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Specific effects of reactive thiol drugs on mitochondrial bioenergetics

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Abstract In this minireview, the more recent findings about the effects of peculiar reactive thiol drugs on mitochondria are presented. These include the following compounds: metallo *meso-tetrakis* porphyrins, palladacycles, telluranes and phenothiazines. Metallo *meso-tetrakis* porphyrins can exhibit both beneficial and deleterious effects on mitochodria that are modulated by the central metal, cell location, and availability of axial ligands. Therefore, these compounds have the versatility to be used for cell and mitochondria protection and death. The antioxidant activity of manganese porphyrins is related to a glutathione peroxidase-like activity. By attacking exclusively the membrane protein thiol groups without glutathione depletion, palladacycles are able to induce mitochondrial permeability transition (MPT) and cytochrome *c* release in the absence of oxidative stress. In hepatoma cells, the mitochondrial action of palladacycles was able to induce apoptotic death. As opposed to palladacycles, telluranes and phenothiazines are able to conjugate the capacity to promote the MPT in a dose-dependent manner in association with efficient antioxidant activity toward lipids. These studies demonstrated that the action of drugs on mitochondrial bioenergetics can be modulated by peculiar reactivity with thiol groups. Therefore, they contribute to studies of toxicity as well as the design of new drugs.

Keywords Thiol reactivity · Mitochondrial permeability transition · Porphyrins · Palladacycles · Telluranes · Phenothiazines

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Introduction

Thiol functional groups of proteins and peptides, also referred to as SH or sulfhydryl, are present in the lateral chain of cysteine residues and play key roles in the structure and function of these biomolecules. Thiol groups contribute to the stabilization of the tertiary and quaternary structures of proteins by forming respectively intra and inter-chain disulfide bonds (Wedemeyer et al. 2000; Welker et al. 2001; Bruce et al. 2008; Arnesano et al. 2004). An important functional contribution of thiol groups refers to the catalytic activity of cystein-proteases, proteolytic enzymes responsible for terminal protein degradation in lysosomes, apoptosis, MHC class II immune responses, prohormone processing, extracellular matrix remodeling important to bone development, parasite infection, collagen and elastin degradation at sites of inflammation in diseases such as atherosclerosis and emphysema (Lipton et al. 2002; Chapman et al. 1997).

Also, thiol groups play a key role in the cell redox balance, and their functions include involvement in the antioxidant system and cell signaling (Foyer 2005; Moran et al. 2001). The interaction of sulfhydryl-rich proteins and GSH in the cell redox balance and signaling is particularly effective for soluble proteins. Transmembrane proteins can exhibit sulfhydryl groups not accessible to S-thiolation and constitute a particular system in the cell redox balance. The redox status of thiol groups of mitochondrial membrane proteins is particularly important due to the pivotal role of this organelle in the cell oxidative and nitrosative stress with repercussions in cell aging and death (Pai et al. 2007).

Thiol oxidation and the mitochondrial permeability transition

The redox balance of the thiol content of mitochondrial membranes has been related to the occurrence of the mitochondrial permeability transition (MPT) process (Moran et al. 2001; Petit et al. 1996; Rodriguez-Enriquez et al. 2004), characterized by matrix swelling, uncoupling, and calcium release and may precede necrotic and apoptotic cell death (Green and Reed 1998; Kroemer 1999; Kowaltowski et al. 2001). In fact, the thiol cross linkage (Fagian et al. 1990; Valle et al. 1993; Castilho et al. 1995) between thiol groups of mitochondrial membrane proteins promoted by free radicals or thiol oxidant agents in the presence of calcium seems to be central at the MPT onset (Bernardes et al. 1994; Kowaltowski et al. 1997; Kowaltowski and Castilho 1997). Despite strong evidence of the relationship between oxidative stress and the MPT via direct and indirect changes in the redox state of thiol groups, the exact molecular composition of the mitochondrial permeability transition pore (PTP) remains unclear. However, as discussed herein, the different degrees of mitochondrial protein aggregation that accompany the PTP opening suggest that the oxidation of some thiol groups is enough to stabilize the pore structure via cross-linkage of the protein content involved in the PTP structure.

The effect of several chemical agents on mitochondrial bioenergetics has been used as a tool to study the structure of the PTP and its functional characteristics. ANT has been considered for a long time as a central PTP component, mostly due to sensitivity of the MPT to physiological (ADP) or non-physiological ligands. The studies with thiol reactive agents indicated that ANT exhibits two conformational states, named as *c* and *m*. Indeed, the reactivity of the hydrophobic thiol reagent NEM with ANT-Cys56 (Halestrap and Brenner 2003; Beyer and Nuscher 1996) coincident with the well documented capacity of this thiol reagent to inhibit mitochondrial swelling (Kowaltowski et al. 1997; Novgorodov et al. 1990) suggests the oxidation of Cys56 residue of the translocase as a critical step for PTP opening (Pestana et al. 2009). It has been shown that the cross linkage of ANT sulfhydryl groups involves Cys56 that protrudes into the matrix space in the *m*-state but not in the *c*-state of the protein (Beyer and Nuscher 1996; Majima et al. 1995).

Other thiol reactants have been used in studies of mitochondrial permeabilization associated with PTP opening, such as diamide, a pro-oxidant molecule that also reacts with ANT Cys56 leading to the opening of PTP (Costantini et al. 2000) and phenylarsine oxide (PhAsO), a dithiol reagent that causes oxidation of SH groups and mitochondrial swelling (Kowaltowski and Castilho 1997). In that study it was proposed that calcium stimulates the MPT induced by PhAsO by acting at the external side of the inner mitochondrial membranes. In addition, the mechanisms of MPT induction by PhAsO were investigated comparatively to t-butyl hydroperoxide as oxidative stress inducer and in both cases the oxidation of thiol groups of reactive cysteines in ANT is involved (Halestrap et al. 1997). In fact, it was shown that the MTP triggered by PhAsO was associated to severe hypercontracture, and irreversible membrane injury associated with increased cytoplasmic free calcium (Korge et al. 2001). Also, it was demonstrated that the mitochondrial permeabilization induced by the thiol reactant 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) is mediated by its interaction with membrane proteins. It was not accompanied by the oxidation of pyridine nucleotides or significant lipid

peroxidation (Bernardes et al. 1994). Interestingly, literature data have shown that organotellurium and organosellenium compounds exhibit both reactivity with thiol groups and antioxidant properties (de Avila et al. 2006).

Recent findings of our group concerning the study and comprehension of the PTP opening mechanism by using a variety of chemical reactants of thiol groups are reviewed and discussed herein.

1- Reaction with thiol groups associated with oxidative stress: the versatility of *meso-tetrakis* porphyrins

Literature data report that *meso-tetrakis* porphyrins exhibit both pro- and antioxidant properties for biological systems, being evident that the pro-oxidant actions are responsible for the toxicological effects of these compounds (Pessoto et al. 2009). Mechanistic studies revealed that the biological effects of these porphyrins are modulated by the *meso* ligands, the metal center and the microenvironment. The structural characteristics of four studied *meso-tetrakis* porphyrins (i.e., the cationic Fe[III] and Mn[III] *meso*-tetrakis 4-*N*methyl pyridinium porphyrins [TMPyP] and the anionic Fe[III] and Mn[III] *meso-tetrakis* [*para-sulfonatophenyl*] porphyrins [TPPS4]) determined two crucial characteristics for their biological effects: the catalytic mechanism of peroxide cleavage (i.e., homolytic and heterolytic scission of *O-O* bond) and catalytic efficiency in the biological environment. For the manganese *meso-tetrakis* porphyrins, this latter characteristic is strongly related with the capacity of the peroxide-generated Compound II (oxomanganese IV) intermediate to use reducing agents such as glutathione (GSH) and thiol-rich proteins as reducing agents for the completion of the peroxidase-like catalytic cycle (Araujo et al. 2010; Pessoto et al. 2009).

The antioxidant activity of manganese porphyrins has been attributed to the capacity of these compounds to mimic the mitochondrial superoxide dismutase (SOD) mechanism (Ferrer-Sueta et al. 2003) and the pro-oxidant activity of iron porphyrins to the generation of free radicals due to the homolytic cleavage of peroxides.

Different from the cationic Mn(III)TMPyP (Inada et al. 2007), the anionic Mn(III)TPPS4 exhibits no protector effect against Fe(citrate)-induced lipid oxidation, and it is unable to protect mitochondria against endogenous hydrogen peroxide. The protective effect of the anionic manganese porphyrin is restricted to a delay of the swelling caused by *tert*-BuOOH and calcium. The iron parent porphyrin, Fe^{3+}TPPS4, exacerbates the swelling caused by *tert*-BuOOH, and both porphyrins present no significant effect against Fe(II)citrate-induced mitochondrial swelling. A systematic comparative study of the catalytic properties of *meso*-tetrakis porphyrins modulated by the porphyrin metal center and *meso* ligands (Araujo et al. 2010) revealed that Fe(III)TPPS4 promotes predominantly homolytic cleavage of peroxides. This iron porphyrin exhibits catalytic efficiency tenfold higher than Mn(III)TPPS4, which cleaves peroxides predominantly by heterolytic scission of the *O-O* bond. The corresponding cationic Fe(III) and Mn(III) porphyrins exhibited a similar catalytic efficiency, though significantly lower than the anionic partners. In comparison with the iron parent porphyrin, the lower catalytic efficiency of Mn(III)TPPS4 can be assigned to two factors: (1) the low reactivity of hydroperoxides as the oxygen donor to manganese(III) porphyrins and (2) the incapacity of oxomanganese to use peroxide as a reducing agent for recycling. In accordance with the above proposition, the presence of high GSH concentrations duplicated the peroxide consumption by Mn(III)TPPS4. In cell conditions, a significant catalytic efficiency of Mn(III) is expected due to the availability of axial ligands for the metal center and reducing agents such as GSH and proteins necessary for Compound II (oxomanganese IV) recycling of the resting Mn(III) catalyst. Therefore, the use of thiol-reducing agents leads to GSH depletion, proteins' oxidation, and consequently, biological damages. In contrast to what was observed for the anionic manganese partner, GSH was an efficient reducing agent of the resting Mn(III) TMPyP, thereby impairing peroxide consumption and free radical generation by the cationic porphyrin. Altogether, peculiar reactivity of TMPyP with thiol groups is in accordance with the antioxidant activity described for this porphyrin. Large amounts of GSH could lead to the reduction of porphyrin Mn(III) to Mn(II) followed by rapid recycling of the oxidized form by molecular oxygen in a futile cycle contributing to GSH depletion and peroxide consumption. The efficiency of GSH as a contributor for the manganese porphyrin antioxidant activity is limited by its capacity to react significantly with the resting Mn(III) form. Furthermore, the increase of porphyrin efficiency leads to the increase of the free radicals' production that, in turn, contributes to GSH depletion. GSH can efficiently reduce the Mn(III)TMPyP, and this process impairs the peroxide consumption and the consequent free radical production by TMPyP.

2- Reaction with thiol groups not accompanied by whole mitochondrial oxidative damages: the efficiency of paladacycles

Organometallic compounds derived from platinum are largely used as chemotherapeutic agents against several types of

cancer due to the impairment of cellular replication via DNA binding (Rozencweig et al. 1981). Such binding is related to the high reactivity of these compounds with reduced thiol groups, and thiol reactants are able to modulate the antitumor action (Lemma et al. 2000; Sadowitz et al. 2002). However, the use of these substances as anticancer drugs is limited by the side effects that include nephrotoxicity (Goldstein and Mayor 1983). The toxicity seems to be related to the thiol oxidation, impairment of mitochondrial functions, and the MPT (Custódio et al. 2009).

Similarly, organometallic compounds derived from palladium have been raised as potential chemotherapeutic agents (Caires 2007). A cyclopalladated compound derived from N,N-dimethyl-1-phenethylamine dppe[1,2-ethanebis (diphenylphosphine)] (dmpa), complexed to 1,2-ethanebis (diphenylphosphine) (dppe) ligand, named complex 7a, showed a potent antitumor activity *in vitro* and *in vivo* against B16F10-Nex2 murine melanoma cells of low immunogenicity implanted subcutaneously in mice (Rodrigues et al. 2003). In the same study, an extracellular acidification was observed, suggesting that the impairment of mitochondrial respiration contributes to the observed effects. Also, cyclopalladated dmpa complexed to bis (diphenylphosphine)ferrocene (dppf) was found to inhibit cathepsin B, associated with tumor progression and metastasis (Bincoletto et al. 2005) and presented cytotoxic action in K562 leukemic cells *in vitro* (Oliveira et al. 2009). Recently, it was shown that the palladacycle compound R(+) dmpa:dppe (1:1) PdC presented a high reactivity to the mitochondrial membrane protein thiol groups related to the onset of the MPT in isolated mitochondria. A mechanistic approach was conducted and presented new insights to the requirement of Ca^{2+} and the role of specific oxidation of thiol groups on the MPT in the absence of oxidative stress (Santana et al. 2009). PdC induced an extensive mitochondrial swelling at extremely low concentrations accompanied by the dissipation of mitochondrial transmembrane potential, uncoupling of the oxidative phosphorylation, disruption of the mitochondrial calcium homeostasis, cytochrome *c* release, and apoptotic death in hepatoma cells. The thiol-reducing agent dithiothreitol (DTT), but not the chelating calcium agent EGTA, was able to prevent completely all these processes, showing that the reactivity of PdC with thiol groups of mitochondrial membrane proteins determines the MPT onset independent of the presence of calcium.Interestingly, PdC did not promote oxidative stress and did not react with the hydrophilic thiol group of GSH. Also, it was not observed extensive protein aggregates distinguishable in SDS-PAGE as observed in the MPT triggered by oxidative stress conditions. N-ethylmaleimide (NEM), a monothiol reagent which covalently blocks hydrophobic sulphydryl groups at physiological pH and inhibits the MPT (Novgorodov et al. 1990), prevented only partially the PdC-induced MPT. Such findings indicate that the reactivity of PdC thiol with groups of mitochondrial membrane proteins exhibit a specificity probably due to its preferential partitioning into the biological membranes that restrict its effects on critical thiol groups of membrane proteins.

Considering that mitochondrial functions are profoundly affected by the redox state of thiol groups (Halestrap et al. 1997; Le Quoc et al. 1976; Costantini et al. 1996) and that the occurrence of the MPT is associated with the oxidation of thiol groups of mitochondrial membrane proteins (Fagian et al. 1990; Castilho et al. 1995), the formation of the disulfide bond due to cross-linkage of reduced thiol groups was suggested indirectly by using DTNB (Dorta et al. 2003, Pessoto et al. 2007). FT-IR analysis of membrane proteins isolated from mitochondria in the presence of PdC showed the formation of disulfide bonds associated with mitochondrial permeabilization by the appearance of a characteristic band in the infra-red region attributed to S–S vibration, simultaneously to the disappearance of a –SH vibration. Around this frequency range (~2500 cm^{-1}), only a limited number of vibrations are known to absorb, of which the S–H of the cysteine thiol is the most abundant in proteins (Bare et al. 1975; Ataka et al. 2003). The vibrational spectra did not demonstrate characteristic vibrational bands of S–Pd species, corroborating that the decrease in the reduced thiol content measured with DTNB is due to the formation of disulfide. Recently, the high effectiveness of the stereoisomer compound 7a was demonstrated against *T. cruzi*, the causative agent of Chagas' disease (Matsuo et al. 2010) that could be related to the capacity of PdC to promote the MPT.

3- Reaction with thiol groups associated to partial and total protection of mitochondria against oxidative stress: the amazing characteristic of organotelluranes and phenothiazines

The effects of six organotelluranes (IV) RT-03, RT-04 and RT-07 derivatives, named RT-07A, RT-07B, RT-07C, and RT-07D on the mitochondrial bioenergetics were studied. The results obtained with these compounds brought insights on the requirement of oxidative stress and the role played by Ca^{2+} in the MPTP opening that has been characterized as a key mechanism underlying both necrotic and apoptotic cell death (Kim et al. 2003). The studied organotelluranes were able to promote mitochondrial Ca^{2+}-dependent swelling inhibited by cyclosporine A (CsA). These compounds also promoted a decrease of membrane thiol content

accompanied by proportional crosslinkage of mitochondrial membrane proteins characterizing the opening of the classical mitochondrial permeability transition pore (MPTP). Despite the reactivity of the studied organotelluranes with membrane thiol groups (Pessoto et al. 2007; Cunha et al. 2005), the RT-07 derivatives did not promote glutathione depletion. The studied teluranes were also able to protect the mitochondrial matrix from the oxidative stress promoted by *tert*-butylhydroperoxide as assessed by the DCF fluorescence. Some particularities characterize the effects of RT-03, RT-04 and RT-07 derivatives. RT-03 and RT-04 are characterized by a well-defined, dose-dependent effect. At the nanomolar range, RT-03 and RT-04 did not cause any mitochondrial dysfunction and further exhibited exclusive antioxidant activity. At the concentration range of 5–10 μM, RT-03 and RT-04 promoted the Ca^{2+}-dependent opening of the (MPT) pore, regulated by CsA. At the concentration range of 15–30 μM, the swelling was not sensitive to CsA. In addition, a significant decrease of the respiratory control ratio was observed in the absence of Ca^{2+} due to concomitant phosphorylation impairment and uncoupling, transmembrane potential disruption, depletion of mitochondrial-reduced thiol groups, and alterations in the bilayer fluidity. At higher concentrations (i.e., above 100 μM), the organotelluranes caused complete inhibition of the respiratory chain. The RT-07 derivatives have the peculiarity to promote differentiated effects on mitochondrial bioenergetics in the presence and in the absence of Ca^{2+}. As depicted above, in the presence of Ca^{2+}, RT-07 derivatives promote the classical MPTP opening. In the absence of Ca^{2+}, loss of mitochondrial $\Delta\Psi$ was observed concomitant with a decrease of respiratory control due to an increase of the state-4 respiration rate not accompanied by mitochondrial swelling and extensive thiol oxidation. In the absence of Ca^{2+}, RT-03 and RT-04 promoted inhibition of the respiratory chain but not the loss of $\Delta\Psi$. The differentiated effects of telluranes that were observed in the presence and in the absence of Ca^{2+} are probably caused by the exposure of specific reactive protein thiol groups modulated by Ca^{2+}-sensitive changes in the membrane structure. Figure 1 shows the correlative dose-dependent effects of RT-03 on mitochondrial swelling and SH content.

These effects can be rationalized by the capacity of telluranes to react with thiol groups of mitochondrial proteins and lead them to the pore assembly. When Ca^{2+} was absent, the pore remained occluded by cyclophylin D, and swelling was not observed. However, the attack to thiol groups could affect respiratory chain components and/or ANT, a putative component of the MPTP (Kowaltowski et al. 2001; Bernardi 1999; Kim et al. 2003) leading to a decrease of respiratory control.

Besides their reactivity with thiol groups, the telluranes also present hydrophobicity and thus the capacity to affect the inner bilayer structure. For RT-03 and RT-04, increase of ANS fluorescence was observed at the concentration range coincident with the ability to promote CsA-insensitive MPT. Changes in lipid bilayers were also observed in PCPECL liposomes where proteins are absent, suggesting that alterations in the membrane lipid bilayer organization should be involved in mitochondrial dysfunction promoted by these compounds.

Several studies have correlated oxidative stress with the MPTP opening (Kowaltowski et al. 2001). In fact there are strong points of evidences for correlation between the NADPH redox status and the occurrence of the MPT. However, other studies have also demonstrated the opening of the MPT by the amphipathic peptides mastoporan and MP-14, in a dose-dependent manner (He and Lemasters 2002). However, contrary to the studied organotelluranes, these peptides do not exhibit any antioxidant properties and could not provide a condition in which the pore could be formed concomitantly with efficient antioxidant protection.

The CsA-sensitive MPTP opening by organotellurane in concentrations unable to promote physical and chemical damages on the lipid bilayer as well as oxidative stress in the mitochondrial matrix points to remarkable evidences that the MPTP opening could be promoted exclusively by cross-linkage of proteins associated to the mitochondrial inner membrane. These results suggest that the lipid damage concomitant with PTP opening by oxidative stress should be only a side effect and not a decisive event.

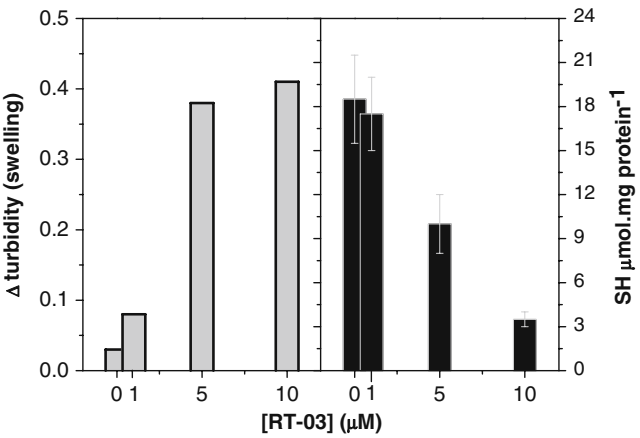

Fig. 1 Mitochondrial swelling (Δ turbidity after 20 min incubation, shown in *left panel*) and the corresponding SH content (*right panel*) promoted by different concentrations of RT-03 (0, 1, 5, and 10 μM)

Besides the capacity to induce the MPTP opening and mitochondrial dysfunction not accompanied by oxidative stress, the organotelluranes exhibited antioxidant activity against classical inducers of mitochondrial oxidative damage. It is known that organyl diselenides and ditellurides compounds exhibit potent antioxidant properties due to the inherent reactivity of dichalcogen bonds (Nogueira et al. 2004). Unlike organotellurides, organotelluranes exhibit high reactivity with sulphydril groups, and it is expected that the products of these reactions retain antioxidant properties, since they have mixed dichalcogen bonds. This mechanism could be responsible for the exceptional antioxidant activity RT-03 and RT-04 exhibited at the nanomolar concentration range. The studied organotelluranes were able to protect the lipid fraction and/or matrix from oxidative stress.

Studies involving PTP opening concomitantly with efficient antioxidant protection was also addressed by using phenothiazines as tools. For a long time, PTZ derivatives have been used in the treatment of schizophrenia, psychosis, and anxiety (Lehmann and Ban 1997) as anti-emetic drugs (Bhargava and Chandra 1963; McCabe and Maraveyas 2003) and even in the treatment of chemotherapy-induced emesis (Allan 1987). Also these molecules present interesting chemical features and biological properties (Rodrigues 2007). Numerous studies have described trifluoperazine, a piperazinic PTZ derivative, as an MPT inhibitor (Broekemeier et al. 1985; Pereira et al. 1992). At low concentrations (10 μM), PTZ exhibit important antioxidant activity characterized by inhibition of the accumulation of mitochondria-generated $O_2^{\bullet-}$, Fe^{2+}/citrate-mediated lipid peroxidation of the mitochondrial membrane, Ca^{2+}/t-butyl hydroperoxide (t-BOOH)-induced mitochondrial permeability transition (MPT)/protein-thiol oxidation, and cytochrome *c* release in isolated rat liver mitochondria (Rodrigues et al. 2002). On the other hand, at higher concentrations (100 μM), PTZ derivatives thioridazine (TR), fluphenazine (FP) and trifluoperazine (TFP) triggered MPT, characterized by an extensive mitochondrial swelling associated to the oxidation of the mitochondrial membrane protein thiol groups (Cruz et al. 2010). However, the TR-induced SH oxidation was not accompanied by the detection of large amounts of high molecular protein aggregates in SDS-PAGE and by the GSH oxidation. Interestingly, even at higher concentrations, PTZ maintain their antioxidant effect, protecting mitochondrial membranes from oxidative damage and showing that the PTZ-induced MPT is associated with a specific thiol oxidation but not to a generalized oxidative damage. Additionally, in a homogeneous medium, PTZ were not able to react directly with thiol groups of GSH and other thiol-containing substances. It has been previously proposed that PTZ-derived free radicals may be generated chemically by the action of peroxidases (Eghbal et al. 2004), and when photoexcited, PTZ generates PTZ-derived cation radicals (Rodrigues et al. 2006) able to oxidize mitochondrial membranes (Rodrigues et al. 2005). It was shown that the PTZ cation radicals photochemically generated were able to react with thiol groups of a model protein and that the inhibition of mitochondrial peroxidases by using cyanide prevented the TR-induced MPT (Cruz et al. 2010). Additionally, the pre-incubation of mitochondrial suspension with EGTA prevented the mitochondrial swelling but not the thiol oxidation, showing a cause-consequence relationship between the two processes. Calcium is only necessary to the latter. The observed specificity in relation to the oxidation of thiol groups of mitochondrial membrane proteins but not other thiols such as GSH is given by the hydrophobicity of these drugs that become partitioned preferentially in membranes (Hendrich et al. 2002), resulting in the modification of specific thiol groups involved in the formation of PTP, despite their ability to protect mitochondrial membranes from lipid peroxidation.

In order to study the structural requirements for the induction of the MPT by PTZ derivatives, the effects of the phenothiazine nucleus (PHT) on isolated rat liver mitochondria were studied. The PHT was able to prevent mitochondrial swelling and thiol oxidation promoted by Fe^{2+}/citrate but not by *t*-BOOH that promotes oxidative stress, because it is decomposed by glutathione peroxidase (GPx) with a concomitant depletion of GSH. In this situation, organic peroxides generate high valence states of respiratory cytochromes that could attack both lipid and thiol groups. The PHT cannot inhibit the peroxidase activity of either GPx or respiratory cytochromes or the consequent direct oxidation of mitochondrial thiol groups. However, considering that the PHT was able to act as a trap of prooxidant species, the free radicals generated by Fe^{2+}/citrate are accessible and rapidly trapped by the PHT resulting in the inhibition of the MPT (Borges et al. 2010). Different from PTZ, the PHT at higher concentrations was not able to cause mitochondrial swelling

Conclusions

The thiol groups of proteins contribute to protein structure and functions. Particularly for mitochondria, oxidation and cross-reaction of thiol groups are related to the assembly of membrane proteins to induce the mitochondrial permeability transition that may result in cell death. Therefore, cells exhibit mechanisms responsible for the redox balance of thiol groups that include the use of the peptide glutathione (GSH) and thioredoxin (Trx) via NADPH-dependent redox cycles. Both oxidation and cross-reactions of thiol groups may or may not involve the participation of oxidative stress promoted by free radicals. In the present minireview, it was

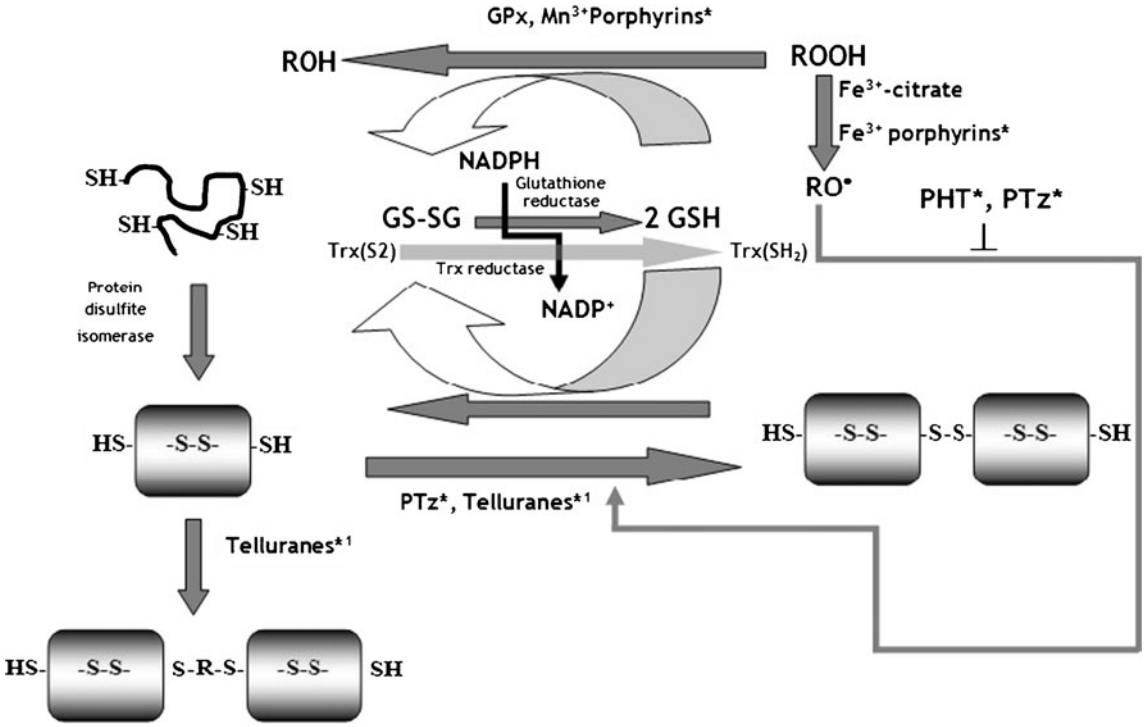

Fig. 2 Biological roles of protein thiol groups and the effects of SH reactives. In this Figure it is shown the participation of SH groups in the tertiary structure (*left side*) of proteins (*right side*) as well as in the aggregation promoted by thiol reactives like telluranes that could intercalate between two thiol groups (*lower left side*) or act as a thiol oxidant promoting the formation of inter-chain S-S bridges and protein aggregation (*right side*). Protein aggregation can also be promoted by free radicals (indicated by the thin *gray line*). The prooxidant effect of free radicals in the mitochondrial SH content can be prevented by the antioxidant action of enzymes like GPx and cationic manganese porphyrins by using GSH as reducing agent. The prevention of protein thiol groups oxidation by free radicals can also be prevented by the scavenger action of PTZ and PHT. Despite the mechanism, the protein oxidation can be reversed by the use of GSH and Trx that, in turn, are recycled by using NADPH. Compounds discussed in this minireview are labeled with *. *Label 1* indicated that for a discussion about possible mechanisms of tellurane effects, see Pessoto et al. (2007)

shown that different classes of chemicals have their beneficial or deleterious effects on mitochondrial bioenergetics related to the reactivity with thiol groups. The effects of meso-tetrakis porphyrins on mitochondria are modulated by the central metal, type of *meso* substituent and availability of axial ligands. The cationic Mn^{3+} *meso-tetrakis* porphyrins exhibits protective effects in rat liver mitochondria by acting as a GSH peroxidase, while the Fe^{3+} partner as well as the anionic form act as peroxidase-generating free radicals from the scission of peroxides. The deleterious effects of free radicals include the oxidative attack to thiol groups of mitochondrial membrane proteins leading to an inter-chain crosslink and the MPT. In rat liver mitochondria, the phenothiazine nucleous (PHT) can prevent oxidative damage by peroxide-derived free radicals generated by Fe^{3+}-citrate but not by cytochromes. In a different way, palladacycles, telluranes and phenothiazine-derivatives have their effects on mitochondrial bioenergetics, directly or indirectly related to the reaction with thiol groups not accompanied by oxidative stress. As a paradox, telluranes and PTZ in a concentration-dependent way promote the MPT via a crosslink of thiol groups concomitant with efficient antioxidant properties on lipids. The mechanism of a protein crosslink exhibited by telluranes and phenothiazines deserves future investigations. Figure 2 summarizes topics outlined from this minireview.

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