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MOLECULAR PHARMACOLOGY OF CHEMO-RESISTANT LEISHMANIA

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ABSTRACT

Infectious diseases leishmaniosis among them, constitute a leading cause of death world wide, especially in the developing world, where they remain as an important cause of concern and has become a serious problem because of the everyday enhanced risk of co infection with HIV and the increasing frequency of resistance development of the parasites to the drug agents. Emergence of drug resistance is usually associated with changes in the expression of an specific membrane P-glycoprotein, but also includes physiological responses with high complexity. In the present review we summarize results which emphasize that the comprehension of the molecular pharmacology of drug-resistant phenotype must include, as a way for identifying new strategies for the control of the disease, the understanding of the multiple biochemical and functional parasite mechanisms involved.

Key Words: Infectious, Leishmaniosis, Resistance.

INTRODUCTION

Leishmaniosis is a syndrome with a variety of clinical manifestations classically named as visceral, cutaneous and mucocutaneous leishmaniosis and chemotherapy remains as the therapeutic approach normally used for controlling this disease (Barret et al. 1999; Hirst and Stapley, 2000; WHO, 2002). Although an enormous effort has been done in the last 20 years to a) design alternative less toxic therapies, b) describe additional valid drug targets against this disease, c) decrease the conditions which encourage the persistence of Leishmania in the vertebrate host, d) control the enhanced risk of co-infection with HIV and parasites causing visceral leishmaniosis, and e) understand the lack of response of some strains of Leishmania sp. to various drugs (Barral et al. 1991; Barret et al. 1999; WHO, 2002), these issues are still not controlled.

Sodium stibogluconate, the most frequently used antileishmanial drug was empirically developed more than 80 years ago, it is extremely toxic, should reach the intramacrophage stage of the parasite, and in order to be effective, should be delivered over 20 or more days, either by i.v. inoculation, for visceral leishmaniosis, or into single lesions for cutaneous manifestations (WHO, 2002). Alternative to this therapy, amphotericin B, allopurinol, pentamidine, paramomycin and more recently, inhibitors of the sterol biosynthesis such as miltefosine (Jha et al. 1999), are employed. Almost all these treatments remain unsatisfactory and the development alternative treatments both for visceral leishmaniosis and the topic

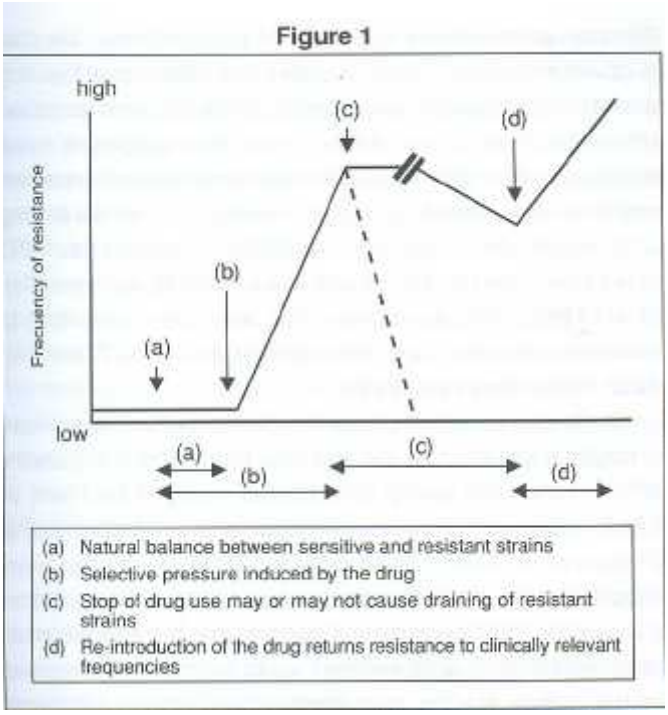
treatment of cutaneous leishmaniosis must be addressed. Additionally, the emergence of chemo-resistance, an effect that could be the result of natural resistance against the drug or acquired resistance developed when the parasites are exposed to sub-optimal drug doses **(Figure 1)** (Cohen, 1992; Sereno and Lemesre, 1997), constitute one of the main problems of chemotherapy. A rational way to address this issue should be based on a better knowledge of the biology of the parasite and its host, the responses developed during the host-parasite interaction and the elucidation of the molecular pharmacology of antileishmanial drug resistance.

LIFE CYCLE AND THE ROLE OF THE PLASMA MEMBRANE

The Leishmania life cycle is diheteroxene with two well described developmental forms, the intracellular amastigote, inside the reticuloendothelial cells of the mammalian hosts, and the extracellular promastigote, in the gut of a phlebotomine sandfly (WHO, 2002). As the physical and chemical differences among the hosts where Leishmania survives, impose a continuous stress on the organism seldom observed in nature, the plasma membrane should be fundamental for the successful host cell-parasite interaction. Unfortunately almost all the transport systems described up to now in Leishmania have been characterized in the promastigote stage and the studies have seldom used the intracellular amastigote stage relevant for the human disease.

For example, rapid and efficient plasma membrane transport systems activated by the different physical environments where the parasite lives have been demonstrated. That is, a) various membrane ATPases are associated with intracelullar calcium regulation, pH maintenance and osmotic homeostasis (Bakker-Grunwald, 1992; Jiang et al. 1994; Marchesini et al. 2002); b) an amiloride sensitive iso-osmotic Rb^+ transport system, that releases 1.3% of the intracellular Rb^+ from late-log phase Leishmania (L.) donovani promastigotes is up-regulated in a medium of reduced osmolality (Blum, 1992); c) proliferating L. donovani promastigotes exhibit a Rb^+ uptake that is partially inhibited by blockers of ion-translocating ATPases such as N,N‘-dicyclohexylcarbodimide (DCCD) and N-ethyl maleimide (NEM) (Suffia et al. 1997); d) the resulting current obtained by the incorporation of purified membrane vesicles from Leishmania (L.) mexicana into lipid bilayers was found to resemble current steps typical of ion channels (DiFranco et al. 1995), and e) pharmacological data have demonstrated that L. mexicana are sensitive to voltage-dependent K^+ channel blockers, TP-**b**inding-**c**assette (ABC) transporter blockers, Na^+ channel and Na^+/H^+ antiporter blockers, and chloride channel blockers (Ponte-Sucre et al. 1998). These data indicate that besides the metabolite transporters which have been characterized in Leishmania (Glaser and Mukkada, 1992; TerKuile and Opperdoes, 1993; Blum et al. 1999; Burchmore and Barret, 2001), ion-translocating plasma membrane transporters and various ATPases are present in the surface membrane of the parasite and are responsible for the maintenance of cell homeostasis.

More interestingly, as above mentioned, these parasites are adapted to stressful conditions through their life cycle such as extreme temperature and pH (Zilberstein and Shapira, 1995; Ullman, 1995), parameters that in vitro induce the promastigote transformation into the "amastigote like" parasite (Turco and Sacks, 1991). Very few data on how these physiological events are influenced by drug resistance and how drug resistance interferes with the events that trigger and regulate the parasite differentiation through their life cycle have been described (Sereno and Lemesre, 1997). Due to the significance of the in vivo transmission of drug-resistant parasites by Phlebotomine, the answer to this question is extremely relevant.

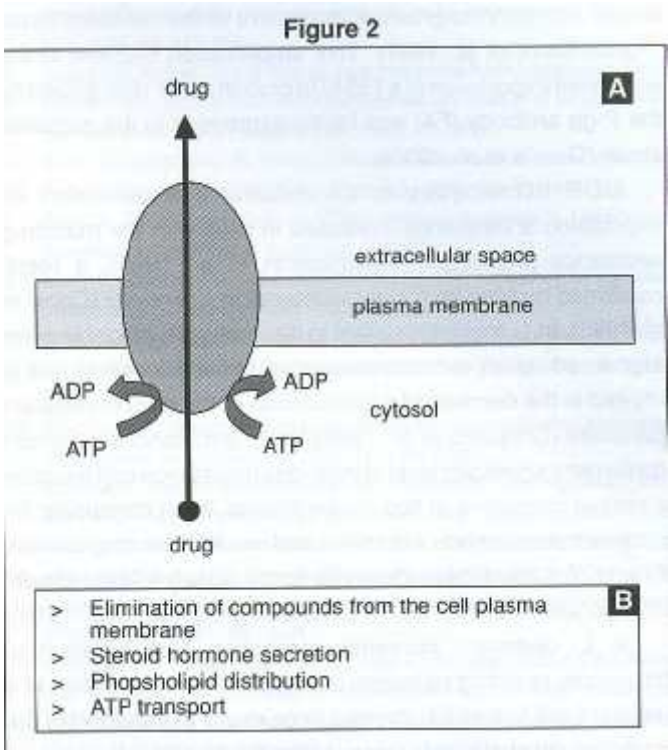


Natural equilibrium between drug-sensitive and drug-resistant strains can be disrupted by the continuous presence of the drug and its consequences. The emergence of chemo-resistance then could be the result of natural resistance against the drug or acquired resistance developed when the parasites are exposed to sub-optimal drug doses.

THE MOLECULAR PHARMACOLOGY OF CHEMO-RESISTANCE

The expression of a conserved type of membrane protein called P-glycoprotein (P-gp) (**Figure 2A**) has been one of the most consistent changes detected in many chemo-resistant cells including tumor cells (Higgins, 1992). It is a member of the ABC -for TP inding assette-transporter family, (Doige and Ames, 1993). P-gp expression seems to increase as a result of chemotherapy but it is also involved in the elimination of compounds from the plasma membrane, steroid hormone secretion, phospholipid distribution and ATP transport (Orlowski and Garrigos, 1999) (**Figure 2B**) of normal cells, a fact which indicates that they should have additional physiological and pathological meanings relevant on whether and how they can be targeted to improve therapy (Bradshaw and Arceci, 1998).

On the other hand, altered membrane partition models for decreased drug accumulation in chemo-resistant cells have become popular (Roepe, 2000). In fact, although the most popular models suggest the use of the energy released through the hydrolysis of ATP for the translocation of drugs out of cells by P-gp, some data suggest that over expression of the P-gp perturbs the electrical membrane potential and/or intracellular pH and indirectly alters translocation and intracellular retention of cationic, weakly basic, hydrophobic drugs (Roepe and Martiney, 1999). Alternatively it has also been proposed that the protein alternates between drug pump and Cl- channel (or channel regulator) conformations, implying that both direct and indirect mechanisms of altered drug translocation may be catalyzed by MDR protein (Roepe and Martiney, 1999).



2A) ABC transporters extrude drugs from the cell against their concentration gradients through a conserved type of protein called P-glycoprotein.

2B) P-gp is involved in the elimination of compounds from the cell plasma membrane, steroid hormone secretion, phospholipid distribution and ATP transport.

THE MOLECULAR PHARMACOLOGY OF LEISHMANIA CHEMO-RESISTANCE

Similar to chemo-resistant tumor cells, the increased expression of P-gp like proteins have been frequently associated with decreased cellular accumulation of the used compounds in chemo-resistant Leishmania. In fact, the data suggest that Leishmania ABC-type multidrug transporters can be included in the P-gp (Higgins, 1992) and the multidrug resistance-associated protein (MRP) (Cole et al. 1994) plasma membrane transporter.

MRP homologues in Leishmania include ltpgpA, an extrachromosomal circle (H-circle) amplified in a methotrexate resistant L. tarentolae promastigote cell line (Ouellette et al. 1991) as well as a group of genes whose gene products confer low levels of resistance to vinblastine, arsenite and trivalent antimonials in L. tarentolae and L. major (Ellenberger and Beverly, 1989; Ouellette and Borst, 1991; Légaré et al. 1994; Papadopolou et al. 1994). The transfection of ltpgpA into sensitive parasites, triggers the decrease in the accumulation of pentostam (Mukhopadhyay et al. 1996; Haimeur and Ouellette, 1998; Haimeur et al. 1999; 2000). In L. mexicana resistant to general blockers of ABC transporters, the amplification of a fragment which hybridized to ltpgpA and shows size polymorphism was evident in the resistant strain (Ponte-Sucre et al. 1997). This amplification resulted in the enhanced expression of a 185 kDa protein band, recognized by the P-gp antibody (F4) and faintly expressed in the sensitive strain (García et al. 2000).

MDR homologues in Leishmania include mdr1; its expression is frequently increased in cells with the multidrug resistance phenotype (Hendrickson et al. 1993), a result confirmed by transfection experiments in L. enriettii (Chow et al. 1993). In L. tropica resistant to

daunomycin, ltmdr1 is over-expressed as an extrachromosomal circular location and is implied in the decreased accumulation of the drug in resistant parasites (Chiquero et al. 1998). In L. amazonensis, lamdr1 conferred a significant level of multi-drug resistance and encodes a protein consisting of two similar halves, each containing six putative transmembrane domains and one ATP-binding domain, 91 and 78% identical respectively to the closely related ldmdr1 in L. donovani and lemdr1 in L. enriettii (Katakura et al. 1999).

In L. donovani parasites, selection and isolation of tubericidin-resistant parasites lead to the characterization of a resistant cell line which showed impairment in transport of the drug through nucleoside transporters expressed in the plasma membrane (Hendrickson et al. 1993; Vasudevan et al. 2001). Alternative studies have shown that methrotexate-resistant L. donovani are deficient in the folate-methotrexate transporter (Kaur et al. 1998). In pentamidine-resistant cell lines, this phenotype has been shown to be linked to a decreased accumulation of the compound, accompanied by decreased transport of structurally unrelated molecules such as pyrimidine nucleotide and alterations of the polyamine levels (Basselin et al. 1997).

But, are there additional physiological functions involved in the expression of chemo-resistance in Leishmania? Recently described data suggest that alternative genes not related to drug transport, can be also over expressed as a result of chemo-resistance. Of note these physiological responses cannot explain drug resistance by the membrane partition models and suggest novel mechanisms for Leishmania chemo-resistance.

For example, resistant L. tarentolae strains that accumulate less arsenite than wild type cells (Dey et al. 1994) do not show difference in the rate of arsenite accumulation in everted plasma membrane-enriched vesicles prepared from wild type and arsenite-resistant cells has been found (Dey et al. 1996). Of note, an increased synthesis of trypanothione, the thiol involved in the arsenical conjugation and extrusion of the drug and of the conjugated transporter substrate has been described (Mukhopadhyay et al. 1996). These data suggest a novel mechanism of drug resistance through a multigene mechanism which involves ltpgpA, an enzyme involved in the rate limiting step of gluthathione biosynthesis (GSH) (Grondin et al. 1997) and a transporter which actively efflux As(III) glutathione (Dey et al. 1996), This mechanism has also been described in vinblastine resistant Leishmania (Wong et al. 1994; Chow et al. 1993; Henderson et al. 1992).

L. donovani made resistant to arsenite had similar levels of a tubulin expression as the wild type promastigotes, parasite differentiation into axenic amastigotes changed the levels of tubulin expression and phosphorylation between the two strains (Prasad et al. 2000; Prasad and Dey, 2000). As tubulin plays important roles in proliferation, cell shape and differentiation (Chan et al. 1991these results suggest that the expression of drug resistance could alter either the tubulin proteins themselves or the events leading to cytoeskeletal changes that occur during parasite differentiation (Prasad et al. 2000; Prasad and Dey, 2000).

In L. donovani resistant to amphotericin-B, changes in the membrane transport of the drug and a lower membrane micro-viscosity have been demonstrated (Mbongo et al. 1998). The change in micro-viscosity has been associated with changes in the prevalent lipids present in resistant L. donovani cell plasma membrane, instead of the ergosterol normally found in wild type parasites, saturated fatty acids and an ergosterol precursor cholesta-5,7,24-trien-3 b-ol were found in resistant. Finally, terbinafine-resistant L. major had an increased expression of ergosterol biosynthetic intermediates (Cotrim et al. 1999) and in pentamidine-resistant L.

amazonensis alterations in membrane fluidity, lipid content and loss of pentamidine binding sites have been described (Basselin and Robert-Gero, 1998).

Infectivity is affected by drug resistance in L. donovani (Chan et al. 1991), Leishmania (V.) guyanensis (Gazola et al. 2001) and L. mexicana (García et al. 2000; Silva and Ponte-Sucre, 2001). This effect correlated with a decreased agglutination pattern for lectins in L. (V.) guyanensis (Gazola et al. 2001) and L. mexicana (Camacho, N. personal communication), a decreased expression of the L. mexicana meta-1 protein (Camacho, N. personal communication), a decreased activity of L. mexicana acid phosphatase (Garcia et al. 2000), alterations in lipophosphoglycan expression in pentamidine resistant parasites (Basselin and Robert-Gero, 1998) and an altered pattern of serine residue phosphorylation (García et al. 2000).

Cellular ATP and rate of respiration are fundamental for cell survival. Although in pentamidine and antimycin-A -resistant parasites, a modification of mitochondrial activity (Basselin and Robert-Gero, 1998) and a point mutation in the mitochondrial apocytochrome b (Cyb) gene (Schanufer et al. 2000) have been demonstrated; in arsenite resistant L. mexicana amazonensis, significant changes in the rate of respiration and cellular ATP content between chemo-resistant and wild type cells were not observed (Singh y Lee, 1999), but in L. mexicana resistant to ABC transporter-blockers, the metabolite preferences were found to be altered and the production of glycolytic derived pyruvate precursors for the Krebs cycle and the expression of the glucose transporter were found to be significantly decreased (Uzcategui, N. and Figarella, K., personal communication).

CONCLUSION

Drug resistance is one of the most serious problems in the control of infectious diseases. For leishmaniosis it is becoming a common problem in many endemic areas. This phenomena emerge from the ability of the parasites to adapt to the anthropogenic pressure. Although over expression of membrane-bound ABC transporters occurr, their function cannot explain many of the relevant features related to the expression of drug resistance. The data presented herein suggest that besides the classical increase in the expression of P-gp, the development of drug resistance affect fundamental parasite’s functions such as infectivity, incorporation of metabolites considered to be fundamental for the parasite survival, xenobiotics conjugation and extrussion, host-parasite interaction, cell shape, differentiation and oxidative phosphorylation, mechanisms that may be central to impair the successful treatment of leishmaniosis. Due to the significance of the in vivo transmission of drug-resistant parasites, attention should then be given to understand the biology of the resistant parasite, including the alternative mechanisms involved in this phenomena, as well as their pharmacological meaning related to the function of P-gp in the context of drug resistance phenotypes.

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