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REVIEW ARTICLE

Role of Polyamines in Parasite Cell Architecture and Function

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Abstract: In the absence of accessible, effective vaccines, the fight against parasitic disease relies mostly on chemotherapy. Nevertheless, the considerable side effects, high costs and growing number of refractory cases comprise substantial drawbacks. Thus, the search for new antiparasitic compounds remains a high priority. The polyamine biosynthesis, conversion and transport pathways offer different targets for selective chemotherapy. Polyamine analogues and other antagonists may provide tools in the search for new lead compounds. Light and electron microscopy techniques may encompass valuable approaches to elucidate the possible mechanisms of action of different antiparasitic compounds, allowing the identification of subcellular target compartments, presumably establishing the basis for a more rational drug design and/or planning of therapeutic strategies.

Keywords: Polyamines, Electron microscopy, chemotherapy, putrescine, parasitic disease, therapeutic strategies.

1. INTRODUCTION

Parasitic diseases still comprise the etiology of huge morbidity and mortality in many parts of the globe, particularly low resource countries. Thus, citizens of wealthy nations, whenever infected in developing countries, for military, commercial or touristic purposes, must join the Third World neglected patients and rely on old-fashioned drugs, often faced with resistant pathogens and associated with severe side effects [1-3]. Nevertheless, Chagas disease and leishmaniasis are spreading with the wider distribution of the vectors led by global warming [4] and host migrations [5]. Furthermore, tropical diseases such as malaria may be acquired in airplanes or airport neighborhoods. Nowadays microbes fly much more rapidly and longer, reaching distant nations in a few hours. In a globalized world, we must consider a globalized epidemiology.

Polyamines (PA) such as spermidine (N-(3-aminopropyl)butane-1, 4-diamine), spermine (N, N'-bis(3-aminopropyl)butane-1, 4-diamine) and the diamine putrescine (1, 4-diaminobutane) are ubiquitous, biogenic, low molecular weight, aliphatic polycations that play pivotal and pleiotropic roles in processes such as genome transcription, translation, protein regulation, orchestrating cell division, differentiation and general functioning [6-7].

Spermidine (Spd) and spermine (Spm) were named after their early discovery in the human semen. Anthony van Leuwenhoek (1632-1723) observed Spm crystals in human semen [8]. Putrescine (Putr) and cadaverine (pentane-1, 5-diamine) were so termed for their detection in decaying (putrid) material such as corpses (cadaveric tissues). However, these rather versatile molecules are strongly associated to living organisms [6-7], rather than being putrid material, PA are particularly abundant in proliferating cells [9], which are, by definition, viable.

This fact accounts for the remarkable interest in PA biology in cancer cells [10] and parasites [11]. Tumor cells and parasitic organisms share a number of features [12-13], including evasion mechanisms' invasive and proliferation capacities and tumors may be considered metabolic parasites [14].

Many of these properties are largely regulated by polyamine levels, leading to the successful use of anticancer drugs in antiparasitic chemotherapy [15] and *vice versa* [16-18]. Thus, polyamine conversion and transport pathways are studied in both cancer and parasitic disease models.

2. POLYAMINES IN CELLS ARCHITECTURE AND FUNCTION

Conciderable differences in PA distribution are observed among mammalian tissues and organs as well as among species [6], but most of its biological roles are evolutionarily conserved.

PA electrostatic binding to anionic sites on cell membranes may stabilize cell surfaces and cytoplasmic organelles such as mitochondria, lysosomes *etc.* [19]. PA binding to phospholipid polar head groups and anionic domains of proteins can stabilize red blood cell plasma membranes and membrane skeleton, reducing deformability and fragmentation [8, 20]. PA also regulate vesicle trafficking [21], mast cell granule fusion [22-23], but PA *per se* are also able to promote membrane fusion [24]. Polyamines can bind to anionic sites in proteins and were shown to modulate the activity of ion channels such as glutamate receptors [25], Na^+, K+-ATPases, phospholipases [19] *etc.*

Microtubule structure is highly conserved in eukaryotes, but their functioning is largely modulated by post-translational modification, such as acetylation or phosphorylation (also observed in other proteins), detyrosination/tyrosination, polyglutamylation, and polyglycylation [26]. The covalent binding of spermidine and putrescine to proteins, termed as "polyamination", has been known for over three decades [27], but its biological significance was then mostly speculative. Although this intriguing, recently disclosed post-translational modification remains largely overlooked, it is recognized as a modulator of microtubule stability and resistance to Ca^{2+} as well as low temperatures [28]. Transglutaminase-catalyzed polyamination of several glutamine residues on both α and β tubulins stabilizes microtubule in nerve cells. Transglutaminases such as TG2 help nucleate and stimulate tubulin polymerization, promoting

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axon microtubule stability, neurite growth [29], as well as stabilization of neuronal connections [30].

The functional role of these polycations in cytoskeleton functioning [31-33] may explain at least in part the role of PA in theregulation of cell migration and microvascular sprouting [32, 34]. DFMO was shown to inhibit epithelial cell migration and this effect was reversed by exogenous PAs [35]. PA effects on cell migration may be brought by cell attachment to extracellular matrix [36], focal adhesion kinase signaling [35], cytoskeleton functioning influencing both microtubules [33, 37] and actin microfilaments [38-39] as well as Rho-dependent responses [35].

Interestingly, polyamine deprivation alters the ER cisternae and Golgi apparatus organization affecting actin microfilaments but not microtubules [40]. Cell migration may be also modulated by potassium channels [35, 41]. Spermidine/spermine N1-acetyltransferase (SSAT) binds to the cytoplasmic portion of α9β1 integrins, where it catalyzes the PA acetylation and consequent degradation or excretion. This metabolic event can revert the PA-mediated blockage of Kir 4.2 inward-rectifier potassium channels and therefore the inward K^+ movement can promote cell migration [42].

Sharp increases in polyamine levels usually precede the cell proliferation onset, including cancer, embryo tissues and parasites [8]. Polyamine biosyntesis increases are preceded by abruptly enhanced activity of enzyme ornithine decarboxylase (ODC, EC 4.1.1.17), required for liver regeneration [43] and pancreatic integrity [44]. The amino acid L-ornithine may be internalized from the extracellular milieu through surface transporters or obtained from L-arginine by the action of arginase (EC 3.5.3.1) (Fig. **1**). The short-lived ODC catalyzes the rate-limiting decarboxylation of ornithine giving rise to Putr, which receives an amminopropyl group from decarboxylated S-adenosyl-L-methionine (dc-AdoMet), forming Spd. The addition of another amminopropyl gives rise to Spm. These reactions are catalyzed by aminopropyltransferases termed as spermidine synthase (EC 2.5.1.16) and spermine synthase (EC 2.5.1.22), respectively. The rapid turnover of ODC, its antizyme and antizyme inhibitor as well as transport and conversion systems allow the fine-tuning of polyamine levels [6, 45]. High

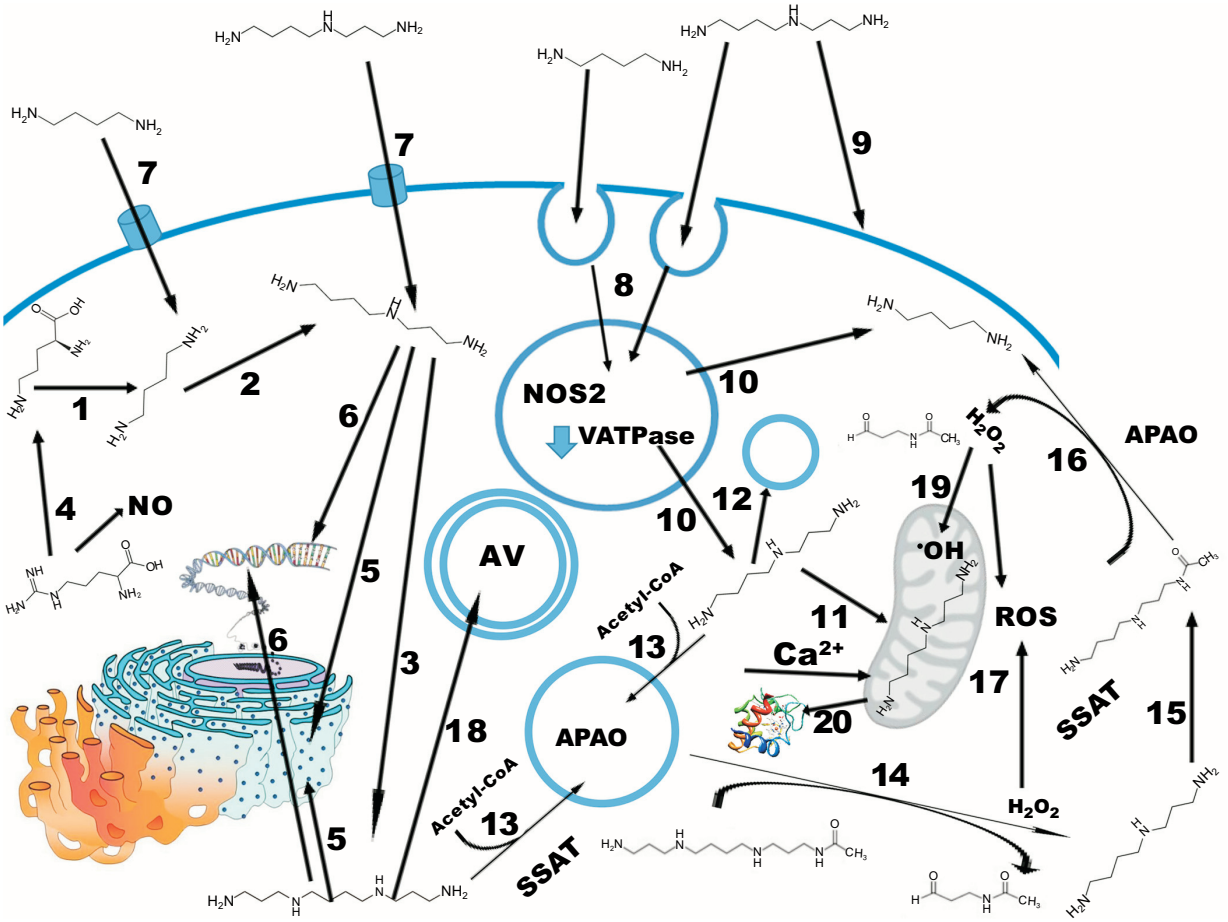

Fig. (1). Polyamines pathways within the cell.

Biosynthesis begins with the decarboxylation of internalized L-ornithine by ODC (1), giving rise to Putr, which receives an aminopropyl group form AdoMet in a reaction catalyzed by Spd synthase (2). The formed Spd receives another aminopropyl via Spm synthase (3) forming Spm. Alternatively ornithine may be generated from L-arginine by arginase (4). This amino acid may also be a substrate in the NO synthesis. The cytoplasmatic PAs bind to RNA (5) and DNA (6). Preformed extracellular polyamines are transported via surface permeases (7), or ingested through caveolae and addressed to acidic vesicles in the cytoplasm (8) and bind to surface proteins and lipids (9). The PA are released from receptors by the action of NOS2 and exit the vesicles by pH elevation resultant of VATPase inhibition (10). Cytoplasmic PA bind and are incorporated by mitochondria regulating Ca^{2+} accumulation (11). PA bind to lysosomal membranes (12). PA are acetylated by spermidine/spermine N^1-acetyltransferase (SSAT) and transferred to peroxisomes (13). The peroxisomal enzyme N^1-acetylpolyamine oxidase (APAO) catalyzes the oxidation of N^1-acetylated Spm (14) and Spd (16) generating Spd and Putr, respectivelly. The acetylation of Spd by SSAT forms the substrate for APAO (15). The oxidation of N^1-acetylpolyamines by APAO (14, 16) produces aldehydes such as 3-acetoaminopropanal and H_2O_2 and the latter in turn leads to the formation of ROS (17). Under stress conditions PA promote the formation (18) of autophagic vacuoles (AV). The H_2O_2 may lead to the formation of hydroxyl radicals via Fenton-like reactions (19). This may cause MPT and thus trigger apoptosis via cytochrome *c* release (20).

cellular polyamine levels upregulate the antizyme that inactivates ODC, which is targeted to ubiquitin-independent degradation by the 26S proteasome [46].

The polyamine oxidase[1]* (PAO)-dependent interconversion pathway may also furnish Putr and Spd from the higher polyamines, *i.e.* Spd and Spm, respectively [45, 47, 48].

Mice *ODC* and *AdoMet decarboxylase* gene knockouts are lethal at early embryonic development. Spermidine synthase deficiency causes severe disorders (*vide infra*), reduced longevity, whereas spermine and spermidine may promote longevity reducing aging outcomes [49]. Also, nona/centenarians display higher spermidine and spermine blood levels [50] and it has been proposed that in addition to endogenous biosynthesis, the polyamines required for longevity maybe obtained from food [51], and from gut bacteria [50, 52]. Nevertheless, probiotic microrganisms such as *Lactobacillus rhamnosus* GG can via arginine deiminase diminish PA biosynthesis by gastric cells, preventing neoplastic transformation of the mucosa [53]. Thus, different human diseases are produced by unbalanced polyamine metabolism (*vide infra*), demonstrating that polyamines are required for normal mammalian physiology [54-55].

Although polyamines are usually associated with rapidly proliferating cells, non-dividing villus tip enterocytes display high ODC activities [56], indicating different functions for these small versatile molecules. These authors suggested a role for the endogenous polyamines in the mitochondrial function (*vide infra*). In this regard putrescine may function as energy source for *Escherichia coli* [57] via ɤ-glutamylputrescine synthetase [58].

The amine positive charges of these molecules at physiological pH mediate their electrostatic binding to acidic sites on macromolecules including nucleic acids and proteins, as well as phospholipids, accounting for their involvement in multiple phenomena of remarkable relevance in the living cell [6, 59].

Cytochemical staining revealed polyamine binding to condensed chromatin in dividing cells [60-61], indicating the role of these polycations in chromatin compaction via charge neutralization [62]. Later polyamines were demonstrated by immunocytochemistry in nuclei and nucleoli of different cell types [63].

The DNA-binding via phosphate groups lead to the formation of DNA-PA aggregates which may play a role in DNA physiology [64-66] and may account for its properties in gene expression regulation and a "polyamine modulon" was described in *Escherichia coli*, but similar functions are played in mammalian genes including *c-Myc* and *c-Jun* [54], activating stress response sequences by induction of stress responsive regulons. The polyamine regulon activates the production of several transcription factors and other molecules required for cell growth [6]. Chromatin condensation is modulated by DNA-binding by Pas [67] and ODC activity is able to regulate histone acetyltransferase leading to chromatin histone hyperacetylation and influencing gene expression and possibly carcinogenesis [68-69].

Spd is the precursor of hypusine, involved in hypusination, a post-translational modification of the elongation factor eIF5A, regulating protein synthesis [70].

Most of the polyamine molecules may be found in association with transporter and ribosomal RNA [6] and these polycations can regulate the functioning of tRNA, rRNA and mRNA [71].

PA bound to free and endoplasmic reticulum (ER)-bound ribosomes were revealed by electron microscopy immunolocalization to be mainly found in rough ER cisternae [72-74], whereas the nuclei were not labelled, indicating a role in active ribosome stabilization, thus regulating protein synthesis, regulating the aminoacyl tRNA formation, initiation, extension and translation fidelity [8]. In this regard, Spd modulates ribosome subunit dissociation [75]. Both Mg^{2+} and Putr may influence RNA stability [76]. The biological role of Spm is largely unclear, but it may comprise a Spd storage molecule.

3. TRANSPORT

The regulation of cytoplasmic PA relies largely on cell surface transporters in both microbial [77] and mammalian cells [78]. In mammals, the polymine source is mostly nutritional. Foods such as meat are rich in spermine whereas cheese and fruit are rich in putrescine [79].

Enteric microbiota also comprises a major PA source and its synthesis by murine intestinal bacteria such as *Bifidobacterium animalis lactis* were shown to delay senescence [52], and PA can prevent memory impairment in a autophagy-dependent mechanism [80-81]. Therefore, diverse organisms evolved PA transport systems. PA permeases were characterized in *Trypanosoma cruzi*, *Leishmania* (*vide infra*) and yeast cells [82].

Different mammalian cell types employ multiple PA transport mechanisms. These polycations may be incorporated both via cell membranes solute carrier transport system [83] and via lipid raft caveolae [84].

PA were shown to bind strongly via electrostatic charges to proteoglycans such as the glycosylphosphatidylinositol-anchored surface heparan sulfate glypican-1, which mediate their uptake by mammalian cells [85-86]. PA histochemical detection revealed intense cytoplasmic labeling of human granulocytes and secretory cells [87-88]. Soulet *et al*., using BODIPY- and [^{3}H]-labelled Spd, demonstrated that PA are incorporated via receptor-mediated endocytosis [89] and accumulated in acidic membrane-bounded compartments also largely stained by the lysosome probe lysotracker. Afterwards the incorporated PA is released from a putative receptor by the action of NO and then transferred to the cytoplasm upon vacuolar ATPase inhibition-mediate pH elevation [90]. Amine accumulation within lysosomes cause osmotic imbalance resulting in water entry into the organelle and the pressure exerted on the organelle lipid bilayer may culminate in lysosomal disrupture, triggering distinct cell death subroutines (*vide infra*). Furthermore, lysosomes generate ROS via Fe^{2+} or Fe^{3+} in the presence of H_2O_2 by the Fenton reaction (*vide infra*) as well as via electron carrying in a redox chain involving NADH, FAD, ubiquinone and cytochrome b, keeping the reduced state and proton gradient of the organelle [91].

Neuron mitochondria and lysosomes were not immunocytochemically stained for PA [72-73]. Nevertheless, mitochondria uptake PA (*vide infra*) and mast cell granules which are considered ''secretory lysosomes'' accumulate polyamines [23]. It is noteworthy that PA are able to regulate membrane fusion [24, 92] and mast cell secretion [93]. In order to act in mammalian neuromodulation upon N-methyl-D-aspartate receptors, Spd and Spm are transponted into vesicles in neurons and astrocyres via a vesicular polyamine transporter (VPAT), encoded by the *SLC18B1* gene [94]. In addition, besides its anti-oxidant properties, PA may exert lysosomal stabilization [19] accounting at least in part for its anti-inflammatory effects [95]. Furthermore, PA, particularly Spd regulate cystine efflux from lysosomes [19]. In addition, proteomic analysis showed that polyamines are pumped into the acidocalcisomes, organelles conserved from bacteria to humans [96] and at least in a protozoan parasite may be related to lysosomal compartments (*vide infra*).

Remarkably, Putr [97] and PA [98] analogues may be lysosomotropic. Different amines [99] and amine-containing drugs [100] were reported to be lysosomotropic and amines were detected in compartments formed by the endosome-lysosome fusion [101]. Synthetic and endogenous amines, including the polyamine me-

[1]*More recently termed N1-acetylpolyamine oxidase (APAO)

tabolite 3-aminopropanal (3-AP) may be trapped within lysosomes [101-102]. The 3-AP accumulation may lead to lysosomal disruption and therefore either apoptotic or necrotic cell death [69-103], including human cells [104]. In this regard, the polyamine oxidase inhibitor MDL-72, 527 induces apoptosis of hematopoetic cells by lysosomotropic effects [105].

PA may enter the cytoplasm via gap junctions regulating cell functions such as astrocytic coupling [106], epithelial cells proliferation and stress response [107] and skin pattern formation in the zebrafish *Danio rerio* [108]. Interestingly the microtubule function influenced by PA may promote gap junction formation [37]. It is noteworthy that PA are required for actin filaments and microtubules organization [33, 38]. The role of PA such as Spm on cancer cell migration may rely not only on the polycation effects on cytoskeleton, but also mitochondrial function [109].

4. POLYAMINES IN MITOCHONDRIA

A new field in biomedical sciences, termed mitochondrial medicine [110-112] exploits the understanding of this pivotal organelle functioning in health and pathologic conditions as a strategy in designing and developing innovative therapeutic interventions [29, 113, 114].

The polyamine role in mitochondrial function may be inferred from the transport of these cations in and out the organelle [115] and preferential effects of antagonists upon this compartment. DFMO causes mitochondrial damage which is reverted by spermine addition [56]. Methylglyoxal bis(guanylhydrazone) (MGBG), a competitive inhibitor of S-AdoMet decarboxylase, blocks PA biosynthesis affects mitochondrial function [59], and thus diminishes cell proliferation [116]. In addition mitochondrial polyamine transporters may be targeted by polyamine-conjugated compounds [117-119].

PA were shown to stabilize mitochondrial membranes [120], by different mechanisms *i.e.* crosslinking anionic groups at phospholipids and proteins [121], quenching ROS [122] and inhibition of the the activity of phospholipase A2, so Spd can repair rat liver mitochondria [123]. PA also protect the organelle nucleic acids, as demonstrated by the depletion of mitochondrial DNA by the PA analogue N1, N12-bis(ethyl)spermine [124].

PA antioxidant activity may regulate mitochondrial function and it was also shown that spermine can scavenge free radicals within the organelle [121] promoting oxidative stress resistance (*vide supra*). PA also regulate the Ca^{2+} and phosphate transport into the organelle [19]. Cytoplasmic Ca^{2+} homeostasis is regulated by endoplasmic reticulum, mitochondria and mitochondria-associated ER membrane (MAM), wich plays several roles in cell signaling and function, including Ca^{2+} transport, ER stress, mitochondrial morphology, autophagy, apoptosis and inflammatory signaling [125]. Spm and Spd may enhance Ca^{2+} accumulation from the cytoplasm and even regulate mitochondrial permeability transition (MPT) [126] and ODC inhibition may trigger apoptosis [127].

The polyamine effects on mitochondria may orchestrate the cell death by triggering multiple apoptotic pathways in mammalian cells. Polyamines and its oxidation products may respectively prevent and promote MPT [115]. Besides cytochrome c, MPT Resulting from megachannel opening leads to the release of endonuclease G and Apoptosis Inducing Factor (AIF) [115].

It is noteworthy that MPT is a common event in autophagic, apoptotic and necrotic cell death processes [128]. The diamine agmatine is incorporated by mammalian mitochondria modulating the MPT and thus apoptosis [129]. Polyamine biosynthesis prevents tumor necrosis factor α-induced apoptosis by reducing the oxidative stress [130]. Nevertheless polyamine deficiency was reported to inhibit the release of cytochrome c, preventing camptothecin [131] and ischemia-induced apoptosis [132].

Tyrosine kinases such as epidermal growth factor receptor and Src family kinases were shown to translocate to mitochondria in response to different proliferative stimuli. These processes are regulated by mitochondrial PA and oxidative stress as phosphotyrosine phosphatases may be inhibited by ROS production [133].

Polyamines appear to be necessary for mammalian cell mitochondria functioning and integrity [56], control the mitochondrial metabolic rates [126] protect *Saccharomyces cerevisiae* from oxygen toxicity and preserve mitochondrial function of grown anaerobically yeast cells [134].

PA also regulate mitochondrial protein synthesis as Spm promotes fMet-tRNA binding to mitochondrial 55S ribosomal particles leading to initiation of RNA translation in the organelle [135]. Spm molecules bind to ATP molecules [136], keep high ATP cellular levels by down modulating the F1-ATPase activity [115]. PAs were preferentially found in the mitochondria-rich midportion of spermatozoa [137]. Nevertheless, polyamines may also down regulate mitochondrial function [138]. Putr addition enhanced the ATP synthesis in DFMO-treated mammalian cells [139-140].

Different lines of evidence indicate that mitochondria comprise valuable targets in chemotherapy for different disorders, including cancer [141-142], ischemia-reperfusion injury [143] and parasitic diseases [144-146]. Therefore, a better understanding of the mechanisms underlying mitochondrial transport systems and their role in cell homeostasis may elucidate the action of lead compounds and furnish new strategies for rational drug design, based on the parasite cell biology [147-150]. In this regard, the leishmanial drug resistance is a complex, multifactorial phenomenon *loc. cit.* [151], but it is worth mentioning that *Leishmania mexicana* resistance to pentamidine involves exclusion of the drug from the protozoan single mitochondrion [152]. Synthetic naphthoquinones exert trypanocidal effects caused by mitochondrial dysfunction [147]. Mitochondrial damage or destruction was also observed in parasites incubated with sterol biosynthesis inhibitors [153, 154].

5. POLYAMINES IN STRESS

PA are not only implicated in cell proliferation [8], but also can regulate cell death by regulating programmed cell death in animal and plant organisms [155]. Several cell death mechanisms were discovered and characterized in the last decade [156] and many signaling events build a complex network encompassing cross-talking pathways that ultimately determine cell fate [84, 155, 156, 157]. Polyamines may take part in different steps of these mechanisms namely necrosis [158] and apoptosis [133], which may involve distinct cell organelles [159]. Therefore, PA may be involved in mechanisms of cell stress triggered by lysosomal [160], mitochondrial [133], peroxisomal [161] or endoplasmic reticulum [162] pathways.

PA may also play an important role in programmed cell death mechanisms [6, 163, 164] such as the p53-dependent apoptosis of intestinal epithelial cells [165] or in transgenic pancreatitis apoptosis and necrosis [166, 167] and programmed necrosis [168].

Furthermore polyamine oxidation leading to H_2O_2 production may cause mitochondrial uncoupling and cytochrome *c* release [169] (*vide infra*). Such properties motivate its exploitation for chemotherapy and/or chemoprevention [170-172]. Interestingly polyamine levels can switch cell death from apoptosis to necrosis [173]. In addition, polyamines may trigger autophagy [174] that may either lead to cell death or enhance cellular and organismal lifespan [175-176]. In this regard, Spm was shown to trigger autophagy by p53 activation in HT1080 cells [177].

Polyamines may be considered “stress molecules” [178], playing multiple roles in response to drug-induced [179-181], temperature [182-183], radiation [184], UV irradiation [185] acid [186] or osmotic stress [187] as well as biotic and abiotic stress tolerance in plants [188-189]. In addition, PA were shown to inhibit the assem-

bly of stress granules in intestinal epithelial cells modulating apoptosis [190].

Cells are continuously exposed to oxidative stress as the production of Reactive Oxidative Species (ROS) is not completely counterbalanced by antioxidant mechanisms involving detoxifying molecules such as Glutathione (GSH) and GSH-dependent enzymes (*e.g.* glutathione peroxidase), superoxide dismutase etc. ROS may be endogenously produced by H_2O_2 breakdown. H_2O_2 is produced not only by mitochondria, but also by peroxisomes, endoplasmic reticulum P450 system and cytoplasmic oxidases. In peroxisomes there are several oxidases [91], including polyamine oxidase (PAO).

The H_2O_2 entering lysosomes can in the presence of iron undergo a Fenton reaction leading to the production of hydroxyl radicals ($HO^{\bullet-}$), which may damage lysosomal membrane [91, 160]. Disruption of lysosomal membranes may enhance oxidative stress [191] and cause cell death by both apoptosis [192] and necrosis [193]. Lysosome-released cathepsins B and D may carry out Bid cleavage and so mitochondrial apoptosis. Alternatively, cathepsin D may interact directly with proapoptotic Bax triggering apoptosis [160]. The macrophage cell death induced by the lysosomal membrane permeabilization was reported to be mediated by apoptosis or necrosis, in partial or extensive lysosomal rupture, respectively [194].

The basicity of polyamines at physiological pH indicates its antioxidant properties within living cells and these molecules act as a free radical scavenger quenching ROS [195-197]. Therefore these ubiquitous polycations may play a protective role under oxidative stress conditions, preventing lipid peroxidation [181, 198, 199], in animals [178], plants [200] and microbes [134], including Fungi [201].

Thus, polyamines may be required for aerobic growth as demonstrated in *Saccharomyces cerevisiae* [67], reverting oxygen toxicity [134].

6. POLYAMINES IN HUMAN PATHOLOGIES

Polyamine uptake and metabolism are often associated with stress responses in numerous experimental models on both animal [202] and plant cells [200-203], regulating amino acid metabolism [204].

Changes in PA metabolism was directly linked to solely one human disease, *i.e.* Snyder-Robinson Syndrome (SRS, OMIM #309583), an X-linked Mental Retardation Syndrome (MRXSSR) caused by mutation on chromosome Xp22, associated with a decrease in human Spm Synthase Gene (*hSMS*) expression and so elevated Spd/Spm ratios, SRS may be caused by mutation in *hSMS*.

SRS is characterized by alterations such as mild-to-moderate intellectual disability, facial asymmetry, marfanoid habitus, unsteady gait, thickened lower lip, narrow or cleft palate, nasal dysarthic speech, kyphoscoliosis, osteoporosis, hypotonia *etc*. [205]. Spm deficiency provokes Gy (gyro) phenotype in mice also associated with severe neurological injury [70].

The almost complete absence of PA deficiency mutations indicate these polycations are required for life maintenance. Although there is only one disease directly interrelated to PA metabolism, there is a plethora of diversified connections between PA and numerous diseases. There are over 40 human diseases resulting from oxidative stress or displaying oxidative stress involvement in its pathogenesis and/or etiology [133]. They include pathologies such as cancer, cardiovascular disease [206] HIV Infection [207], neurodegenerative diseases, ulcerative colitis, Down's syndrome [208], autoimmune diseases *etc*. [209]. Altered PA levels were reported in genetic diorders such as psoriasis, Beckwith-Wiedemann Syndrome (BWS) and sickle cell anemia [55].

Elevated systemic PA during pregnancy and malignancy are conceivably associated with cell proliferative activity, whereas hyperpolyaminemia is implicated in a number of disorders such as liver and renal insufficiencies, infections, rheumatoid arthritis, systemic lupus erythematous *etc*. [55].

Enhanced putrescine concentration was reported following brain ischemia and may play a role in cell damage and neurodegeneration [210]. Hypoxia leads to the activation arginase that hydrolyzes L-arginine into urea and L-ornithine. Ischemic events shift arginine consumption to the formation of ornithine, the polyamine precursor, rather than nitric oxide (NO) formation, an adaptation for preventing cell damage. Under low pO2 conditions, endothelial cells display enhanced protein kinase C (PKC)-α activity, which inhinbits nitric oxide synthase (NOS) and stimulates arginine entry to polyamine biosynthesis. The TNFα produced during hypoxia leads to the formation of ROS that inhibit NOS activity and may also stimulate arginase expression. In early reperfusion the polyamines produced trigger Ca^{2+} overload, whereas at late reperfusion, the increased NO production may be associated with low PA biosynthesis, resulting in cell damage [211].

Polyamines may be implicated in the pathophysiology of autoimmune diseases [212], possibly elevated polyamine synthesis can block cellular methylation by competing for S-adenosylmethionine (SAM) impairing normal epigenetic control [213].

The formation of reactive aldehydes such as 4-hydroxy-2-nonenal, malondialdehyde, acrolein are mutagenic causing DNA damage t[1]hat take part in the pathophysiology of different human diseases [214]. Acrolein is enhanced and may comprise biochemical markers for cerebral stroke [215], nephritis [216], neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease [217].

Another human disease linked to PA metabolism is keratosis Follicularis Spinulosa Decalvans (KFSD), an eventually severe dermal disorder affecting skin and eyes, characterized by inflammatory hyperkeratotic lesions in many parts of the tegument.

KFSD is a condition caused by mutations enhancing the activity of the *Sat1* gene coding for spermidine/spermine-N^1-acetyltransferase [54]. Curiously, polyaminergic genes including *sat1* may be associated with mood disorders, anxiety, and even suicide attempts [218-219].

Increased polyamine blood and/or urine levels have been implicated in several pathological conditions [220] and particularly neoplasisc diseases [221]. Polyamines not only take part in tumor cell proliferation but also in metastatic spreading. PA deficiency decreases the expression of matrix metalloproteinases, required for invasion and diminished the lymphocyte function-associated antigen-1 (LFA-1), inhibiting adhesion and tumoricidal, immune responses [222].

7. POLYAMINES IN PARASITES

Different parasitic organisms display remarkably high polyamine levels, reaching the mM range and play multiple roles. PA exert much the same functions in protozoa and metazoan, as well as prokaryotic cells to that in mammalian cells, taking part in cell division and chromatin organization. These organic cations also regulate mitochondrial function and organization (*vide infra*). PA were found in evolutionary conserved acidocalcisomes [96], which may have a lysosome or endosome-related origin in *Leishmania* sp. [153, 154, 223]. Besides parasites (*vide infra*) polyamines may play important roles in free-living amoebae [224].

For comprising stress molecules, PAs may play fundamental roles in regulation of pathogen-host interactions [178]. Several relevant aspects of parasite PA were focused in this Hot Topic Issue as well as in previous comprehensive reviews [225-227].

Microbial polyamines can be instrumental in proliferation [228], stress responses [229-231] and pathogenesis and/or virulence regulation in *Trypanosoma cruzi* [232-233], *Cryptosporidium parvum* [234]; *Trichomonas vaginalis* [235-236] and *Leishmania* sp. [237-239].

In this regard, the *Leishmania*-infected sand flies regurgitate proteophosophoglycans that raise L-arginine catabolism and therefore the synthesis of polyamines required by the rapidly proliferating parasites [240].

Parasite proliferarion relies on deoxyhypusination of eIF5A in *Trypanosoma brucei* cells [241]. The authors also showed that *Tb*eIF5A is required not only for cytokinesis, but also for parasite normal morphology and flagellar-cell body attachment. In *Trichomonas vaginalis*, Putr is required for eIF-5A expression, which was inhibited by the putrescine analogue 1, 4-diamino-2-butanone (DAB).

Interestingly DAB enhances *T. vaginalis* adherence to vaginal epithelial cells *in vitro*, but reduces its cytotoxic activity, by downregulating the expression of parasite 65-kDa (TvCP65) and 39-kDa (TvCP39) cysteine proteinases [236, 242, 243] both implicated in the cytotoxicity. The authors also reported the role of putrescine in the TvCP39 cellular distribution. Interestingly the *Trichomonas vaginalis* intracellular infection with *Mycoplasma hominis* increase Putr levels threefold [244].

The reviewed effects of PA as autophagy stimulants may culminate in significant effects on parasitic diseases. Induction of autophagy leads to diminished macrophage leishmanial invasion [245], but enhanced *L. amazonensis* intracellular proliferation [246]. On the other hand, autophagosome formation promotes host cell parasitism by *T. cruzi* [247], and the autophagosome marker LC3 was detected in the parasitophorous vacuole, whereas PA depletion downregulates autophagy in mammalian cells diminishing *T. cruzi* intracellular survival [248].

8. TRANSPORT SYSTEMS

The cell surface transporter proteins have been implicated in both uptake and extrusion of drugs. In this regard, malaria parasites display versatile transport system. *P. falciparum* Chloroquine (CQ) Resistance Transporter (PfCRT) pumps CQ from the parasitopharous vacuole in a H^+-coupled antiport mechanism also envolved in the uptake of nutrientes such as polyamines and amino acids [249].

Drug efflux pumps have a major role in drug resistance, but there are multiple mechanisms depending on different genes causing resistance to diverse drugs as reported for *Leishmania* parasites [250] and different agents may target distinct sites within the parasite cell, as assessed by electron microscopy [147, 150].

Abnormal *Leishmania* cell organization and cytokinesis may arise from drug-mediated pressure [251]. Interestingly polyamines may regulate the microtubule [37] and microfilament functioning and thus the cytokinetic machinery. Hence there is mounting evidence that polyamine levels regulate microtubule-associated protein 4 (MAP4), impeding microtubule assembly and the microtubule-associated filaments were disorganized in *Leishmania* promastigotes with MDR phenotype [251].

9. POLYAMINE TRANSPORT

Biogenic amine transport systems may comprise valuable targets in antiparasitic chemotherapy [252]. Besides polyamine trasporters or permeases, these organic polycations may be uptaken by endocytosis (*vide supra*), but this processes has not been described in parasitic protozoa. Important contributions have been made towards characterizing parasite polyamine transportes or permeases, however their contribution towards the polyamine content of these cells in situ is poorly understood.

Putr and Spd transport was reported in *Leishmania donovani* and *L. mexicana* [253-254]. PA transport was shown to be pH-dependent and promastigotes and amastigotes transporters display pH optima of 7.4 and 5.5, respectively [254].

The diamidine pentamidine, used as a secondary drug of choice in leishmaniasis chemotherapy, inhibits the putrescine and spermidine uptake non-competitively [254-255]. Not only diamine/polyamine incorporation can lead to drug resistance, but It has also been demonstrated that ornithine incorporation by the *Trypanosoma brucei* amino acid transporter gene, *TbAAT6* (Tb927.8.5450), results in eflornithine resistance [256].

Although mammalian PA transport was identified earlier, the first polyamine transporter identified, was the high-affinity putrescine-spermidine transporter LmPOT1 in *Leishmania major* cloned and characterized by Hasne and Ullman [255]. The transporter was unequivocally detected by immunoflourescence on both the flagellar and cell body plasma membrane of the promastigote surface.

Interestingly parasites cultured with polyamine biosynthesis inhibitors such as DFMO displayed enhanced polyamine transport in a physiological balanced system.

DFMO treatment was reported to enhance putrescine incorporation by mammalian cells [257-258] and parasitic protozoa such as *Trichomonas vaginalis* [259], *Leishmania infantum* [260], *L. mexicana* [261] as well as by the monoxenic trypanosomatid *Crithidia fasciculata* [262].

Multiple mechanisms may be involved in not only the parasite response to polyamines, but also the mode of action of polyamine analogues. Besides the pro-oxidant effects [263], DAB was reported to inhibit ODC activity in different organisms, such as *Entamoeba* sp. [264-265] as well as in *Escherichia coli* and *Dictyostelium discoideum* [266]. This analogue not only blocked *L. amazonensis* ODC, but also impaired [^{3}H]putrescine uptake [267]. Parasite preincubation with the diamine analogue remarkably enhanced [^{3}H]putrescine incorporation in a protein synthesis-dependent mechanism.

Culturing of *T. cruzi* epimastigotes under putrescine-free conditions enhances the diamine transport into parasites by increasing Vmax of TcPOT1.1 and decreasing the kM of TcPOT1.2 [268]. The putrescine transport enhancement can also be mediated by transporter redistribution to the parasite cell surface [268]. The high-affinity TcPOT1-mediated diamine transport is required for massive intracellular parasitism [269]. The polyamine incorporation from the extracellular milieu can play a role in leishmanial *in vivo* infection. Nevertheless, Spd synthase activity was shown to be required for *Leishmania donovani* virulence [237]. Although little is known about the biological role of Putr, exogenous Putr was demonstrated to restore *L. donovani* virulence *in vivo* [238]. Therefore, it is reasonable to infer that polyamine transporter up-regulation also take place during mammalian host infection. Thus, not only ODC activity [270] (see also article by Roberts & Ullman in this issue) but also PA synthesis and transport mechanisms are required for leishmanial virulence.

As *Trypanosoma cruzi* is devoid of both arginase that produces ornithine from arginine and ODC activity [271] as well as of arginine decarboxylase, which produces putrescine from arginine it is auxotrophic for exogenous putrescine, which is incorporated from the extracellular milieu [272]. Thus the DAB effects are presumably caused by transport impairment.

Putr auxothrophy is also observed in *Toxoplasma gondii* [273] and spermidine auxiotrophic metabolism in *Giardia*, *Trichomonas*, *Toxoplasma*, *Cryptosporidium*, *Entamoeba* and Microsporidia, which lost enzymes, involved in spermidine biosynthesis [274]. Therefore, these protozoa rely on transport and/or interconversion mechanisms [275]. In this regard, the macrophages endogenous PA levels are sufficient to support *T. gondii* intracellular proliferation [276].

Trypanosoma cruzi epimastigotes express two putrescine and cadaverine transporters termed TcPOT1.1 and TcPOT1.2 [268]. The receptor molecules were immunolocalized primarily at the flagellar pocket region. Light microscopy detection produced limited *bona fide* localization, whereas the electron microscopy clear immunogold labeling images presented demonstrate the presence of the transporters at the Golgi apparatus, on the contractive vacuole membrane as well as in the spongy associated structure named spongiome. Similarly, *P. falciparum* trophozoites isolated from red blood cells incorporate tritiated putrescine and spermidine [277].

10. PARASITE POLYAMINES AND OXIDATIVE STRESS

Immune responses effector mechanisms are largely mediated by ROS generated mostly by polymorphonuclear leukocytes, eosinophils and macrophages. Bacteria [278], protozoal parasites [279] and helminthes [280] evolved antioxidant mechanisms to evade oxidative stress. Microbial escape from oxidative stress mediated by putrescine/polyamine was reported in *E. coli* [281], *Streptococcus pneumonia* [282]. DFMO and DAB in combination with amphotericin B enhance ROS production and the antibiofilm activity *Candida albicans* [283]. Putrescine was shown to regulate the formation of *Escherichia coli* persister cells resistant to aminoglycoside antibiotic netilmicin [284]. Disruption of the gene-encoding PAO in *odc* mutants of the maize pathogenic fungus *Ustilago maydis* indicates that although not required for cell growth, dimorphic transition, putrescine is necessary for protection against salt and osmotic stress and possibly virulence [285].

Nevertheless, the putrescine accumulation leads to oxidative stress-dependent apoptotic murine myeloma cell death [286]. It was previously reported that putrescine overproduction leads to rat nervous system damage [202] and plant cells hyperexpressing transfected mouse *odc* gene, enhanced putrescine catabolism may lead to reduced glutathione levels and enhanced H_2O_2 production [287]. Therefore, polyamines may play an anti-oxidant role, whereas their metabolites may be prooxidant.

It was previously suggested that polyamines produced in high concentrations by the anaerobic protozoan *Trichomonas vaginalis* could play an antioxidant role [288] and neutralize the vaginal acidic pH [229]. Oxidative stress produced by pro-oxidant compounds such as H_2O_2/Fe^{2+} or nifurtimox may be detoxified by polyamines that prevent lipoperoxidation in *T. cruzi* [230]. Similarly, putrescine exerts protective functions on *E. coli* [289]. Therefore, the oxidative stress and protozoal antioxidant defenses may provide numerous chemotherapy targets [279]. Although PA may protect *T. cruzi* from oxidative stress [230], rather than damaging, the oxidant environment can be useful to some microbes [290] and promotes the *T. cruzi* infection *in vivo* [291].

DFMO treatment alters mitochondrial function in *Trypanosoma rhodesiense* [292] and *T. brucei* [293]. Spermidine and spermine are transported to the mitochondrial matrix, but little is known about the participation of putrescine in mitochondria, but the diamine was shown to protect *T. brucei* respiratory function during mitochondria purification, suggesting a possible stabilizing function [294]. Putrescine-mediated mitochondrial membrane stabilization may comprise a selective strategy for chemotherapy, since in mammalian cells spermine was shown to be more effective [120].

Putrescine may be involved in stress resistance as suggested by the solvent tolerance of *Pseudomonas putida* expressing redundant putrescine catabolism [295]. The diminished lipid peroxidation following down modulated reduction of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), in the DAB-treated trypanosomatid parasites *Trypanosoma cruzi* [296] and *Leishmania amazonensis* [267] maybe, at least partially, explained by the $O_2^{\bullet-}$ radical- mediated DAB pro-oxidant effect [263]. As mitochondria are $O_2^{\bullet-}$-producing compartments [279-297], mitochondrial destruction may be part of an antioxidant strategy, controlling cell damage [298]. The mitochondrial down modulation may take place in mitophagy [299] and/or mitoptosis-like phenomena [300] and the mitochondria-produced ROS regulates the autophagy [301] and may produce matrix degradation, assessed by EM as observed in *T. cruzi* [296] and *L. amazonensis* [267] or in *Aedes aegypti* muscle [302]. In this regard, the analogue could simulate putrescine overproduction triggering the amine oxidase activity and consequent H_2O_2 and toxic aldehtde production as reported in the *Rhodococcus opacus* putrescine homeostasis [303]. It could be associated to reduction of glutathione levels and impaired cytoplasmic Ca^{2+} regulation, causing multifactorial oxidative damage. The mitochondrial membrane location of monoamine oxidase as well as the ROS production by this redox organelle, is potentially a target for DAB, and could explain at least in part the preferential destruction of this compartment. The possibility that DAB effects could be at least in part mediated by polyamine/diamine oxidase(s) is supported by the partially reverted lipid peroxidation by aminoguanidine in DAB-treated *G. lamblia* trophozoites [304]. It remains to be determined whether this analogue was directly oxidized by the enzyme(s) and/or altered putrescine catabolism.

ROS production within mitochondria, cannot only orchestrate cell death, but also inflammatory cytokine production [305]. Thus, chemotherapeutic agents targeting polyamine metabolism may accomplish extensive effects, not only upon the parasites but also on mammalian hosts.

11. POLYAMINE METABOLIC PATHWAYS AS CHEMOTHERAPY TARGETS

The biosynthesis and conversion pathways as well as transport systems differ considerably, between mammalian and parasite cells, with some missing steps in the latter, providing several potential targets for chemotherapy [225-226] (*vide infra*). Similarly, the trypanothione system also poses several possibilities for chemotherapic interventions [306-307].

Contrary to several bacteria and viruses that infect humans, *bona fide* vaccines are generally not available for parasitic diseases. Therefore, chemotherapy remains a crucial tool in controlling these infections. Yet, the drugs used for chemotherapy are often not effective and associated with adverse effects [308]. Thus, innovative approaches such as *in silico* designed and/or combined antiparasitic drugs may reveal novel lead compounds, although some of the hi-tech results have been disappointing in the development of new drugs. PA-based approaches for developing new antiparasitic and antitumor compounds are largely supported by experimental data [309-310].

The polyamine metabolic pathways are considerably distinct not only between human cells and parasitic protozoa [225-226], but also as compared to helminthes [311] and fungi [201], providing potential targets for different chemotherapy interventions [228].

The similar properties observed in cancer cells and parasites [12-13] indicate that different drugs may have dual use for both anticâncer and antiparasitic treatment [312], it should be noted that DFMO is effective for the treatment of HAT [313-316] despite being developed for câncer chemotherapy, where it is still used for prevention of metastasis [310]. Furthermore the inflammatory responses triggered by viruses, bacteria, protozoa [317] and helminthes [317-320] may lead to carcinogenesis.

The striking observation that ODC inhibition by DFMO blocked *Trypanosoma brucei* proliferation and is trypanocidal *in vivo* was seminal in chemotherapy research [321]. Furthermore, this drug was shown to cross the blood-brain barrier and was effective in human infection by *T. gambiense* [322]. Therefore the physicians of the international philanthropic organization Doctors Without Borders/'Médecins Sans Frontières' named the compound "resurrection drug". Nevertheless, its production was discontinuated by the manufacturer due to its elevated costs. Later eflornithine production was restarted for cosmetic purposes [323]. Besides *T.*

brucei, DFMO was shown to inhibit the proliferation of *Giardia lamblia* [324], *Trichomonas vaginalis* [325], *Leishmania infantum* [326] *Plasmodium falciparum* [327-328], *P. berghei* [329] and *Pneumocystis carinii* [330].

The understanding of putrescine synthesis and/or transport is required since this diamine incorporation from the extracellular milieu or production by ODC, is the first and limiting step in polyamine metabolism. As putrescine concentrations in parasites are often much higher than in mammalian tissues this versatile molecule may play varied roles [331-332].

Polyamine metabolism may be involved in leishmanial drug resistance as proteome analysis showed that antimony resistance in *L. panamensis* is associated to SAMS overexpression [333]. Similarly trypanothione and ODC overexpression were reported in drug-resistant *Leishmania* sp. [334]. Thus trypanothione functioning provides possible targets for chemotherapy [335]. In addition polyamines may be involved in *Escherichia coli* multiple antibiotic resistance [336].

A quantitative proteomic analysis indicates that DFMO resistance by *Leishmania donovani* may result from the upregutated expression of PA metabolic enzymes [337]. Parasites of the Trypanosomatidae family present a PA-derivative with a central role in protozoa redox control. The Spd-glutathione adduct bis-glutathionyl Spd, termed trypanothione is a unique species of these pathogens [338], indicating that enzymes such as trypanothione reductase may comprise selective targets for drug development [339].

Interestingly parasites such as *T. cruzi* and *Toxoplasma gondii* are unable to synthesize, so are auxiotrophic for putrescine, rely on the diamine incorporation from the extracellular milieu and or backconversion of internalized Spd/Spm [273]. Thus, chemotherapy interventions may focus PA transport. In addition the polyamine concentrations and presumably function differ considerably. Spm and Spd concentrations reach mM levels in mammals, whereas Putr levels are much lower (~1μM) [115], corroborating that PA and Putr transport and/or synthesis may comprise valuable chemotherapy targets. Arginine metabolism may also furnish useful targets for drug development.

Mammalian host cells both synthesize and uptake arginine, which may be used as substrate in two different macrophage pathways nitric oxide synthase (NOS) and arginase, respectively associated to M1/M2 phenotype dichotomy, and so, Th1- and Th2-dependent responses [340-342]. This dichotomy can determine the fate of the intracellular trypanosomatids *Trypanosoma cruzi* [343-344], *Leishmania* sp. [239, 345] as well of the apicomplexan *Toxoplasma gondii* [346].

Arginase may supply L- ornithine for ODC activity permitting *Leishmania* amastigote proliferation within the parasitophorous vacuole [239]. Visceral leishmaniasis progression is promoted by STAT6-dependent host arginase 1 expression [347]. *Trypanosoma brucei* growth maybe induced by host arginase activity triggered by parasite release of kinesin heavy chain [348].

The increased arginase activity was reported in cutaneous leishmaniasis lesions [349] and may be involved in the poor outcome of *Leishmania*-HIV coinfections [350] and comprise a marker for visceral leishmaniasis progression [351]. Furthermore, natural

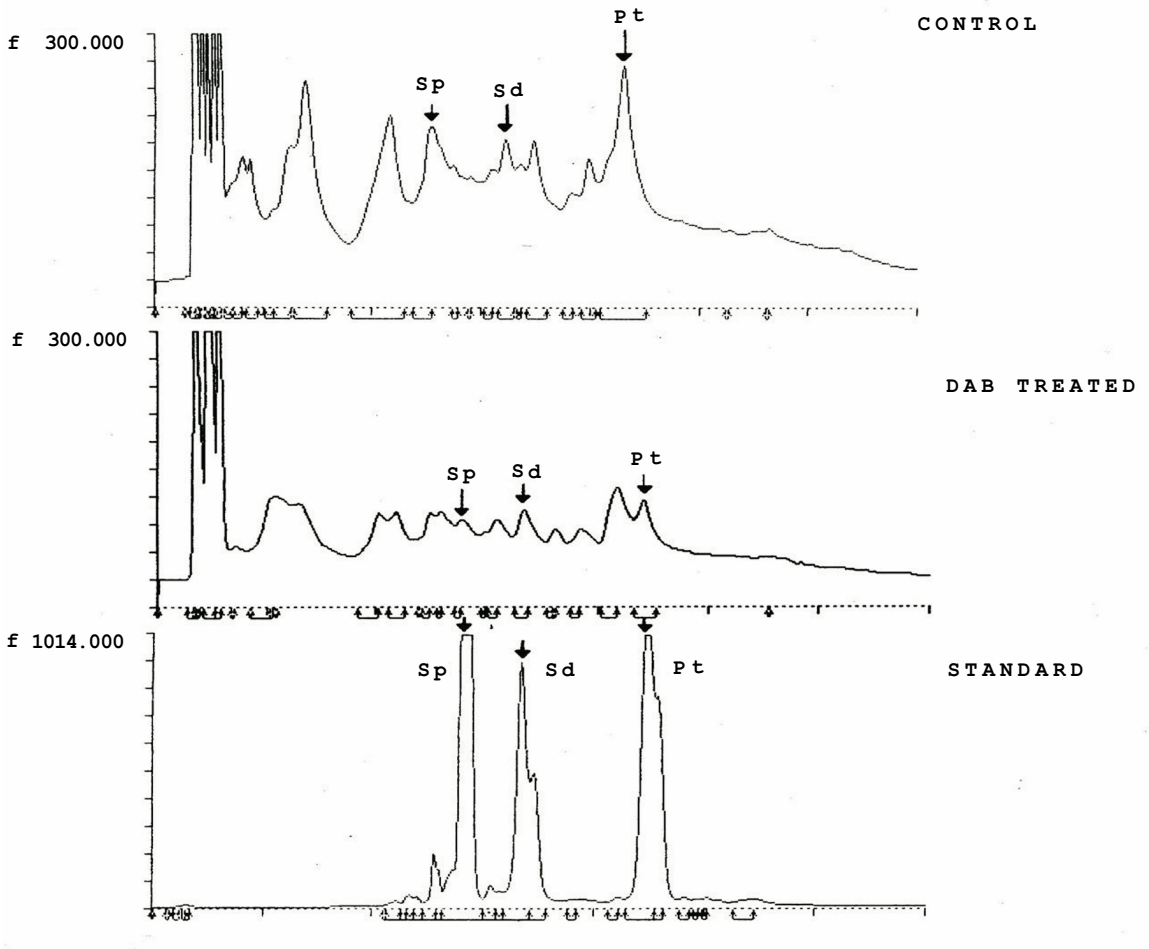

Fig. (2). HLPC of untreated control (upper panel) and DAB-treated (middle panel) *Tritrichomonas foetus* parasites. The standards are shown in the lower panel. Kindly provided by Dr. Nigel Yarlett.

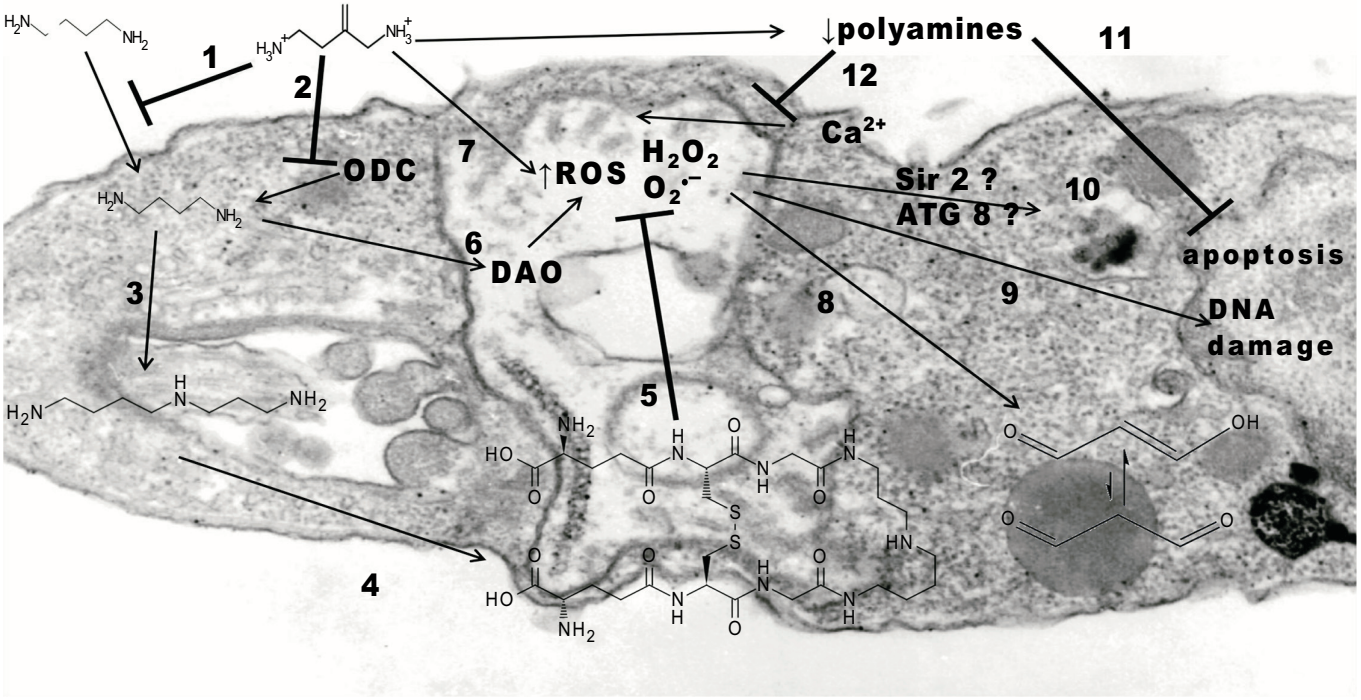

Fig. (3). The Putr analogue 1, 4-diamino-2-butanone (DAB) is able to inhibit both putrescine transport from the extracellular milieu (1) or its biosynthesis (2) by ornithine decarbpxylase (ODC). The diminished spermidine levels (3) may reduce trypanothione production (4), leading to enhanced accumulation of reactive oxygen species (ROS) such as H_2O_2 and $O_2^{\bullet-}$ (5). Putrescine and/or its analogue may be catabolized via mitochondrial diamineoxidase (DAO), giving rise to ROS (6). DAB per se was also shown to produce ROS (7), which may lead to lipoperxidation with formation of malondialdehyde or propanedial (8), ROS-mediated DNA damage (9), autophagy (10). In addition, the diminished polyamine pool can modulate apoptosis triggering (11) as well as Ca^{2+} transport to mitochondria (12).

products with arginase antagonistic activity such as flavonoids purified from *Cecropia pachystachya* or dietary sources may comprise leishmanicidal compounds causing mitochondrial DNA disorganization [352-353].

In the case of the extracellular intestinal parasite *Giardia lamblia* (synm. *G. intestinalis*, *G. duodenalis*) this competition may compromise host cell fuction and tissue architecture. Arginine consumption by the trophozoite reduce the proliferation and differentiation of intestinal epithelial cells [354]. It also diminishes the NO production by enterocytes.

DAB is reported to inhibit the proliferation of fungi [355-356], *Entamoeba invadens* [265], *Entamoeba histolytica* [264] and interestingly the *E. histolytica* ODC is sensitive to DAB, but not to DFMO[357-358]. In an early study we noticed that DAB inhibits the *in vitro* proliferation of the trichomonad parasite *Tritrichomonas foetus* [359], remarkably reducing the cellular levels of Putr, Spd and Spm (Fig. **2**).

Interestingly, DFMO was only effective upon *Trichomonas vaginalis* parasites cultured in a semi-defined medium [288], whereas DAB inhibited the growth of the protozoan even in complex medium [235], presumably because the analogue blocked the surface permeases as reported in *Leishmania amazonensis* [267], in which DAB, not only inhibited ODC activity, but also the [^{3}H]putrescine incorporation. Thus, such analogues comprise useful tools to study putrescine metabolism in pathogens. DAB was also reported to inhibit ODC in *Escherichia coli* [281]. We have noticed that besides *T. foetus* [359]. DAB affects other parasitic protozoa. The analogue also diminished the axenic proliferation of *Giardia lamblia* [304], *L. amazonensis* [267] and *Trypanosoma cruzi* [296], indicating the compound is able to affect both aerobic and anaerobic/microaerophilic protozoa. DAB was also shown to inhibit the fungal differentiation [360], proliferation/growth of fungi such as *Candida albicans* [357].

Although DAB could be termed a "wide-spectrum microbicide" [263] and we have studied its microbicidal activity on different parasite species, little is known about its mechanisms of action upon distinct cell types. It was previously shown that DAB can undergo aerobic oxidation catalyzed by Fe^{2+} and Cu^{2+} ions producing NH_4^+ ion, H_2O_2 and 4-amino-2-oxobutanal (oxoDAB) [263]. The superoxide radical($O_2^{\bullet-}$)-mediated DAB oxidation may explain, at least in part, the extensive mitochondrial damage observed in the DAB-treated trypanomasomatids *T. cruzi* [296] and *L. amazonensis* [267] as part of the oxygen entering this organelle is converted to $O_2^{\bullet-}$ [361]. Therefore, DAB could play a double role both producing ROS and hampering polyamine-mediated antioxidant mechanisms (Fig. **3**).

The polyamine metabolism of *Plasmodium falciparum* may provide useful targets for antimalarial chemotherapy [328]. ODC antagonists such as DFMO used either alone [329, 362, 363] or in combination [364, 365] may show microbicidal activity against *Plasmodium* sp. both *in vitro* and *in vivo* but are mostly cytostatic leading just to growth arrest. It is noteworthy that *P. falciparum* erythrocytes treated with 10 mM DFMO for 73 h presented parasites with numerous hemozoine crystals in the cytoplasm [366], indicating that PA-deficiency may lead to destabilization of the lysosome-like digestive vacuole (or food vacuole), triggering parasite programmed cell death [367].

Thus, the elucidation of *Plasmodium* polyamine biosynthesis and/or transport mechanisms may offer promising new therapeutic strategies for malaria [327, 367].

For all that, the search for effective, low costs anti-parasitic drugs remains a priority.

Electron microscopy was shown to be useful in improving our understanding not only of the protozoan parasite cell biology [149, 368], but also of the mode of action of diverse antiparasitic compounds [147, 150, 369, 370], including natural products and derivatives permiting for the first time a structure-activity relationship at the subcellular level [371]. The ultrastructural analysis permits determining the not only the subcellular target sites, but the fashion by which they are altered may help elucidating mechanisms of action of drugs leads on parasite specific targets and, ultimately, the pathogen death [150, 372]. Interestingly ultrastructural analysis of drug-treated parasites may expand gene expression data, permitting a detailed understanding of action mechanisms at cellular and subcellular levels [373].

CONCLUSION

Taken together the present discussed data indicate that polyamines not only take part in pivotal metabolic pathways, but also play different roles in cellular organization of both mammals and eukaryotic protozoa. It is also demonstrated that ultrastructural analysis may be useful in the elucidation of mechanisms of action of chemotherapeutic agents.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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