II, CN and protein kinase C are involved in the calcium pathway (Mecozzi et al. 2000; You et al. 2013). Another Ca^{2+} − binding proteins, the tegumental allergen-like proteins have been characterized in *S. mansoni* but their roles are less clear (Carson et al. 2018).

Sarco/endoplasmic reticulum Ca^{2+} ATPase pump (SERCA)

SERCA is a Ca^{2+}-pumping ATPase and a member of P-type ATPases which are a large family of integral membrane transporters. P-type ATPases include in addition to Ca^{2+} transporting proteins, Na^+ and K + -ATPases, H^+ and K^+ protons and heavy metal transporting ATPases as prominent members. Ca^{2+}-pumping ATPases is characterized by forming a phosphorylated (P-) intermediate state during their ion transport cycle. They transport two Ca^{2+} ions from the cytoplasm across cell membranes at the expense of the hydrolysis of one ATP molecule. It has been classified into three major classes which differ in their structures, their kinetic properties and their location within the cell. These classes are plasma membrane Ca^{2+}ATPases (PMCA), sarco/endoplasmic reticulum Ca^{2+}ATPases (SERCA) and Golgi secretory pathway Ca^{2+}ATPases (SPCA). SERCA pumps free calcium ion from the cytoplasm into intracellular SR/ER against a large concentration gradient of nearly10000-fold (Toyoshima 2009).

Types of Ca^{2+} ATPases in *Schistosoma*

The homologues of the SERCA in *S. mansoni* SMA1 and SMA2 had been cloned (de Mendonca et al. 1995; Cunha et al. 1996; Talla et al. 1998). The full-length gene *SMA1*corresponding to SMA1 is located on chromosome 1 (Chr1), at the Smp_007260.1 locus (GeneDB) and *SMA2* gene is located on Chr3 at the Smp_136710 locus (Talla et al. 1998).

SMA1 and SMA2 are found to be very close to each other regarding the gene and the protein sequence. They are slightly differing in length and in the sequence of the nucleotide binding domain and have the same affinity to bound calcium. In terms of sensitivity to the two specific SERCA pumps inhibitors, SMA1 and SMA2 have somewhat similar sensitivity to cyclopiazonic acid but different sensitivities to thapsigargin (Talla et al. 1998; Berriman et al. 2009; Maréchal et al. 2018). SMA1 belongs to SERCA and PMCA subclasses while SMA2 is a SERCA subclass (de Mendonca et al. 1995; Talla et al. 1998). The third type, SMA3 is detected in the membranes of the adult tegument belonging to the secretory pathway subclass (Da'dara et al. 2001).

Physiological role of SERCA

Role of SERCA in Ca^{2+} homeostasis

Ca^{2+} ions are present in low concentrations in the cytoplasm and with a similar level in the nuclear and the mitochondrial matrix while the extracellular concentrations are high. Calcium movements along these concentration gradients are vital for enormous cell process. Ca^{2+} stores, mainly the SR/ER, can accumulate Ca^{2+} and maintain a higher Ca^{2+} level than the cytoplasm. SERCA is the only active Ca^{2+} transporter from the cytosol to the SR/ER, therefore maintaining Ca^{2+} homeostasis. It presents high affinities for Ca^{2+} and has a low pumping rate which makes it suitable to respond to modest elevations in cytosolic Ca^{2+} and to re-establish the resting Ca^{2+} level. SERCA pumps calcium in an ATPase activity-dependent manner and affecting capacitative calcium entry where the Ca^{2+}filling state of SR/ER regulates the plasma membrane Ca^{2+} channels (Bagur and Hajnóczky 2017).

SERCA and its relation to protein synthesis and apoptosis

In addition to the role of SR/ER in securing cell homeostasis and its crucial role in calcium sequestering and signaling, it is also concerned with protein synthesis, control of protein post-translational modification, their folding and intracellular translocation. High concentrations of Ca^{2+} ions are required for enzymes and chaperone proteins residing in the SR/ER lumen which are involved in these processes (Corbett and Michalak 2000). Maintaining a high calcium concentration at the SE/ER is controlled by molecular chaperones that bind and buffer calcium, channels that release calcium from the SR/ER to the cytosol and calcium importing mechanisms which are mainly driven by SERCA. The crosstalk between these mechanisms determines maintenance of a steady state calcium level within the SR/ER (Berridge 2002).

SR/ER stress can be induced by depletion of Ca^{2+} pool, inhibition of protein glycosylation and reduction of disulfide bonds. The stress affects the efficiency of protein folding and

leads to accumulation of unfolded and misfolded proteins in the SR/ER (Ron and Walter 2007). Correctly folded proteins only enter the secretory pathway in the SR/ER and Golgi apparatus (Wang et al. 2015). Prolonged inhibition of SERCA activity can disrupt the SR/ER function and thereby leading to prolonged SR/ER stress which can affect the efficiency of protein folding and hence leads to apoptosis. Therefore, maintenance of calcium homeostasis in the SR/ER by SERCA pump is vitally important for its functional integrity (Ushioda et al. 2016).

Role of SERCA in Ca^{2+} transfer from the SR/ER to the mitochondria

Ca^{2+} entering the cell during depolarization triggers Ca^{2+}release from SR/ER via the IP_3R and RyR channels. SR/ER and the adjacent mitochondria share sites of close apposition known as mitochondria-associated membranes (MAM) creating a microdomain of high Ca^{2+} upon release of Ca^{2+} from SR/ER. This microdomain allows the rapid uptake of a large amount of $Ca^{2+}$by mitochondria which is another reservoir of Ca^{2+}. Within the highly specialized SR/ER-mitochondrial region, Ca^{2+} moves between these organelles through a toolkit consisting of IP_3R and RyR on the SR/ER side and the voltage-dependent anion channels and mitochondrial Ca^{2+} uniporter (MCU) on the mitochondria which moves the Ca^{2+} from the mitochondrial intermembrane space to the mitochondrial matrix. SERCA re-uptake Ca^{2+} to the SR/ER, and the mitochondrial Na+/Ca^{2+} exchanger extrude Ca^{2+} from the mitochondria. Therefore, the mitochondrial Ca^{2+} uptake from this region is under control of SERCA (Rizzuto et al. 2012) (Fig. 1).

The transfer of Ca^{2+} to the mitochondria is critical for cell survival, regulation of metabolism, signal transduction as well as cell death. However, mitochondrial Ca^{2+} overload is a hallmark of the initiation process of apoptosis through opening of the mitochondrial permeabilization transition pores leading to release of cytochrome *c* and other pro-apoptotic factors. Thus, low level of SR/ER-mitochondria Ca^{2+} transfer maintains cell survival while excessive Ca^{2+} release from ER/ER to mitochondria results in mitochondrial Ca^{2+} overload and apoptosis (De Marchi et al. 2014). It was found that reduction of SERCA activity by resveratrol leads to mitochondrial Ca^{2+} overload and subsequent cancer cell death (Madreiter-Sokolowski et al. 2016).

Regulation of SERCA activity in *S. mansoni* and the relation to PZQ effect

Exposure of *Schistosoma* to PZQ is accompanied with elevation of the intracellular Ca^{2+} leading to activation of a number of distinct signaling pathways. These pathways influence gene expression of certain proteins including Ca^{2+}/calmodulin-dependent protein kinases (Ca^{2+}/CaMK) and CN which are concerned with regulation of SERCA (Aragon et al. 2009; You et al. 2013).

When cytosolic Ca^{2+} concentrations are at low level, phospholamban and sarcolipin (the main regulators of SERCA) interact with SERCA and decreases the pump's apparent affinity for Ca^{2+} (Vangheluwe et al. 2005). However, at high Ca^{2+} concentrations, phosphorylation of these inhibitors by calmodulin kinase II (CaMKII) promotes dissociation of the complex and this inhibitory function is alleviated (Bhupathy et al. 2009). Moreover, SERCA is found to be transcriptionally regulated by CaMKII indirectly through activation of nuclear activated factor κB (NFκB) (Park et al. 2018).

SERCA of *S. mansoni* has been shown to be negatively regulated by *Saccaromyces cerevisiae* yeast CN (Talla et al. 1998). Rossi et al. (2004) suggested that in schistosomes there is an indirect mechanism responsible for the CN-mediated inhibition of SERCA pumps. Furthermore, different protein kinases have also essential functions in *S. mansoni*. They are important for adaptations of the worm in response to diverse environments during the parasite development, vector interaction, and host infection (Andrade et al. 2011). Calmodulin (CaM) is implicated in egg hatching, miracidia transformation and is essential for sporocyst development (Thomas and Timson 2018a).

Relation of SERCA to multi drug resistant transporters

The ATP-binding cassette (ABC) transporter proteins are a large family of membrane proteins that have many cellular functions in living organisms. Their main function is exportation of xenobiotics from the cell. They also participate in normal physiological processes including transport of diverse compounds as peptides, ions, steroid hormones and cholesterol. Some members of this family as P-glycoprotein (P-gp) and the multidrug resistance proteins transport drugs and their importance stems from the fact that overexpression is commonly accompanied with the phenomenon of multidrug resistance (Higgins 1992).

It was suggested that P-gp-mediated multi drug resistance may be related to intracellular calcium homeostasis, however, the direct mechanism of this relationship is still unknown (Sulová et al. 2009). More specifically, the relation between P-gp and SERCA was investigated by Gutheil et al. (1994) who found that the resistance to thapsigargin (an inhibitor of SERCA) can involve not only alterations in P-gp expression but also SERCA isoform expression. Bottova et al. (2010) suggested that P-gp may play a role in Ca^{2+} homeostasis by regulating cellular concentration of protons. It was found that SERCA counter-transports protons to produce changes in Ca^{2+} levels and the expression of P-gp has been associated also with outward proton movement. Furthermore, Šereš et al.

(2008) had detected differences in contents of several proteins of the endoplasmic reticulum involved in calcium homeostasis including SERCA in P-gp expressing cells.

In *S. mansoni* genes had been predicted to encode ABC transporters (Kasinathan et al. 2014). PZQ may interact with these transporters as either a substrate or as an inhibitor of transport mediated by them (Kasinathan and Greenberg 2012). The ATPase activity that provide energy for these efflux pumps is found to be Ca^{2+} dependent (Pinto-Almeida et al. 2015) and Ca^{2+}elevation itself was found to increase the SERCA expression (Wu et al. 2001). It is worthy to mention that these two membrane proteins, SERCA and P-gp, are elevated by activated CaMKII via NFκB (Luo et al. 2013; Park et al. 2018).

Praziquantel (PZQ)

PZQ is the only drug currently used to control schistosomiasis. It is safe, effective against the three major schistosome species, administered orally, inexpensive and has relatively minimal side effects (Vale et al. 2017). Because of these advantages, PZQ has become effectively the only antischistosomal commercially available drug. Repeated rounds of mass drug administration using PZQ as a corner stone against schistosomiasis for many decades in several countries made the prospect of emerging resistance particularly worrisome (Vale et al. 2017; Abou-El-Naga 2018). Alarmingly, growing concerns are emerging regarding possible PZQ drug resistance in the field (Doenhoff et al. 2002). Furthermore, it is possible to experimentally induced PZQ resistance under laboratory conditions in mice (Fallon and Doenhoff 1994) and also after intra-molluscan exposure of the parasite to the drug (Couto et al. 2011; Abou-El-Naga et al. 2019).

The drug has bimodal activity with two distinct phases against the worms, based on the developmental stage present at the time of administration. The drug has limited efficacy against the very early stage (first few days after infection) and a progressive insensitivity down to very low levels around 3–21 days after infection, a major concern in regions with high reinfection rates. From this point on, worms gradually regain susceptibility until they become fully susceptible only when egg production begins, approximately 6 weeks following the infection (Xiao et al. 2010).

Although PZQ is the mainstay treatment for schistosomiasis and despite decades of extensive use, much remains unknown about its exact mode of action(s) and molecular target(s), as does the reason for its differing efficacy against juvenile and mature worms (Aragon et al. 2009). Praziquantel affects different developmental stages of *S. mansoni*. The drug alters the membrane permeability of the adult parasites, leading to vacuolation of the tegument. The initial effects of PZQ against schistosomes include intense muscle contraction and later paralysis which co-inside with the influx of extracellular calcium. The effect of PZQ on the tegument has been linked to one mode of action that is disruption of calcium ion homeostasis and in spite of that, the exact mechanism associated with this phenotype is unknown. The contracted and relaxed worms lose their binding capacity and are drawn into the liver where they are eliminated (Cioli and Pica-Mattoccia 2003). Matsuda et al. (1988) found that treatment with PZQ leads to hatching of the eggs in the host tissues. Exposure of the miracidia to PZQ leads to contraction of the middle part giving the appearance of an unequal dumbbell (Coles 1979). Mattos et al. (2006) found that although the sporocyst exposed to PZQ remained alive and in motion, marked contraction of its musculature and damages of the tegument were observed. PZQ also influences the cercariae tegument leading to morphological and biological changes (Xie et al. 2013).

Praziquantel (PZQ) [(RS)-2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazin-[2,1a]-isoquinolin-4-one] is a chiral molecule, has an asymmetric center in its structure, therefore, it exists as two enantiomers, the "levo" *R*(−) isomer which possesses anthelmintic activity and the inactive one; "dextro" *S*(+) that is responsible for the bitter taste of the drug and some side effects. The commercial preparation is a racemic mixture (RS)-PZQ composed of equal parts of the two isomers (Andrews 1985).

PZQ has some limitations, as low activity towards juvenile stages of the worm and reduced efficacy after prolonged use (Aragon et al. 2009; Abou-El-Naga et al. 2019). The drug also has low solubility and high permeability in the gastrointestinal tract, so the dissolution rate is the limiting factor of its absorption and one of the main reasons for the high doses (Passerini et al. 2006). Therefore, researches for development of novel drugs, new PZQ analogs and a second generation of antischistosomal drugs by changing the PZQ structure are necessary given that the PZQ structure offers several positions susceptible to chemical substitutions (da Silva et al. 2017).

Several approaches have been used to discover novel antischistosomal agents. One of these strategies is the molecular modeling which has been increasingly integrating the use of computational and experimental methods. Computational methods for drug design can be classified into two major categories; structure based and ligand based. The structure-based drug design utilizes information on the structure and functional character of a potential drug target. In the ligand-based drug design, knowledge of the biological and physicochemical properties of bound ligands that have been considered relevant for a given target disease are required (Mafud et al. 2016).

In schistosomiasis drug discovery different studies using the molecular modeling approach have recently been applied. Several analogues of PZQ were synthesized through modification of its aromatic ring, but each of these analogues was less effective compared to the parent drug (Ronketti et al.

2007). Recently by using the model predictions, da Silva et al. (2019) proposed 28 new PZQ derivatives and showed that these molecules have the potential to be more active than the known PZQ derivatives but these derivatives still need biological evaluation. A design of new hybrids derived from 10-hydroxy PZQ/artemisinin has activity not only against adults, but also against PZQ-refractory immature worms (Duan et al. 2012). In recent years there has been a trend towards integrating the two methods of the molecular modeling. These studies have been particularly useful in the discovery of novel inhibitors of Thioredoxin glutathione reductase enzyme of *S. mansoni* (Angelucci et al. 2009).

The Conception that PZQ acts primarily through disruption of calcium ion transport was given credence by a series of experiments. It was suggested that PZQ sensitivity is conferred on schistosomes through altering the function of a unique channel β subunit of VGCC (Greenberg 2005). However, there has also been a note of doubt whether Ca^{2+} influx through these channels is crucial to the schistosomicidal effect of PZQ as the gene encoding this subunit is equally regulated in PZQ-insensitive juvenile and adult sensitive schistosomes (Valle et al. 2003; Aragon et al. 2009).

Therefore, it has been hypothesized that calcium influx is only one component in a series of molecular events leading to the antischistosomal activity of PZQ and some downstream constituents of the cascade that is initiated by this interaction differ between juvenile and adult worms, and between isolates with different degrees of susceptibility to PZQ. Thomas and Timson (2018b) proposed that PZQ disrupts the link between a Ca^{2+} transport protein and one of its regulatory subunits. Nevertheless, this suggests that the constituents of calcium homeostasis and calcium signaling pathways in the worm are worthy for further studies as potential molecular targets of PZQ (Greenberg 2005; Pica-Mattoccia et al. 2008; Salvador-Recatalà and Greenberg 2012). It was proposeded that the drug acts on several pharmacological targets and the synergetic effect on these targets leads to its schistosomicidal effect (Thomas and Timson 2018b).

Apoptosis is associated with the schistosomicidal action of PZQ. *Schistosoma* worms showed up-regulation of several genes involved in apoptosis after exposure to PZQ and the difference in responses of juvenile and mature worms to PZQ is suggested to be due to differential expression of genes that regulate apoptosis (Hines-Kay et al. 2012).

PZQ induced apoptosis could be explained by the effect of the drug in increasing cytosolic Ca^{2+} and in inducing oxidative stress. High cytosolic Ca^{2+} is accompanied by increase Ca^{2+} influx into the mitochondria. Under normal physiological range Ca^{2+} activates mitochondrial metabolism. However, when there is Ca^{2+} overburden in the cytoplasm, mitochondrial Ca^{2+} accumulation can switch from a physiologically beneficial process to cell death signal. Increase mitochondrial Ca^{2+} uptake leads to opening of the mitochondrial permeability transition pore. This process is accompanied by release of cytochrome *c* which triggers apoptosis via caspase activation (Pacher and Hajnoczky 2001). Furthermore, Aragon et al. (2009) had demonstrated a transcriptomic response similar to that induced in oxidative stress after exposure of adult *S. mansoni* worms to PZQ.

Resistance to PZQ

The lack of a definite mechanism of action of PZQ on *Schistosoma* worms makes the possibility of emerging PZQ resistance especially daunting. With no other availability of treatment options, and as PZQ has been widely used, there are significant concerns that the effect of the drug may be diminished or the resistance may emerge. Currently there is little convincing data that PZQ resistance constitutes a major problem in the field but several reports of worm isolates exhibiting reduced PZQ sensitivity following drug exposure had appeared in Egypt (Ismail et al. 1999), Senegal (Fallon et al. 1995) and Kenya (Melman et al. 2009). Propagation of the parasite life cycle from patients in these areas showing reduced PZQ sensitivity could not be maintained in the laboratory beyond a few generations (William et al. 2001; Melman et al. 2009). However, resistance has been generated in the labs by propagation of the parasite in mice with exposure to increasing sublethal doses of PZQ over several generations (Coeli et al. 2013; Pinto-Almeida et al. 2015). Moreover, schistosomes with reduced sensitivity to PZQ was also generated by exposure of the parasite in the intra-molluscan phase to the drug (Couto et al. 2011; Abou-El-Naga et al. 2019).

In resistant *Schistosoma* strains neither changes in primary structure nor in the expression levels of the unique channel β subunit of VGCC were found, indicating that they have not exploited altered channels as a means of acquiring PZQ resistance (Valle et al. 2003). However, there may be different pathways for acquiring PZQ resistance and possibly this may be through multiple mechanisms. Some features might be relevant to the resistance including structural change in the drug target, change the accessibility of the drug to the effectors sites in addition to the clearance of the drug through an up-regulation of antioxidant enzymes (James et al. 2009). Pica-Mattoccia et al. (2009) considered the PZQ metabolism in the schistosomes is a central feature in the development of resistance rather than being the results of changes of the molecular drug target. They also found that resistance to the drug is a quantitative feature, referring to the presence of different targets of the drug. Chan et al. (2013) suggested that the decreased sensitivity to PZQ could be independent from alterations in the primary drug target and could be due to changes in the pathways both upstream involving drug handling constituents and downstream of the effector components.

The resistant mechanism of *Schistosoma* to PZQ has been associated with the activity of ABC transport proteins that

export the drug outside the worm (Kasinathan and Greenberg 2012). A recent proteomic study highlighted over expression of SERCA and Hsp70 in schistosomes with decreased sensitivity to PZQ (Abou-El-Naga et al. 2019; Abou-El-Naga 2020). As the mechanism of action of PZQ is not yet completely understood, subsequently, the mechanism of PZQ resistance is still unclear.

SERCA and decreased sensitivity of *S. mansoni* to PZQ (Fig. 2)

SERCA gene was found to be expressed in both *S. mansoni* and *japonicum* among the genes affected by PZQ and was up-regulated in *S. mansoni* isolate with decreased sensitivity to PZQ (You et al. 2013; Almeida et al. 2015; Abou-El-Naga et al. 2019). Up-regulation of SERCA in PZQ resistant *Schistosoma* worms due to the repeated exposure of the parasite to the drug could be attributed to the different mechanisms. The increase Ca^{2+} influx by itself can lead to increase SERCA expression (Wu et al. 2001). Additionally, exposure of the worms to PZQ can influence CaMKII gene expression (You et al. 2013). Activation of CaMKII can up-regulate SERCA via NFκB activation (Park et al. 2018). SERCA can also be over expressed after exposure of the worms to the drug due to SR/ER stress generated from the oxidative stress (Hetz 2012).

Salvador-Recatalà and Greenberg (2012) proposed that some downstream signaling pathways that are activated by Ca^{2+} should differ between resistant or juvenile worms which are far less sensitive to PZQ and susceptible adult worms. Therefore, as SERCA pump is one element of Ca^{2+} pathway, its participation in the mechanism of PZQ resistance could be indirectly associated with other Ca^{2+} related regulatory elements in this orchestrated scenario.

As regards the complex relation between SERCA, B cell lymphoma 2 (Bcl-2) proteins and P-gp, it was found that the anti-apoptotic Bcl-2 gene was highly expressed in juvenile than adult worms after exposure to sub-lethal dose of PZQ (Hines-Kay et al. 2012). Bcl-2 proteins act at the SR/ER to regulate intracellular calcium homeostasis through interaction with R_YR and IP_3R and/or SERCA thus contributes to blocking of Ca^{2+}-dependent apoptosis. These tightly regulated proteins are important in the signal pathway involved in the process of cell death. This multi-functional signaling highlights the effect of altered calcium flux across the SR/ER via IP_3R and R_YR or SERCA on the efficiencies of various apoptotic stimuli (Dremina et al. 2004; Eckenrode et al. 2010). In resistance to drugs, overexpression of both drug-efflux pumps as P-gp and the anti-apoptotic Bcl-2 family proteins are commonly linked (Szakács et al. 2006). It is found that activation of SERCA is mainly responsible for the resistance to metabolic stress-triggered apoptosis in cancer cells and SERCA inhibitors are effective in the treatment of multidrug resistant leukemic cells (Park et al. 2018). The complex sequences following elevated cytosolic Ca^{2+} after exposure to PZQ can lead to over expression of SERCA, activation of CamKII and increasing the activity of the ATPase associated with P-gp (Wu et al. 2001; Luo et al. 2013).

The above complex interacting processes can clarify the role of over expression of SERCA in decreased sensitivity of *Schistosoma* worms to PZQ. Over expression of SERCA can decrease cytosolic Ca^{2+}, increase the worm capacity to restore Ca^{2+} homeostasis, prevent the rise in Ca^{2+} concentration in the mitochondria and decrease apoptosis in addition to

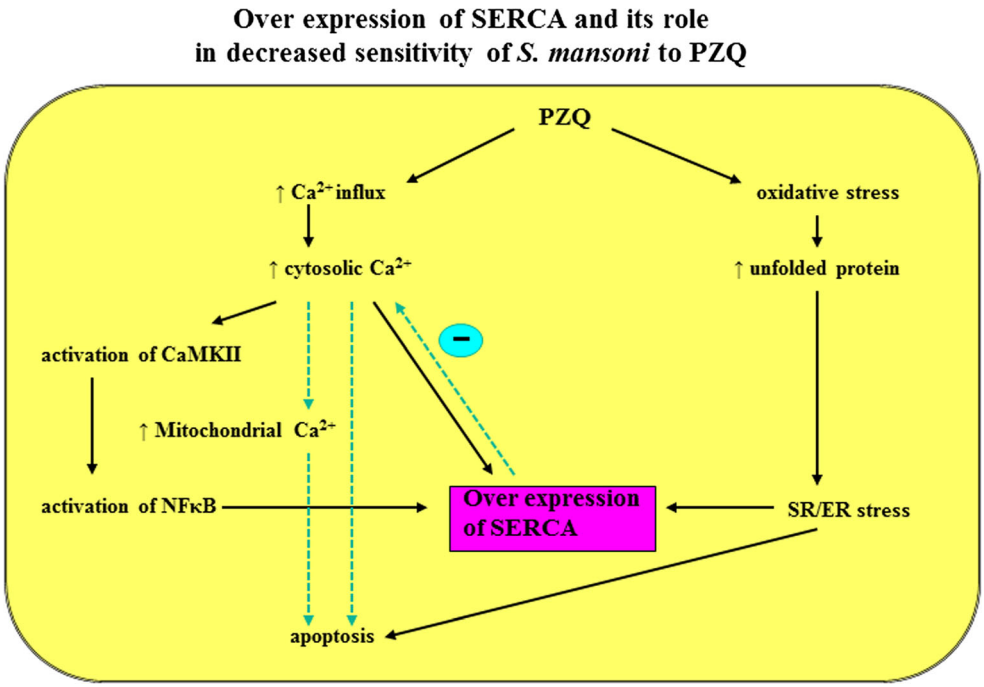

Fig. 2 Diagram illustrating the mechanism of up-regulation and role of SERCA in decreased sensitivity of *Schistosoma mansoni* to PZQ Up-regulation of SERCA is due to increase Ca^{2+} influx which by itself can lead to increase SERCA expression and also due to activation of CaMKII followed by activation of NFκB. The role of SERCA in decreased sensitivity of *S. mansoni* to PZQ is related to increase the worm capacity to restore Ca^{2+} homeostasis, decrease cytosolic Ca^{2+}, prevention of the rise in Ca^{2+} concentration in the mitochondria and decrease apoptosis. CaMKII: calmodulin kinase II, NFκB: Nuclear factor kappa B, PZQ: praziquantel, SR/ER: sarco/endoplasmic reticulum, UPR: unfolded protein response

its relation to P-gp. These sequences oppose the effect of PZQ and increase the resistance of the worm.

In schistosomes with reduced sensitivity to PZQ, it is possible that ATP-dependent multidrug transporters maintain low level of PZQ in the parasite. The agitations produced by Ca^{2+} influx and the downstream component of the cascade that is initiated by this interaction may be opposed by upregulation of SERCA and possibly by certain elements of Ca^{2+} signaling which modulate the process determining cells entrance in the apoptotic state.

In conclusion, reduced susceptibility in field isolates of *S. mansoni* has been found in different loci as a result of repeated use of praziquantel, the mainstay drug of schistosomiasis control. This work reveals the complex interacting processes leading to up-regulation of SERCA in *S. mansoni* worms with reduced sensitivity to praziquantel. The elevation of SERCA could be due to increase cytosolic Ca^{2+}, activation of calmodulin kinase II or induction of SR/ER stress. The possible mechanisms by which up-regulation of SERCA could counter act the low sensitivity to the drug are proposed. SERCA up-regulation increases the worm capacity to restore Ca^{2+} homeostasis, reduces cytosolic Ca^{2+} followed by lowering mitochondria $Ca^{2+}$and consequently inhibition of apoptosis. The relation of SERCA and the P-glycoprotein is discussed. The study of SERCA as one component of the calcium regulatory system in *S. mansoni* is of interest since it may provide clues about praziquantel's mode of action. Understanding the detailed molecular mechanisms of PZQ reduced sensitivity in *S. mansoni* is essential to recognize new therapeutic targets.

Revealing the principal mechanisms of up-regulation of SERCA in adult *S. mansoni* with reduced sensitivity to PZQ and its significance in reducing the schistosomicidal effect of the drug could lead to possible strategies to reverse drug resistance or develop alternative therapies to antagonize the reduced tolerance to PZQ specially with the advance in molecular modeling approaches.

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Compliance with ethical standards

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