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The biological functions of polyamine oxidation products by amine oxidases: Perspectives of clinical applications

Review Article

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Summary. The polyamines spermine, spermidine and putrescine are ubiquitous cell components. If they accumulate excessively within the cells, due either to very high extracellular concentrations or to deregulation of the systems which control polyamine homeostasis, they can induce toxic effects. These molecules are substrates of a class of enzymes that includes monoamine oxidases, diamine oxidases, polyamine oxidases and copper containing amine oxidases. Polyamine concentrations are high in growing tissues such as tumors. Amine oxidases are important because they contribute to regulate levels of mono- and polyamines. These enzymes catalyze the oxidative deamination of biogenic amines and polyamines to generate the reaction products H_2O_2 and aldehyde(s) that are able to induce cell death in several cultured human tumor cell lines. H_2O_2 generated by the oxidation reaction is able to cross the inner membrane of mitochondria and directly interact with endogenous molecules and structures, inducing an intense oxidative stress. Since amine oxidases are involved in many crucial physiopathological processes, investigations on their involvement in human diseases offer great opportunities to enter novel classes of therapeutic agents.

Keywords: Polyamines – Amine oxidases – Multidrug resistance – Colon adenocarcinoma – Acrolein – Mitochondria

Abbreviations: AdNT, adenine nucleotide translocase; ALDH, aldehyde dehydrogenase; AO, amine oxidase; BSAO, bovine serum amine oxidase; CHO, Chinese hamster ovary; Cu-AO, copper containing amine oxidases; DAO, diamine oxidase or histaminase; FAD-AO, flavin-adenin-dinucleotide dependent AO; GABA, γ-aminobutyric acid; GSH, reduced glutathione; LoVo, human colon adenocarcinoma; MAO, monoamine oxidase; MDL 72527, N^1,N^4-bis(2,3-butadienyl)putrescine; MDR, multidrug-resistant; METC, mitochondrial electron transport chain; MPT, mitochondrial permeability transition; PAO, polyamine oxidase; PBS, phosphate-buffered saline; PC, pentose phosphate cycle; PEG, polyethylene glycol; ROS, reactive oxygen species; SAO, serum amine oxidase; TOPA, trihydroxyphenylalanine; TPQ, 2,4,5-trihydroxyphenylalanine quinone

Introduction

The metabolic pathways responsible for the interconversion and elimination of biogenic amines, including di- and polyamines, are essentially the same in all living organisms. The oxidation of polyamines plays an important role in polyamine metabolism in that not only it is responsible for the formation of catabolytes to be excreted, but it also represents the main process by means of which polyamines are irreversibly converted into molecules having different physiological functions. Some of these functions have been widely investigated, others have not yet been fully defined and some others are probably still not known at all. As polyamines are becoming very important in medical biochemistry, an ever increasing interest has been growing around the homeostatic mechanisms which regulate their intracellular concentration.

Oxidation represents a crucial reaction in polyamine metabolism, by means of which these polycations enter the catabolic routes for irreversible inactivation and elimination. Mono-, di- and polyamines, as well as several N-acyl amines, are oxidatively deaminated by amine oxidases (AOs) in a reaction consuming O_2 and H_2O, either by the removal of one primary amino group or by the cleavage of the molecule containing one or two secondary amino groups at one of the secondary nitrogen atoms at a time, and oxidation of the terminal

carbon of the remaining molecule to an aldehyde group during the same catalytic cycle. Oxidative deamination produces the aldehyde, the removed amine moiety and H_2O_2 in stoichiometric amounts according to the following equations:

1. $R\text{-}CH_2\text{-}NH_2 + H_2O + O_2 \rightarrow R\text{-}CHO + NH_3 + H_2O_2$
2. $R\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}R + H_2O + O_2 \rightarrow R\text{-}CHO + NH_2\text{-}CH_2\text{-}R + H_2O_2$

1. Cleavage at the primary amino group (operated by both copper and FAD-dependent AOs)
2. Cleavage at the secondary amino group (operated by the FAD-dependent AOs)

Classification and occurrence of amine oxidases in living organisms

Amine oxidases (amine: oxygen oxidoreductases) (AOs) are ubiquitous enzymes occurring in all prokaryotic and eukaryotic organisms.

AOs are enzymes showing not only heterogeneity in structure, but also differences in substrate and inhibitor specificity, mechanism of substrate oxidation as well as different subcellular localization. A criterion of classification can be that of dividing AOs into two subclasses (Mondovì et al., 1989), depending on the redox cofactor(s) they use: those containing flavin-adenin-dinucleotide (FAD; EC 1.4.3.4), as a cofactor, namely cytosolic enzyme polyamine oxidase (PAO) and mitochondrial monoamine oxidase (MAO), and those having copper and a second organic prosthetic group identified as 2,4,5-trihydroxyphenylalanine quinone (TPQ, Cu-AOs; EC 1.4.3.6) (Table 1).

Mitochondrial monoamine oxidases are primarily involved in the metabolism of the biogenic monoamine neurotransmitters and exogenous aryl-alkylamines. They therefore play a role of secondary importance in the oxidation of acetylated polyamines. In mammals, two different forms of MAOs are present in most tissues, namely MAO-A and MAO-B (Abell et al., 2001), classified on the basis of their selective inhibition by clorgyline and deprenyl, respectively. Isoenzyme A occurs in cathecholaminergic neurons, while isoenzyme B is present in neurons and glial cells of the human brain and also in other different cell types. This different localization suggests that the two isoforms have different physiological functions. In fact, A and B MAOs are probably related to psychiatric and neurological disorders such as depression and Parkinson's disease, respectively (Checkoway et al., 1998). It is well known that MAO-A preferentially deaminates aromatic monoamines such as the neurotransmitters noradrenaline and serotonin, whereas MAO-B oxidizes preferentially phenylethylamine and benzylamine (Tipton, 1994). Tyramine, dopamine, and tryptamine appear to be substrates for both isoforms.

To the Cu-AOs belong numerous enzymes, that have been purified to homogeneity and characterized, from a variety of microorganisms, including fungi and bacteria, plants and animals (Mondovì et al., 1989). Plants Cu-AOs have been isolated from several sources, but only some of them, i.e. *Pisum sativum* (Kumar et al., 1996), *Lens esculenta* (Medda et al., 1995) and *Euphorbia characias* (Padiglia et al., 1998), have been highly purified and characterized. It was observed that their spectroscopic features are similar to those of the animal Cu-AOs. The most representative, and most widely investigated, Cu-containing AOs are those from the serum of some animal species (SAO) and the one classified as diamine oxidase (DAO, histaminase).

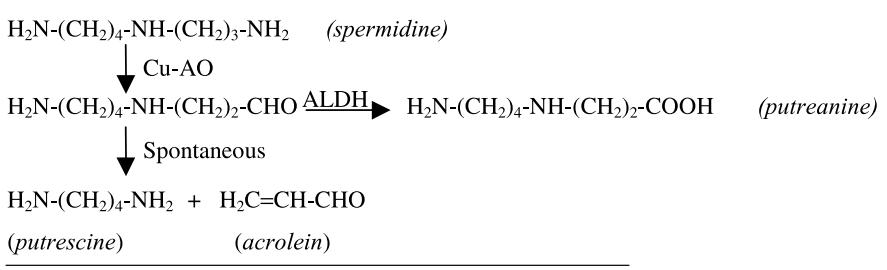
The SAOs preferentially oxidize spermine and spermidine at the primary amino group as well as some aliphatic and aromatic monoamines; DAO acts mainly on diamines and on the important biogenic amine histamine.

Among mammalian Cu-AOs, those from pig plasma (Yadav and Knowles, 1981), bovine serum (Morpurgo et al., 1992; Klinman and Mu, 1994), equine plasma (Carter et al., 1994) and pig kidney (DAO) (Steinebach et al., 1995) have been extensively investigated. All enzymes are dimeric, with 33 fully conserved residues close to the catalytic site and a similar molecular organization of the strongly interacting subunits (Li et al., 1998). The active site, consisting of one copper and one organic cofactor (TPQ) connected by a water molecule,

Table 1. Nomenclature, abbreviations, cofactor(s) and tissue distribution of the main amine oxidases

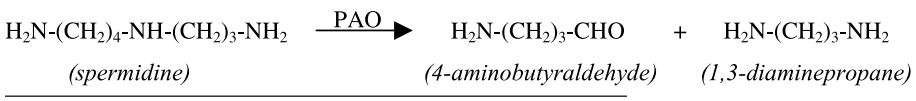
Monoamine oxidase	(MAO)	FAD	All tissues (outer mitochondrial membrane)
Polyamine oxidase	(PAO)	FAD	All tissues (cytoplasm, peroxisomes)
Serum amine oxidase	(SAO)	Cu, TPQ	Serum (some species)
Diamine oxidase	(DAO)	Cu, TPQ	Tissues (cytoplasm, microsomal fraction), biological fluids

is located inside each subunit and communicates with the solvent through a hydrophobic channel. The redox-active carbonyl cofactor of eukaryotic copper-amine oxidases is the 2,4,5-trihydroxyphenylalanine quinone, also known as 6-hydroxydopa (abbreviated as TPQ or TOPA). TOPA quinone (TPQ) is covalently bound to the polypeptide chain of the protein (Janes et al., 1990) and is part of a consensus sequence Asn-TPQ-Asp/Glu of the polypeptide chain, which is common to all copper amine oxidases analysed so far (Janes et al., 1992). Topa quinone (TPQ) is derived from the copper-catalysed oxidation of a post-translationally modified tyrosine residue within the active site (Cai and Klinman 1994; Matsuzaki et al., 1995).



inal oxidation and interconversion reactions. As a general rule, the former reaction is accomplished by the Cu-dependent AOs, and the latter one by the FAD-enzymes. However, this distinction is not absolute: in fact, spermine and spermidine, oxidized by Cu-AOs, may undergo spontaneous degradation when not previously further oxidized by aldehyde dehydrogenases, forming spermidine and putrescine, respectively, with formation of the aldehyde acrolein.

Moreover, some AOs isolated from bacteria and plants, which cleave polyamines (Polyamine Oxidase, PAO), at the secondary nitrogen form 1,3-diaminepropane as reaction product instead of putrescine, which is not liable to being reutilised for resynthesis of higher polyamines.



Amine oxidases and polyamine oxidation

The widespread occurrence of AOs in different organisms and organs accounts for an undoubtedly relevant biological function in biogenic amine metabolism. The structural and biological functions differ depending on the source of the enzyme. AOs have a physiological role in nutrient metabolism, removing potentially toxic biogenic amines from blood plasma and regulating intracellular spermine and spermidine concentration. In prokaryotes, the AOs allow the microorganisms to use amines as carbon and nitrogen sources, whereas in plants and mammals detoxification processes and regulation of fundamental cellular processes such as tissue differentiation, cell growth and programmed cell death are the main roles (Averill-Bates et al., 1993; Dove et al., 2000). In eukaryotes, it is believed that AOs influence cell growth, signalling and development (Green et al., 2002).

From a physiological point of view, two possible catabolic pathways are distinguishable for polyamines: terminal oxidation and interconversion reactions. As a general rule, the former reaction is accomplished by the Cu-dependent AOs, and the latter one by the FAD-enzymes. However, this distinction is not absolute: in fact, spermine and spermidine, oxidized by Cu-AOs, may undergo spontaneous degradation when not previously further oxidized by aldehyde dehydrogenases, forming spermidine and putrescine, respectively, with formation of the aldehyde acrolein.

Disregarding these exceptions of minor importance, this distinction of the polyamine oxidative reactions suggests a different physiological significance for the FAD- and Cu-dependent enzymes in tissues and biological fluids. FAD-dependent PAO, which is integrated into the interconversion pathway, was purified from several sources. PAO is present at high activity in nearly all cells and organs of the vertebrate organism. It is found in both the cytosolic and peroxisome fractions. In contrast, Cu-AOs activity is present at considerably different levels varying the tissues and organs. Cu-AOs have both intracellular and extracellular localization, being present in serum and in some biological fluids.

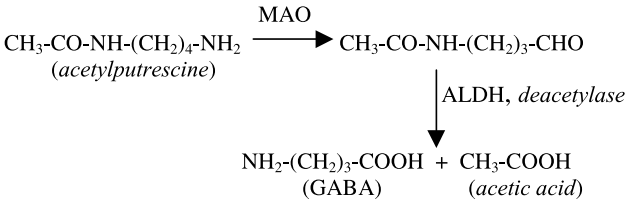
Under physiological conditions, Cu-AO activity is present in the serum of swine, bovine and other animals, but was not detected in horse and human. In man, serum amine oxidase activity rises during pregnancy or under some pathological conditions, such as in cancer patients.

Serum Cu-AOs oxidatively deaminate diamines and polyamines. Bovine serum AO catalyzes the oxidative

deamination of spermine and spermidine (Gahl and Pitot, 1982). Swine serum AOs deaminates diamines, and exhibits histaminase activity (Buffoni, 1966). Instead, in human pregnancy serum it seems to be present only as an enzyme able of deaminating both diamines and polyamines (Gahl et al., 1982).

The Cu-AOs that exert preferential substrate specificity for diamines are termed diamine oxidases (DAO) (Bach et al., 1988). The AOs isolated from several animal sources, able of oxidizing histamine as substrate, are seldom still called histaminases. DAO activity can also be detected in almost all animal tissues with few exceptions (Parchment et al., 1990), but is especially elevated in rapidly proliferating tissues, such as placenta, fetal tissues and organs undergoing hyperplastic, hypertrophic or neoplastic processes (Maslinski et al., 1985; Perin et al., 1985). DAO is abundant in the kidney cortex of several animal species, including man. Its presence in the proximal convoluted tubules epithelium (Argentu-Cerù and Autuori, 1985) would imply a role as biogenic amine scavenger. DAO acting mainly on putrescine, which represents the precursor of higher polyamines, seems to have a leading role in the homeostatic control of intracellular biogenic amine levels.

MAOs show a uniform distribution in the different tissues and organs. PAO activity is present in all tissues and appears to be localized in the cytosol. N^1-acetylspermine and N^1- acetylspermidine are the best substrates, but at a lower rate the unconjugated polyamines are also oxidized (Seiler, 1985). Mitochondrial MAO seems to have a major role in the vertebrate brain (Seiler and Al-Therib, 1974) where the Cu-AO activity is very low, and by catalysing a terminal oxidation reaction MAO achieves in the brain the role proper to Cu-AOs in other organs.



With the exception of cadaverine and putrescine, combined MAO and PAO substrate specificity patterns cover all the free and acetylated polyamine pool (Table 2) (Mondovì et al., 1989).

It is important to cite that the *in vivo* substrate specificity of AOs could be somehow different than that found under standard experimental conditions with purified enzymes. For instance, alteration of mitochondrial membrane-bound MAO occurs under different situations which induce concomitant accumulation of lipid peroxides (Gorkin, 1983). The presence of endogenous inhibitors (Buffoni et al., 1983) and the effect of different environmental conditions such as *in vivo* association with membranous structures (Schmutzler et al., 1969) should be taken in account.

Table 2. Substrates of the most representative AOs

	BSAO	SKDAO	RLPAO	MAO
putrescine		*		
acetylputrescine		*		*
cadaverine		*		
acetylcadaverine		*		*
spermidine	*		*	
N^1acetylspermidine		*	*	
N^8acetylspermidine	*	*		*
spermine	*		*	
N^1-acetylspermine		*	*	
N^1,N^{12}acetylspermine			*	

* oxidized; blank space: not oxidized

BSAO, bovine serum amine oxidase; *SKDAO*, swine kidney diamine oxidase; *RLPAO*, rat liver polyamine oxidase; *MAO*, A and B mitochondrial monoamine oxidase

Note that relative substrate affinities are not considered; oxidation rates of AOs differ widely varying the substrate

Amine oxidases in physiopathological conditions and their therapeutic potential

Studies on AO activity in pathological conditions are of notable interest in that they provide useful information on the state of activity of polyamine metabolism.

Since AOs are involved in many crucial physiopathological processes, investigations on their involvement in human diseases offer great opportunities to enter novel classes of therapeutic agents.

DAO activity was assayed in the regenerating rat liver after partial hepatectomy. The enzyme was found to increase with a peak of activity between 16 and 48 hrs. The return to normal values occurs about one week after the operation (Sessa et al., 1982).

In the rat heart, the development of isoproterenol-induced cardiac hypertrophy provoked an increase of DAO activity. After suspension of drug administration, the enzyme activity regressed to the basal value, together with the cardiac hypertrophy (Perin et al., 1983).

A high DAO activity was also found in a human endocrine tumor, medullary thyroid carcinoma. The enzyme activity was increased not only in the neoplastic tissue, but, for some patients with this kind of pathology and

especially those showing metastases, also in blood (Baylin et al., 1972).

Increased DAO activity was associated with another human tumor which often shows endocrine cell properties, small cell carcinoma of the lung (Baylin et al., 1978). However, other lung cancers of different histologic types had also increased DAO levels (Berger et al., 1981). In medullary thyroid carcinoma and lung cancers, increased DAO activity was also found in metastases as compared to surrounding normal tissue.

An increased DAO activity was also noticed in ascites and pleural fluids of patients having various other types of cancer. Among the others, ovarian carcinoma cancer appeared to be the source of the DAO present in the ascites fluids (Ettinger et al., 1980).

A direct relationship between DAO activity and tumor promotion was demonstrated by Kusche et al. (1988). An induction of tumors in rats was observed upon azoxymethane (AOM) treatment associated with the inhibition of DAO by aminoguanidine (AG). After 52 weeks, none of the animal treated with the sole AOM resulted to have a tumour. In the rats treated with AOM and AG together, tumor was not observable after 24 weeks, while 15 tumors were detected in 10 rats out of 15 after 53 weeks.

A high Cu-AO activity has been associated to mortality in patients suffering from hearth disease, and it has been recognized as a potential risk factor in vascular disorders associated with diabetic complications (Obata, 2002; Yu et al., 2002). Cu-AOs are considered scavengers of circulating toxic amines. Cu-AOs substrates remarkably increase transport of glucose, thus mimicking the effect of insulin. This finding suggests that compounds having high Cu-AO affinity may be potential anti-diabetic agents. Cu-AO is also involved, at the cellular level, in the inflammatory process (Dalfó et al., 2003). Cu-AO inhibitors may be employed as drugs in the treatment of inflammatory diseases (in particular chronic inflammatory conditions, such as chronic arthritis, inflammatory bowel disease, and chronic skin dermatosis), and in vascular diseases (Smith et al., 2002). The relationship of Cu-AOs with the vascular disorders is consistent with its role as a vascular adhesion protein (VAP-1) in the process of inflammation. It was suggested that Cu-AO activity is a characteristic function of the VAP-1 adhesion molecule in vascular endothelium, and that the involvement of VAP-1/Cu-AO in the extravasation process can be explained by local oxidation of currently undefined lymphocyte cell-surface associated substrates.

As concerns the physiological role, bovine serum amine oxidase (BSAO) has been recognized to take part in protein post-translational modification (Mondovì et al., 2003). At the cellular level, BSAO was showed to increase significantly the current of K^+ channels in N1E-115 neuronal cells, thus resulting as a modulator of the electric properties of the ionic channels. At the organ level, BSAO shows an anti-arrhythmic effect on ischemic isolated hearts at reperfusion. Furthermore, BSAO behaves as a free radical scavenger, and protects the isolated heart against dangerous effects of the reactive oxygen intermediates.

The cytotoxic effect induced by BSAO and spermine was examined in human tumor cell lines, either drug-sensitive or multidrug-resistant (MDR). The study has suggested a new strategy to overcome MDR of human cancer cells by using BSAO, which generates cytotoxic products from spermine oxidation, H_2O_2 and aldehyde(s), and acrolein (Calcabrini et al., 2002) (see next paragraph for details).

PAO has an important role in cell growth, differentiation, and gene expression. MDL 72527 is a selective enzyme-activated irreversible PAO inhibitor, endowed with anti-cancer and contragestational effects. The anticancer activity of MDL 72527 is due to a profound depletion in the polyamine pools of tumor cells, whereas the capability to induce apoptosis was ascribed to its lysosomotropic properties (Seiler et al., 2002). Oxidation of polyamines by serum PAO increased multiple phase 2 genes by activating Nrf2 pathway, likely through the metabolite acrolein (Kwak et al., 2003).

MAO-A and MAO-B inhibitors are successfully employed in the treatment of psychiatric and neurological disorders (Andrews and Nemeroff, 1994). MAO-A inhibitors, which modulate the neurotransmitters serotonin, adrenaline, noradrenaline and dopamine, are mainly indicated in the clinical management of mental depression. MAO-B inhibitors are used in Parkinson's disease, a neurodegenerative syndrome for which the main therapy regards the amelioration of the symptoms with *L*-DOPA and/or DA agonists (Drukarch and Van Muiswinkel, 2000). MAO-B is involved in the apoptotic process. The MAO-B inhibitor selegiline combined with *L*-DOPA induced neuronal apoptosis at high doses. In contrast, at low doses selegiline behaves as a neuroprotector agent by stopping the apoptotic event (Walkinshaw and Waters, 1995).

Biological effects of polyamines and their enzymatic oxidation products

a) Polyamines, their oxidation products and cell death

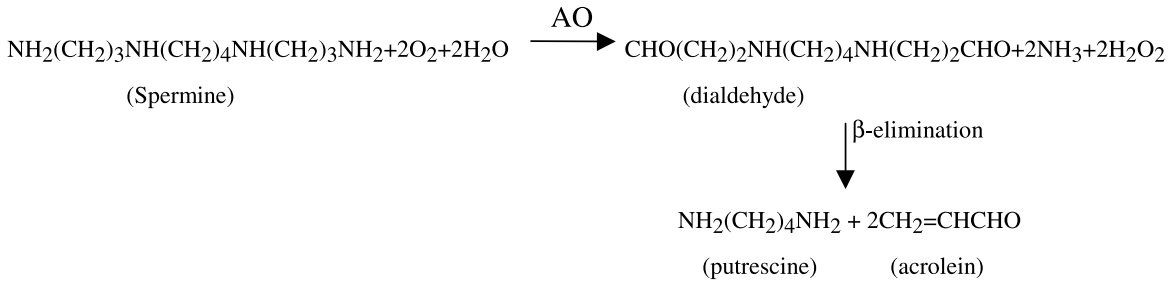
The polyamines spermine, spermidine and putrescine are ubiquitous metabolites. If they accumulate excessively

within the cells, due to either very high extracellular concentrations or deregulation of the systems which control their homeostasis, they can induce toxic effects. AOs are important because they contribute to regulate the levels of polyamines, catalyzing their oxidative deamination. The oxidation products, hydrogen peroxide and aldehyde(s), have been implicated in programmed cell death, induction of cytotoxicity, inhibition of cell division (Bachrach et al., 1987; Henle et al., 1986) and can cause apoptosis in several cell types (Lindsay and Wallace, 1999). Previous studies showed that cytotoxicity of polyamines and their acetyl derivatives, in cell cultures that require the presence of serum, correlates with their properties as substrates of serum amine oxidases (Morgan, 1988): spermine, spermidine, N^1-acetylspermine and N^8-acetylspermidine are about equipotent; putrescine, N^1-acetylputrescine, N^1-spermidine and N^1,N^{12}-diacetylspermine are not cytotoxic. It was observed that cytotoxicity is inhibited by aminoguanidine (Henle et al., 1986), or 3-hydroxy-benzyloxyamine. This was confirmed by some studies showing that cytotoxicity was also caused by purified amine oxidase from bovine serum and exogenous spermine in Chinese hamster ovary (CHO) cells (Averill-Bates et al., 1993). The copper enzyme BSAO (EC 1.4.3.6; MW 170 kDa) oxidatively deaminates spermine and spermidine containing primary amino groups. The products are H_2O_2, the corresponding aldehyde and ammonia.

These compounds generally may undergo either spontaneous modification or further transformation by aldehyde-metabolizing enzymes. The aminoaldehydes can be oxidized to carboxylic acids in an enzymatic reaction involving aldehyde dehydrogenase or, alternatively, they may be reduced to alcohols by alcohol dehydrogenase. In the case of spermine, an unstable dialdehyde liable to undergo spontaneous degradation with production of acrolein (CH_2=CHCHO) is generated (Alarcon, 1970) (Scheme 1).

H_2O_2 was considered responsible for cytotoxicity by some authors (Parchment et al., 1990), while others rather ascribed cytotoxicity to aldehydes (Morgan, 1987). Other studies suggested that H_2O_2 is only in part responsible for cell killing since catalase only affords partial protection (Averill-Bates et al., 1993; Calcabrini et al., 2002). These results demonstrated that both aldehyde(s) and H_2O_2, formed in the oxidative deamination reaction of exogenous spermine by an AO, are involved in cytotoxicity in CHO cells and in human colon adenocarcinoma (LoVo) cell lines, on both sensitive wild type (WT), and their MDR variant, selected by continuous exposure to colchicine (Lord-Fontaine et al., 2001) or doxorubicin (DX) (Calcabrini et al., 2002), respectively. The findings provide direct evidence for the role of AOs in converting spermine into a toxic agent, and several studies describe attempts to clarify the role of aldehydes, acrolein, and H_2O_2 in cytotoxicity. Cytotoxicity, determined in PBS-1% bovine serum albumin occurred when cells were exposed to BSAO (6.54×10^{-3} U/mL) in the presence of different concentrations of spermine (0–340 μM). Fetal bovine serum, usually present in the incubation medium, was omitted in these conditions. In the presence of BSAO and exogenous spermine, the percent cell survival decreased during 60 min of incubation at 37°C. The presence of catalase in the incubation mixture partially inhibited cytotoxicity. This means that H_2O_2 is not the sole toxic agent and that other species, such as aldehydes, contributed to the cytotoxic effect.

This was also supported by the protective effect of exogenous NAD-dependent aldehyde dehydrogenase (ALDH, EC 1.2.1.5) (Averill-Bates et al., 1994). The activities of exogenous catalase and ALDH, simultaneously added to the incubation mixture, were high enough to transform the products of the amine oxidase catalyzed reaction at a rate that prevents their accumulation to cytotoxic levels (Averill-Bates et al., 1994; Calcabrini et al., 2002). Evaluation of the dose response curve for exogenous H_2O_2 and acrolein cytotoxicity in CHO cells supports the sequential involvement of H_2O_2 and other(s) products in cytotoxicity and suggests acrolein



Scheme 1. Reaction scheme for spermine oxidation in the presence of BSAO

as a likely second toxic product of spermine. H_2O_2 was cytotoxic to cells at lower concentrations than acrolein.

It was also observed that cytotoxicity induced by spermine, at very low concentration (6 μM), is higher in MDR cells, LoVo DX, than in their sensitive counterparts, LoVo WT (Calcabrini et al., 2002). This finding was previously observed on chinese hamster ovary (CHRC5) cells resistant to colchicine (Lord-Fontaine et al., 2001), using higher concentrations of spermine (340 μM). On CHO cells, this phenomenon was attributed to a different content of GSH in MDR cells when compared to the parental sensitive ones. Instead, in human colon adenocarcinoma cells, the assay according to Anderson (1989) and flow-cytometric studies revealed a similar fluorescent signal in control LoVo WT and LoVo DX cells, and in the samples treated with BSAO/spermine enzymatic system or exogenous H_2O_2. The fluorescent signal was due to the presence of the intracellular thiol groups (Calcabrini et al., 2002).

b) Death mechanism of tumoral cells

To clarify the mechanism of cytotoxicity induced by H_2O_2 and aldehyde, formed in the enzymatic oxidative deamination of polyamines, several studies were carried out.

H_2O_2 generated by the oxidation of biogenic amines and polyamines is able to cross the inner membrane of mitochondria and directly interacts with endogenous molecules and structures: as result, under appropriate conditions, it induces an intense oxidative stress (Fig. 1) (Mathai and Sitaraman, 1994). H_2O_2 can be reduced to H_2O plus O_2 by catalase, a defence mechanism that, however, seems to be present only in heart mitochondria (Radi et al., 1991). H_2O_2 is also reduced to H_2O by glutathione peroxidase and thioredoxin peroxidase with the production of oxidized glutathione and oxidized tioredoxin that in turn are both reduced by glutathione reductase and thioredoxin reductase. This reaction requires oxidation of NADPH to $NADP^+$. The NADH, which is available in amounts regulated by respiration and Bcl-2, then reduces $NADP^+$ to NADPH with NAD^+ by transhydrogenase which induces H^+ uptake. The reaction catalyzed by transhydrogenase is supported by ATP hydrolysis or high electrochemical gradient and provides the increasing of NADPH in the mitochondrial matrix (Jackson, 1991).

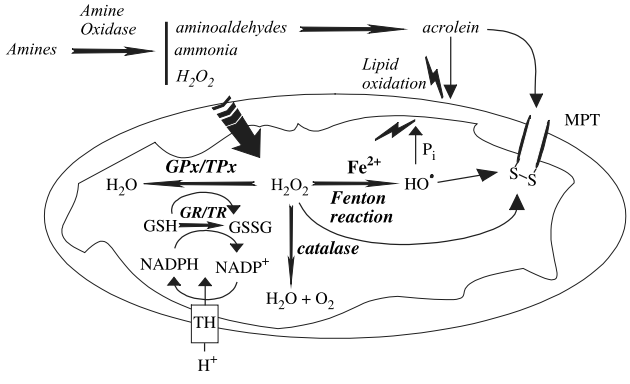


Fig. 1. Mitochondrial ROS production by amines oxidation, their implications on membrane damage and defence mechanisms. The reaction products of amines oxidation, hydrogen peroxide, hydroxyl radical, aldehydes and acrolein, are able to cause the mitochondrial permeability transition and lipid membrane oxidation. H_2O_2 can be removed by catalase and $HO^\cdot$ by mannitol. The defence mechanisms by GPx/GR and TPx/TR systems and nicotinamide nucleotide TH, are also shown. *GPx*, Glutathione peroxidase; *GR*, Glutathione reductase; *MPT*, Mitochondrial permeability transition; *TH*, Transhydrogenase; *TPx*, Thioredoxin peroxidase; *TR*, Thioredoxin reductase

When H_2O_2 removal pathways are lacking or inactivated, H_2O_2 accumulates in large amount and by interacting with Fe^{2+} of Fe/S centres located in the respiratory chain, raises the highly reactive hydroxyl radical ($HO^\cdot$) by means of Fenton reaction. The radical $HO^\cdot$ oxidizes some critical thiol (SH) groups of the adenine nucleotide translocase (AdNT), belonging to the mitochondrial permeability transition (MPT) pore complex, leading to pore assembly and opening. H_2O_2 alone can be able to oxidize the same SH groups leading to the MPT. Furthermore, also endogenous pyridine nucleotides are oxidized by the reactive oxygen species (ROS). It is not clear whether this oxidation is directly implicated in pore opening or it is a consequence of the process. Alternatively, $HO^\cdot$ may also promote membrane permeabilization through lipid oxidation, a process strongly stimulated by phosphate. All these observations clearly point out that MPT is related to the redox state of mitochondria and can be caused by ROS generated by biologically active amines oxidation too, while NADPH produced by the activity of transhydrogenase plays a central role in mitochondrial defence against oxidative stress. The indirect antioxidation effect of the energy dependent transhydrogenase might represent an evolutionary protective mechanism against oxidative stress since high membrane potential stimulates ROS generation (Korshunov et al., 1997).

The proteins involved in the pore architecture are not well determined, although it is commonly believed that AdNT can play a key role (Brustovetsky and Klingenberg, 1996; Brdiczka et al., 1998). In this regard it has also been observed that a critical cysteine residue of AdNT is oxidized in isolated and in *in situ* mitochondria which underwent MPT (Costantini et al., 2000).

Notably, it has been reported very recently that the ADP/ATP translocator is not essential for the MPT pore opening, but it would have a regulatory role (Kokoszka et al., 2004).

Investigations by transmission electron microscopy and fluorescence techniques, on the effects of the enzymatic oxidation products of 6 μM spermine, showed more pronounced ultrastructural modifications in LoVo MDR than LoVo WT cells. The structural changes mainly observed in the mitochondrial organules might cause functional alterations that were not reflected in damage of DNA, i.e. condensation of chromatin and/or fragmented nuclei, typical signals of apoptotic death (Calcabrini et al., 2002). In fact, Henle et al. (1986) showed that spermidine added to the culture medium containing fetal bovine serum resulted in extensive DNA damage and that the amount of damage increased rapidly as a function of the polyamine concentration (higher than 30 μM). The data supported that the enzymatic oxidation of spermidine resulted in the formation of both H_2O_2 and aldehyde(s), or acrolein. The aldehyde(s) toxicity may not be involved in DNA damage, but only contribute to the decreasing of cell survival.

The ultrastructural alterations might be associated with a depolarization of the mitochondrial membrane, that was immediately observed when the cells were treated with AO in the presence of spermine. Living cells previously labelled with the lipophilic cationic probe 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide, JC-1 dye (Cossarizza et al., 1993), allowed to reveal alterations of mitochondrial membrane potential by flow cytometry technique. The mitochondrial membrane depolarization was more evident in LoVo DX colon adenocarcinoma cells than in LoVo WT ones. The greater cytotoxic effect induced in LoVo MDR cells was not attributed to a lower number of mitochondria, determined by the analysis of the mitochondrial mass. Therefore, this finding suggested different structural and/or functional properties of the mitochondria that were present in sensitive and MDR cell lines. The study of the mitochondrial functionality showed a hyperpolarization status of the mitochondria of MDR cells. Since the mitochondrial membrane potential is related to the activity of the mitochondrial electron transport chain (METC), it is supposed that LoVo DX cells can exhibit a higher METC activity than LoVo WT ones. Jia et al. (1996) observed an increased METC activity in several MDR cell lines as compared to their sensitive counterparts. Thus, it was hypothesized that MDR cells present an increased METC activity because they highly express ATP-dependent P-glycoprotein (P-gp) (Jia et al., 1999). The METC activity led to the formation of ROS such as superoxide radical, H_2O_2 and hydroxyl radical (Sohal, 1997) which are usually removed by cells (Kowaltowsky and Vercesi, 1999). The treatments with AO/spermine enzymatic system showed an increased basal ROS production in LoVo DX cells that was not removed by cellular defences, resulting more sensitive than LoVo WT. The accumulation of these molecules induced a higher impairment of the mitochondrial structure and function in LoVo DX than LoVo WT cells.

Besides H_2O_2, several authors emphasized that also aldehydes can have a prominent role in inducing the oxidative stress in mitochondria in the presence of phosphate (Kowaltowski et al., 1996). In this regard, phosphate catalyzes aldehyde tautomerization (Indig et al., 1988) that in the presence of Fe^{3+} leads to the formation of aldehydes in triplet state (triplet carbonyls) (Nantes et al., 1995) which exhibit peroxidative activity (Kowaltowski et al., 1996).

The oxidation of spermine by PAO produces aminodialdehyde, H_2O_2 and ammonia. Aminoaldehyde generates acrolein spontaneously. Spermidine oxidation, instead, produces 3-aminopropanal which also gives acrolein (Sharmin et al., 2001), responsible of severe cytotoxicity. Other aldehydes (formaldehyde, acetaldehyde and propionaldehyde) were less toxic than acrolein. Thus, it is deducible that acrolein is a major toxic compound produced from spermine and spermidine by polyamine oxidase (Sharmin et al., 2001).

It has been observed that acrolein is able to deplete reduced glutathione level in liver causing an imbalance in the antioxidant defence. This leads to lipid peroxidation with the result of membrane damage, particularly at the mitochondrial level. Consequently, a loss of membrane integrity of mitochondria affects the activity of the Krebs cycle enzymes, cytochromes function and ATP synthesis (Arumugam et al., 1999). Furthermore, it has also been observed that acrolein inhibits state III respiration in brain mitochondria without reducing the activity of complexes I–IV and altering calcium transporter activity (Picklo and Montine, 2001). Recent observations indicate that acrolein is able to induce the MPT (Salvi and Toninello, manuscript in preparation). Acrolein is also a major cytotoxic product of lipid peroxidation which is elevated in brain in several neurodegeneratives diseases (Picklo and Montine, 2001; Pocernik and Butterfield, 2003). In this regard, acrolein adduction to neuronal proteins has been demonstrated in patients with Alzheimer's disease (Picklo and Montine, 2001). Furthermore, a study reports that

acrolein in Alzheimer's disease decreases the activity of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase in a dose-dependent manner (Pocernik and Butterfield, 2003). It has been found that acrolein, in order to exhibit this effect, binds to lipoic acid, a cofactor of both the cited enzymes, and also interacts with NAD^+ in such a way as to decrease the production of NADH (Pocernik and Butterfield, 2003). In conclusion, acrolein toxicity generally leads to impairment in mitochondrial functions and functionally contributes to the neurodegenerative diseases.

To learn more about the cellular response to toxic species produced during the enzymatic oxidation of polyamines, the role of glucose metabolism and the role of glutathione in the cytotoxicity had been also examined by Agostinelli et al. (1996). The cytotoxic effect of each product was altered to a different extent by carrying out the experiments in the absence of glucose, usually available in the incubation mixture. The results suggest that H_2O_2 and aldehyde(s) or acrolein are likely to cause cytotoxicity via different mechanisms (Agostinelli et al., 1996).

Both glucose metabolism and the level of intracellular glutathione had a role in the cytotoxicity induced on cells by spermine oxidation products. H_2O_2 and aldehyde(s) were toxic to CHO cells at concentrations one order of magnitude apart. Glucose protected cells against cytotoxicity in the presence of BSAO at low spermine concentration, where H_2O_2 was the sole species responsible for the cytotoxic effect. Only in the presence of exogenous catalase, the residual cytotoxicity could be attributed to aldehyde(s) (Agostinelli et al., 1996). An essential role for glucose, metabolized via the pentose phosphate cycle (PC), for the detoxification of H_2O_2 was described (Averill-Bates and Przybytkowski, 1994). When cells were exposed to BSAO and spermine the activity of the PC increased. In contrast, there was no activation of the PC when aldehyde(s) were the only toxic species present. Depletion of intracellular glutathione, obtained by L-buthionine sulfoximine treatment, sensitized the cells to the cytotoxic effects induced by both H_2O_2 and aldehyde(s) derived from spermine oxidation by AO. The metabolism of glucose via the PC and the glutathione peroxidase, in the glutathione redox cycle, have an important role in protection against H_2O_2 generated from spermine enzymatic oxidation. Glutathione appears to have a role in protecting cells against cytotoxicity attributed to spermine-derived aldehyde(s), most likely by conjugation in a reaction catalyzed by glutathione S-transferase, whereas metabolism of glucose via the PC did not. The metabolism of both glucose and glutathione affect the cellular response to H_2O_2 and aldehyde(s) or acrolein derived from spermine, although different pathways are involved (Agostinelli et al., 1996).

Perspectives and clinical applications

The enhancement of AO activity in tissues undergoing pathological proliferative phenomena may reasonably be used in the treatment of neoplastic diseases. It is well known that reduced polyamine and increased H_2O_2 and aldehyde(s) levels exert an inhibitory effect on the cellular growth and division (Bachrach, 1970). The enhanced formation of aldehyde(s) or acrolein derived from endogenous polyamines may increase the effect of currently used antineoplastic therapies such as hyperthermic treatment.

Hyperthermia is a clinical treatment beneficial against certain tumours, the effects of which can be enhanced by some metabolic products of tumour cells, especially when combined with anticancer agents that become much more toxic at elevated temperature. This suggested an investigation of the effect of high temperature (42°C) on the cytotoxicity of spermine oxidation products. A marked enhancement of cytotoxicity was observed at 42°C, i.e. concentrations of BSAO and spermine which were non-toxic at 37°C became cytotoxic at 42°C (Agostinelli et al., 1994). The persisting effect in the presence of exogenous catalase showed that hyperthermic cytotoxicity is due to spermine-derived aldehydes, which behave as thermosensitizers. Such an effect was also observed in pleiotropic MDR cells. Studies showed that spermine-derived aldehydes were more toxic to MDR cells than to their sensitive counterparts (Lord-Fontaine et al., 2001). The thermosensitizing activity of aldehyde(s) formed during BSAO catalyzed oxidation of spermine has potential value for improving the therapeutic effects of clinical hyperthermia. The studies reported using an extracellular application of AOs appear promising. Since polyamines are present at elevated levels in cancerous cells, it is expected that by delivering BSAO into them the toxic products of polyamines oxidation could be produced *in situ* for selective killing of the same cells. Therefore, strategies should be developed to find out how the enzyme could be delivered *in vivo*, for possible clinical application. BSAO was incorporated into liposomal vesicles (Agostinelli et al., 1988) and it was also demonstrated that the enzyme could be bound and internalized by cultured cells (Dini et al., 1991) Thus, endogenous polyamines present in tumor cells could be targeted and oxidized by AOs. In this context, macromolecular anticancer drugs can be conjugated with biocompatible polymers which function as carriers and stabilizers, resulting in decreased drug toxicity and increased

therapeutic efficacy (Maeda et al., 1992). It was shown that by conjugating BSAO with polyethylene glycol (PEG) hydrogels, with the aim of increasing its plasmatic half-life or its targetability under administrable form, the yield of immobilization resulted to be very high (Demers et al., 2001).

In conclusion, these researches might be considered of great interest and suggest future applications of AOs in anticancer therapy, and may prove effective mainly against MDR tumours (Calcabrini et al., 2002).

If the results of further investigations will be up to expectations, we think that the handling of amine oxidase activity, in the presence of polyamines, will undoubtedly turn out to be a powerful tool in the development of new anticancer treatments (Demers et al., 2001).

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References

Abell CW, Kwan SW (2001) Molecular characterization of monoamine oxidases A and B. Progress in Nucleic Acid Res Mol Biol 65: 129–156

Agostinelli E, Riccio B, Mucigrosso J, Befani O, Mondovì B (1988) On the handling of amine oxidase activity as a biochemical tool: perspectives. In: Perin A, Scalabrino G, Sessa A, Ferioli ME (eds) Perspectives in polyamine research. Wichtig Editor, Milan, Italy, pp 17–19

Agostinelli E, Przybytkowski E, Mondovì B, Averill-Bates DA (1994) Heat enhancement of cytotoxicity induced by oxidation products of spermine in Chinese hamster ovary cells. Biochem Pharmacol 48: 1181–1186

Agostinelli E, Przybytkowski E, Averill-Bates DA (1996) Glucose, glutathione and cellular response to spermine oxidation products. Free Rad Biol Med 20: 649–656

Alarcon RA (1970) Acrolein. IV. Evidence for the formation of the cytotoxic aldehyde acrolein from enzymatically oxidized spermine or spermidine. Arch Biochem Biophys 137: 365–372

Anderson ME (1989) Tissue glutathione. In: Greenwald RA (ed) Handbook of methods for oxygen radical research. CRC Press, Boca Raton, FL, pp 317–323

Andrews JM, Nemeroff CB (1994) Contemporary management of depression. Am J Med Chem 97: 24S–32S

Argentu-Cerù MP, Autuori F (1985) Localization of diamine oxidase in animal tissues. In: Mondovì B (ed) Structure and functions of amine oxidases. CRC Press, Boca Raton, FL, pp 89–104

Arumugam N, Thanislass J, Ragunath K, Niranjali Devaraj S, Devaraj H (1999) Acrolein-induced toxicity – defective mitochondrial function as a possible mechanism. Arch Environ Contam Toxicol 36: 373–376

Averill-Bates DA, Przybytkowski E (1994) The role of glucose in cellular defences against cytotoxicity of hydrogen peroxide in Chinese hamster ovary cells. Arch Bioch Biophys 312: 52–58

Averill-Bates DA, Agostinelli E, Przybytkowski E, Mateescu MA, Mondovì B (1993) Cytotoxicity and kinetic analysis of purified bovine serum amine oxidase in the presence of spermine in Chinese hamster ovary cells. Arch Biochem Biophys 300: 75–79

Averill-Bates DA, Agostinelli E, Przybytkowski E, Mondovì B (1994) Aldehyde dehydrogenase and cytotoxicity of purified bovine serum amine oxidase and spermine in Chinese hamster ovary cells. Biochem Cell Biol 72: 36–42

Bach AWJ, Lan NC, Johnson DL, Abell CW, Bembenek ME, Kwan SW, Seeburg PH, Shih JC (1988) cDNA cloning of human liver monoamines oxidase A and B: molecular basis of differences in enzymatic properties. Proc Natl Acad Sci 85: 4934-4938

Bachrach U (1970) Oxidized polyamines. In: Herbest EJ, Bachrach U (eds) Metabolism of polyamines 171(IV). Metabolism and biological functions of polyamines. Ann Acad Sci, New York, pp 939–956

Bachrach U, Ash I, Abu-Elheiga L, Hershkovitz M, Loyter A (1987) Fusion-mediated microinjection of active amine and diamine oxidases into cultured cells: effect on protein and DNA synthesis in chick embryo fibroblasts and in glioma cells. J Cell Physiol 131: 92–98

Baylin SB, Beaven MA, Buja LM, Keiser HR (1972) Histaminase activity: a biochemical marker for medullary carcinoma of the thyroid. Am J Med 53: 723–733

Baylin SB, Weisburger WR, Eggleston JC, Mendelsohn G, Beaven MA, Abeloff MD, Ettinger DS (1978) Variable content of histaminase L-dopa decarboxilase and calcitonin in small cell carcinoma of the lung. N Engl J Med 299: 105–110

Berger CL, Goodwin G, Mendelsohn G, Eggleston JC, Abeloff MD, Aisner S, Baylin SB (1981) Endocrine related biochemistry in the spectrum of human lung carcinoma. J Clin Endocrinol Metab 53: 422–429

Brdiczka D, Beutner G, Ruck A, Dolder M, Walliman T (1998) The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition. Biofactors 8: 235–242

Brustovetsky N, Klingenberg M (1996) Mitochondrial ADP/ATP carrier can be reversibly converted into a large channel by Ca^{2+}. Biochemistry 35: 8483–8488

Buffoni F (1966) Histaminase and related amine oxidase. Pharmacol Rev 18: 1163–1199

Buffoni F, Banchelli G, Ignesti G, Pirisino R, Raimondi L (1983) The presence of an inhibitor of benzylamine oxidase in human blood plasma. Biochem J 211: 767–769

Cai D, Klinman JP (1994) Evidence of a self-catalytic mechanism of 2,4,5-trihydroxyphenylalanine quinone biogenesis in yeast copper amine oxidase. J Biol Chem 269: 32039–32042

Calcabrini A, Arancia G, Marra M, Crateri P, Befani O, Martone A, Agostinelli E (2002) Enzymatic oxidation products of spermine induce greater cytotoxic effects on human multidrug-resistant colon carcinoma cells (LoVo) than on their wild-type counterparts. Int J Cancer 93: 43–52

Carter SR, McGuirl MA, Brown DE, Dooley DM (1994) Purification and active-site characterization of equine plasma amine oxidase. J Inorg Biochem 56: 127–141

Checkoway H, Franklin GM, Costa-Mallen P, Smith-Weller T, Dilley J, Swansons PD, Costa LG (1998) A genetic polymorphism of MAO-B modifies the association of cigarette smoking and Parkinson's disease. Neurology 50: 1458–1461

Cossarizza A, Baccarani-Contri M, Kalashnikova G, Franceschi C (1993) A new method for the cytofluorimetric analysis of mitochondrial membrane potential using the J-aggregate forming lipophilic cation 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1). Biochem Biophys Res Comm 197: 40–45

Costantini P, Belzacq AS, Vieira HL, Larochette N, de Pablo MA, Zamzami N, Susin SA, Brenner C, Kroemer G (2000) Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis. Oncogene 19: 307–314

Dalfò E, Hernandez M, Lizcano JM, Tipton KF, Unzeta M (2003) Activation of human lung semicarbazide sensitive amine oxidase by a low molecular weight component present in human plasma. Bioch Bioph Acta 1638: 278–286

Demers N, Agostinelli E, Averill-Bates DA, Fortier G (2001) Immobilization of native and poly(ethylene glycol)-treated ('PEGylated') bovine serum amine oxidase into a biocompatible hydrogel. Biotechnol Appl Biochem 33: 201–207

Dini L, Agostinelli E, Mondovì B (1991) Cultured epatocytes bind and internalize bovine serum amine oxidase-gold complexes. Biochem Biophys Res Commun 179: 1169–1174

Dove JE, Schwartz B, Williams NK, Klinman JP (2000) Investigation of spectroscopic intermediates during copper-binding and TPQ formation in wild-type and active-site mutants of a copper-containing amine oxidase from yeast. Biochemistry 39: 3690–3698

Drukarch B, Van Muiswinkel FL (2000) Drug treatment of Parkinson's disease. Biochem Pharmacol 59: 1023–1031

Ettinger DS, Rosenshein NB, Parmley TH, Mendelsohn G, Baylin SB (1980) Tumor cell origin of histaminase activity in ascites fluid from patients with ovarian carcinoma. Cancer 45: 2568–2572

Gahl WA, Pitot HC (1982) Polyamine degradation in foetal and adult bovine serum. Biochem J 202: 603–611

Gahl WA, Vale AM, Pitot HC (1982) Spermidine oxidase in human pregnancy serum. Biochem J 201: 161–166

Gorkin VZ (1983) Amine oxidases in clinical research. Pergamon, Oxford, p 121

Green EL, Nakamura N, Dooley DM, Klinman JP, Sanders-Loehr J (2002) Rates of oxygen and hydrogen exchange as indicators of TPQ cofactor orientation in amine oxidases. Biochemistry 41: 687–696

Henle KJ, Moss AJ, Nagle WA (1986) Mechanism of spermidine cytotoxicity at 37°C and 43°C in Chinese hamster ovary cells. Cancer Res 46: 175–182

Indig G, Campa A, Bechara EJH, Cilento G (1988) Conjugated diene formation promoted by triplet acetone acting upon arachidonic acid. Photochem Photobiol 48: 719–723

Jackson JB (1991) The proton translocating nicotinamide adenine nucleotide transhydrogenase. J Bioenerg Biomembr 23: 715–741

Janes SM, Mu D, Wemmer D, Smith AJ, Kaur S, Maltby D, Burlingame AL, Klinman JP (1990) A new redox cofactor in eukaryotic enzymes: 6-hydroxydopa at the active site of bovine serum amine oxidase. Science 248: 981–987

Janes SM, Palcic MM, Scaman CH, Smith AJ, Brown DE, Dooley DM, Mure M, Klinman JP (1992) Identification of topaquinone and its consensus sequence in copper amine oxidases. Biochemistry 31: 12147–12154

Jia L, Kelsey SM, Grahn MF, Jiang XR, Newland AC (1996) Increased activity and sensitivity of mitochondrial respiratory enzymes to TNFα-mediated inhibition is associated with cytotoxicity in drug-resistant leukemic cell lines. Blood 87: 2401–2410

Jia L, Kan-Zhi L, Newland AC, Mantsch HH, Kelsey SM (1999) Pgp-positive leukaemic cells have increased mtDNA but no increased rate of proliferation. Br J Haematol 107: 861–869

Klinman JP, Mu D (1994) Quinoenzymes in biology. Annu Rev Biochem 63: 299–344

Kokoszka JE, Waymire KG, Levy SE, Sligh JE, Cai J, Joned DP, Mac Gregor GR, Wallace DC (2004) The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. Nature 427: 461–465

Korshunov SS, Skulachev VP, Starkov AA (1997) High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. FEBS Lett 416: 15–18

Kowaltowski AJ, Vercesi AE (1999) Mitochondrial damage induced by conditions of oxidative stress. Free Radic Biol Med 26: 463–471

Kowaltowski AJ, Castilho RF, Grijalba MT, Bechara EJ, Vercesi AE (1996) Effect of inorganic phosphate concentration on the nature of inner mitochondrial membrane alterations mediated by Ca^{2+} ions. A proposed model for phosphate-stimulated lipid peroxidation. J Biol Chem 271: 2929–2934

Kumar V, Dooley DM, Freeman HC, Guss JM, Harvey I, McGuirl MA, Wilce MCJ, Zubak V (1996) Crystal structure of a eukaryotic (pea seedling) copper-containing amine oxidase at 2.2 A resolution. Structure 4: 943–955

Kusche J, Menningen R, Leisten L, Krakamp B (1988) Large bowel tumor promotion by diamine oxidase inhibition: animal model and clinical aspects. In: Zappia V, Pegg E (eds) Advances in experimental medicine and biology. Progress in polyamine research. Novel biochemical, pharmacological and clinical aspects. Plenum Press, New York, pp 745–752

Kwak MK, Kensler TW, Casero JR (2003) Induction of phase 2 enzymes by serum oxidized polyamines through activation of Nrf2: effect of the polyamine metabolite acrolein. Biochem Biophys Res Commun 305: 662–670

Li R, Klinman JP, Mathews FS (1998) Copper amine oxidase from Hansenula polymorpha: the crystal structure determined at 2.4 A resolution reveals the active conformation. Structure 6: 293–307

Lindsay GS, Wallace HM (1999) Changes in polyamine catabolism in HL-60 human promyelogenous leukaemic cells in response to etoposide-induced apoptosis. Biochem J 337: 83–87

Lord-Fontaine S, Agostinelli E, Przybytkowski E, Averill-Bates D (2001) Amine oxidase, spermine and hyperthermia induce cytotoxicity in P-glycoprotein overexpressing multidrug resistant Chinese hamster ovary cells. Biochem Cell Biol 79: 1–12

Maeda H, Seymour LW, Miyamoto Y (1992) Conjugates of anticancer agents and polymers: advantages of macromolecular therapeutics *in vivo*. Bioconjugate Chem 3(5): 351–362

Maslinski C, Bieganski T, Fogel WA, Kitler MA (1985) Diamine oxidase in developing tissue. In: Mondovì B (ed) Structure and functions of amine oxidases. CRC Press, Boca Raton, pp 153–166

Mathai JC, Sitaramam V (1994) Stretch sensitivity of transmembrana mobility of hydrogen peroxide through voids in the bilayer. Role of cardiolipin. J Biol Chem 269: 17784–17793

Matsuzaki R, Suzuki S, Yamaguki K, Fukui T, Tanizawa K (1995) Spectroscopic studies on the mechanism of the topa quinone generation in bacterial monoamine oxidase. Biochemistry 34: 4524–4530

Medda R, Padiglia A, Floris G (1995) Plant copper-amine oxidases. Phytochemistry 39: 1–9

Mondovì B, Riccio P, Agostinelli E, Marcozzi G (1989) Oxidation of diamines and polyamines. In: Bachrach U, Heimer YM (eds) The physiology of polyamines (1). CRC Press, Boca Raton, pp 177–201

Mondovì B, Pietrangeli P, Morpurgo L, Masini E, Federico R, Mateescu MA, Befani O, Agostinelli E (2003) Some new functions of amine oxidases. Inflammopharmacology 11: 155–163

Morgan ML (1987) Oxidized polyamines and the growth of human vascular endothelial cells. Biochem J 242: 347–352

Morgan DML (1988) Polyamine oxidases and oxidized polyamines. In: Bachrach U, Heimer YM (eds) The physiology of polyamines (I). CRC Press, Boca Raton, pp 203–229

Morpurgo L, Agostinelli E, Mondovì B, Avigliano L, Silvestri S, Stefancich G, Artico M (1992) Bovine serum amine oxidase: half-site reactivity with phenylhydrazine, semicarbazide, and aromatic hydrazides. Biochemistry 31: 2615–2621

Nantes IL, Cilento G, Bechara EJH, Vercesi AE (1995) Chemiluminescent diphenylacetaldehyde oxidation by mitochondria is promoted by cytochromes and leads to oxidative injury of the organelle. Photochem Photobiol 62: 522–527

Obata T (2002) Semicarbazide-sensitive amine oxidase (SSAO) in the brain. Neurochem Res 27: 263–268

Padiglia A, Medda R, Lorrai A, Murgia B, Pedersen ZJ, Finazzi-Agrò A, Floris G (1998) Characterization of Euphorbia characias latex amine oxidase. Plant Physiol 117: 5193–5198

Parchment RE, Lewellyn A, Swartzendruber D, Pierce GB (1990) Serum amine oxidase activity contributes to crisis in mouse embryo cell lines. Proc Natl Acad Sci 87: 4340–4344

Perin A, Sessa A, Desiderio MA (1983) Polyamine levels and diamine oxidase activity in hypertrophic heart of spontaneously hypertensive rats and of rats treated with isoproterenol. Biochem Biophys Acta 755: 344–351

Perin A, Sessa A, Desiderio MA (1985) Diamine oxidase in regenerating and hypertrophic tissues. In: Mondovì B (ed) Structure and functions of amine oxidases. CRC Press, Boca Raton, pp 179–186

Picklo MJ, Montine TJ (2001) Acrolein inhibits respiration in isolated brain mitochondria. Biochem Biophys Acta 1535: 145–152

Pocernik CB, Butterfield DA (2003) Acrolein inhibits NADH-linked mitochondrial enzyme activity: implications for Alzheimer's disease. Neurotox Res 5: 515–520

Radi R, Turrens JF, Chang LY, Bush KM, Crapo JD, Freeman BA (1991) Detection of catalase in rat heart mitochondria. J Biol Chem 266: 22028–22034

Schmutzler W, Goldschmidt O, Bethge KP, Knop J (1969) The release of guinea pig liver histaminase and some of its properties. Int Arch Allergy 36: 45–55

Seiler N (1985) Acetylpolyamines as a substrates of amine oxidases. In: Mondovì B (ed) Structure and functions of amine oxidases. CRC Press, Boca Raton, pp 21–35

Seiler N, Al-Therib MJ (1974) Putrescine catabolism in mammalian brain. Biochem J 144: 29–35

Seiler N, Duranton B, Raul F (2002) The polyamine oxidase inactivator MDL 72527. Prog Drug Res 59: 1–40

Sessa A, Desiderio MA, Perin A (1982) Diamine oxidase activity induction in regenerating rat liver. Biochem Biophys Acta 698: 11–14

Sharmin S, Sakata K, Kashiwagi K, Ueda S, Iwasaki S, Shirahata A, Igarashi K (2001) Polyamine cytotoxicity in the presence of bovine serum amine oxidase. Biochem Biophys Res Comm 282: 228–235

Smith D et al (2002) Inhibitors of copper containing amine oxidases. PCT 02/02090

Sohal RS (1997) Mitochondria generate superoxide anion radicals and hydrogen peroxide. FASEB J 11: 1269–1270

Steinebach V, Groen BW, Wijmenga SS, Niessen WMA, Jongejan JA, Duine JA (1995) Identification of topaquinone, as illustrated for pig kidney diamine oxidase and Escherichia coli amine oxidase. Anal Biochem 230: 159–166

Tipton KF (1994) Monoamine oxidase inhibition. Biochem Soc Trans 22: 764–768

Walkinshaw G, Waters CM (1995) Induction of apoptosis in catecholaminergic PC12 cells by *L*-DOPA. Implications for the treatment of Parkinson's disease. J Clin Invest 95: 2458–2464

Yadav KDS, Knowles PF (1981) A catalytic mechanism for benzylamine oxidase from pig plasma. Stopped-flow kinetic studies. Eur J Biochem 114: 139–144

Yu P, Wang M, Deng YL, Fan H, Shira-Bock L (2002) Involvement of semicarbazide-sensitive amine oxidase-mediated deamination in atherogenesis in KKAy diabetic mice fed with high cholesterol diet. Diabetologica 45: 1255–1262

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