Mini-review

Alternative electron acceptors: Proposed mechanism of paraquat mitochondrial toxicity

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A B S T R A C T

Paraquat (PQ) is a relatively safe and effective herbicide used all over the world. PQ is very toxic to all living organisms; and many cases of acute poisoning and death have been reported over the past decade. The main suggested potential mechanism for PQ toxicity is the production of superoxide radicals from the metabolism of the PQ by microsomal enzyme systems, and by inducing mitochondrial toxicity.

Mitochondria are considered to be a major source of reactive oxygen species in cells and according to this hypothesis, PQ, through suitable oxidation and reduction processes, is able to participate in the redox system in mitochondria. The potential ability of PQ to accept electrons from complex (I, II, III, IV) leads to rapid reaction with molecular oxygen to yield superoxide anion which can lead to the formation of more toxic reactive oxygen species, e.g., hydroxyl radical, often taken as the main toxicant.

Lipid peroxidation due to PQ has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased mitochondrial components, reduced mitochondrial survival and lipid fluidity.

The biological effect of reactive oxygen species (ROS) is controlled by a wide spectrum of enzymatic and non-enzymatic defense mechanisms such as superoxide dismutas (SOD), catalase (CAT) and glutathione. According to this hypothesis, the chemical cascades lead to the reduction of PQ, which reacts quite rapidly with molecular oxygen to yield superoxide anion. The generation of free radicals and lipid peroxidation are the main factors that lead to mitochondrial damage.

1. Introduction

Paraquat (PQ) is one of the most widely used nonselective dipyridyl herbicides. PQ (1,1'-4,4'-bipyridium dichloride; methyl viologen) is a broad spectrum contact herbicide which is toxic to man and animals (Copland et al., 1974; Murray and Gibson, 1972), and many cases of acute poisoning and death have been reported over the past decade (Bruyndonckx et al., 2002). PQ ingestion is...
one of the leading methods of suicide in countries such as Taiwan, Japan, Malaysia, the West Indies, Sri Lanka and Samoa. In Japan, 1200–1500 deaths per year from PQ ingestions were reported in the 1980s (Onyon and Volans, 1987). PQ poisonings are still common in the UK, where a study of pesticide poisoning between 1990 and 1991 in UK indicates 44 deaths with PQ responsible for 75% (Pesticide Monitoring Unit, Surveillance of Human Acute Poisoning from Pesticides, 1 October 1990–30 September 1993, National Poisons Information Service (Birmingham Centre). A recent annual report of the American Association of Poison Control Centers indicated 61 cases of PQ poisoning with one reported death (Bronstein et al., 2007). The lung is the primary target organ of PQ, and pulmonary effects represent the most lethal toxicity that occurs several days to three weeks after exposure to PQ. Lung injury characterized by edema, hemorrhage, interstitial inflammation and proliferation of bronchial epithelium (Copland et al., 1974; Murray and Gibson, 1972; Bus and Gibson, 1984). The primary mechanism is through the generation of free radicals with oxidative damage to lung tissue (Smith, 1988; Aldrich et al., 1983). According to this hypothesis, PQ selective accumulation in the lung leading to lipid peroxidation or NADPH depletion is thought to be the primary cause of cell death and lung tissue damage. Moreover, the cells associated with alveolitis (macrophage, lymphocytes and neutrophils) may produce other factors that regulate the proliferation, chemotaxis and secretory activity of fibroblasts (Carre and Leoponde, 1993). Gastrointestinal decontamination of PQ poisoning in man usually consists of the administration of highly effective oral adsorbants, namely Fuller’s earth (15% suspension), bentonite (7.5% suspension) and activated charcoal (Suntres et al., 1992). Various other pharmacological approaches, including vitamin E (α-tocopherol), vitamin C (ascorbic acid) desferoxamine (desferrioxamine), clofibrate, low-molecular-weight thiol-containing antioxidants, xanthine oxidase inhibitors, superoxide dismutase and N-acetyl styrene, riboflavin and niacin, selenium, metallothionen, methylene blue, L-cysteine, cyclophosphamide and dexamethasone, monounsaturated fatty acids, lymphokines or cytokines and lung surfactant-stimulating drug, ambroxol have been studied in animals or humans in regard to alleviating PQ toxicity, but so far none of these treatments have been clearly shown to be effective (Dinis-Oliveira et al., 2008; Ghazi-Khansari et al., 2005). Therefore, there is no useful antidote for PQ toxicity in man. Recently, inhibitors of the angiotensin-converting enzyme (ACE), which catalyzes the conversion of angiotensin (I) to angiotensin (II) was reported to decreased pulmonary fibrosis in animal models (Canadan and Alagozu, 2001; Ghazi-Khansari et al., 2007; Mohammadi-Karakani et al., 2006).

The molecular mechanism of PQ toxicity is not well understood. There are two main points of view that have emerged: (1) PQ-related cell toxicity could be mediated by oxygen free radicals coming from the metabolism of the PQ by microsomal enzyme systems, and/or (2) PQ, by inducing mitochondrial toxicity.

Mitochondria are key regulators of cell life and death and play an important role in a wide range of diseases, including cancer, diabetes, cardiovascular disease, and the age-related neurodegenerative diseases including Alzheimer’s disease (Armstrong, 2008; Mancuso et al., 2007).

Mitochondria are candidate targets of PQ toxicity in animal tissues and plants (Taylor et al., 2002). The mechanisms of PQ toxicity are frequently related to the generation of the superoxide anion, which can lead to the formation of more toxic reactive oxygen species (Suntres, 2002).

2. Mechanism of mitochondrial toxicity

Mitochondria are considered to be a major source of reactive oxygen species (ROS) in cells (Cadenas and Davies, 2000) and inhibition of the electron transport chain can increase the steady-state levels of these autoxidizable components and consequently increase reactive oxygen species generation by mitochondria (Han et al., 2001).

2.1. Alternative electron acceptor

The potential ability of PQ to accept electrons from complex (I) has produced conflicting results. Some studies reported that PQ does not inhibit mitochondrial complex (I) in order to mediate its toxic action (Richardson et al., 2005) and some studies reported that PQ induced the production of superoxide with NADH-dependent respiration in bovine heart submitochondrial particles (SMP) (Hasegawa et al., 1997) and these results suggested that NADH-ubiquinone oxidoreductase (complex I) might be related to superoxide production by PQ (Fukushima et al., 2002).

PQ is able to accept electrons from complex (I), and PQ radical formation has been observed in vitro (Fukushima et al., 1993). The site around the 30 kD sub-unit of complex (I) was expected to be the radical formation site (Fukushima et al., 1995) as PQ is highly water soluble and has difficulty entering the mitochondrial inner membrane (Hirai et al., 1992; Shimada et al., 1998). However, this 30 kD sub-unit is transmembrane protein, therefore, there is a possibility that complex (I) can catalyze the NADH-PQ reaction using NADH in the matrix and PQ in the cavity between the outer and inner membranes. Indeed, the effect of acute PQ exposure on complex (I) has already been demonstrated experimentally (Fukushima et al., 1994; Tawara et al., 1996).

On the other hand, PQ enhanced NADH-dependent lipid peroxidation in bovine heart SMP in the presence of ADP-Fe3+ chelate (Sata et al., 1983) and PQ induced the production of superoxide with NADH-dependent respiration in bovine heart SMP (Hasegawa et al., 1997). These results recommended that NADH-ubiquinone oxidoreductase complex (I) (Tomita et al., 2001; Junod et al., 1989; Shimizu et al., 1996; Dawson and Dawson, 2003) might be connected to superoxide production by PQ. Antimycin A and nitrofurantoin, inhibitors of ubiquinol-cytochrome c reductase (complex III) in the electron transfer chain of mitochondria, caused a resistance to PQ. Some studies have reported that PQ at doses of 1 mM causes respiratory system depression through inhibition of mitochondrial complexes (III) and (IV) (Fukushima et al., 1994).

According to this hypothesis, PQ, through suitable oxidation and reduction processes, is able to participate in the redox system in mitochondria. PQ exist in three main oxidation states, namely, PQ2+ ↔ PQ3+ ↔ PQ4+ (Dinis-Oliveira et al., 2008). The PQ divalent cation is colorless, whereas the partially reduced PQ• contains an odd electron and this odd electron is shared by all the nuclear carbon positions in the rings (Calderbank, 1968). This step is completely reversible, such that one equivalent of a reducing agent will reduce more than 50% of PQ2+ to PQ•+ only if its reduction potential is more negative than that of PQ. According to the Ito and Kuwana (1971) reported the potential of the first reduction for PQ2+ as −0.446 V and the second is given as −0.88 V.

In aqueous solution, the PQ divalent cation (PQ2+) accepts electrons, forming the blue PQ radical (PQ•+), which reacts very rapidly with molecular oxygen to yield superoxide anion, O2•− (Fig. 1). Under aerobic conditions, PQ•+ promotes continuous formation of O2•−, undergoing repeated redox cycles (Joaquim et al., 2001). These are substances capable of extracting electrons from intermediates in the respiratory chain, competing with the natural substrates. These substances may also affect the redox cycle, passing electrons back to the respiratory chain at a later point, bypassing sites in the chain essential for energy generation. The second requirement of the proposed mechanism of PQ mitochondrial tox-
Fig. 1. Structural PQ radical undergo redox-cycling. PQ or PQ$^{2+}$: paraquat; PQ$^{+}$: paraquat monocation free radical; O$_2$: oxygen; O$_2$$^•$−: superoxide radical.

Fig. 2. Mechanism of PQ mitochondrial toxicity. The potential ability of PQ to accept electrons from complex (I, II, III, and IV) which reacts rapidly with molecular oxygen and formation of superoxide anion are shown.

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Paraoxon (PQ$^{2+}$) Paraquat Cation radical (PQ$^+$) O$_2$

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icity was that the cyclic reduction–oxidation of PQ initiates lipid peroxidation through subsequent superoxide radical and singlet oxygen intermediates. Finally, the chemical cascades leading to the reduction of PQ, and the generation of free radicals and lipid peroxidation are the main factors that lead to mitochondrial damage (Ghazi-Khansari et al., 2006) (Fig. 2).

2.1.1. Inhibition of manganese-dependent SOD and catalase activity

It was reported that PQ inhibited the processing of human manganese-dependent SOD (hMnSOD), and it was concluded that mitochondrial processing and import of the precursor protein hMnSOD were early events susceptible to dysfunction induced by PQ (Wright et al., 1997). On the other hand, CuZnSOD-deficient cells were more sensitive to oxygen toxicity than were MnSOD-deficient cells (Fig. 3) (Huang et al., 1997). Membranes are highly permeable to hydrogen peroxide (H$_2$O$_2$) (Chance et al., 1979) and PQ toxicity has been attributed to H$_2$O$_2$ production (Farrington et al., 1973; Hassan and Fridovich, 1979).

Catalase is known to protect PQ toxicity as well. Previous study indicated that PQ has been assigned to H$_2$O$_2$ production, and therefore decreased catalase activity due to PQ (Fig. 3) (Ghazi-Khansari and Mohammadi-bardbori, 2007).

2.1.2. Lipid peroxidation

Lipid peroxidation has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased cellular and organelle components, reduced mitochondrial survival and lipid fluidity (Fig. 3) (Aydin et al., 2004; Ghazi-Khansari and Mohammadi-bardbori, 2007; Mohammadi-bardbori and Ghazi-Khansari, 2007).

2.1.3. GSH and GSSG cascade

Among the oxidative stress agents PQ is a thiol-oxidizing agent resulting in fast oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG). GSH is considered the most important mitochondrial antioxidant and its depletion markedly enhances the sensitivity of the mitochondrial structure to the ROS-mediated injury (Fig. 3) (Ghazi-Khansari and Mohammadi-bardbori, 2007).

2.1.4. ATP synthesis and mitochondrial swelling

Another study suggested that PQ uncoupled oxidative phosphorylation by inducing lipid peroxidation and had an inhibitory action on the redox chain and adenosine triphosphate (ATP) synthase activity (Palmeira et al., 1995). Mitochondrial swelling can be initiated by the many agents and spontaneous swelling can be reversed by the addition of adenosine triphosphate (ATP; Lehninger, 1959). PQ treatment can induce mitochondrial swelling and disruption in the organelle interferes with the cell energy charge, thus driving the cell to eventual death (Cappelletti et al., 1996). Mitochondrial depolarization and swelling can also be due to non-specific permeabilization of the inner mitochondrial membrane (Fig. 3) (Castilho et al., 1994).

2.1.5. Ca$^{2+}$-dependent inner-membrane permeability transition

In Ca$^{2+}$-dependent inner-membrane permeability transition it was suggested that oxidative damage to mitochondria was mediated by Ca$^{2+}$-dependent inner-membrane permeability transition, and that the toxic effect of ROS on mitochondria was triggered by the cyclosporin A-sensitive and Ca$^{2+}$-dependent membrane transition (Takeyama et al., 1993). Another report also suggested that PQ caused the opening of the cyclosporin A-sensitive, Ca$^{2+}$-dependent permeability transition pore synergistically with nitric oxide (NO) (Fig. 3) (Costantini et al., 1995).

2.2. NADH-quinone oxidoreductase

Oxygen radicals were produced during NADH oxidation by the mitochondrial outer membrane (Hirai et al., 1992), and the existence of an NADH-dependent system in rat liver mitochondria was
demonstrated which was capable of PQ reduction (Shimada et al., 1998).

The outer membrane fractions also catalyzed rotenone-insensitive NADH oxidation by PQ. Another report also suggested that the NADH-PQ reduction activity was not inhibited by anti-NADH-cytochrome b5 reductase antibody, but was inhibited by p-hydroxymercuribenzenzozate, the inhibitor of NADH-cytochrome b5 reductase (Fukushima et al., 2002). PQ stimulated basal oxygen consumption without influencing the oxygen utilization associated with adenosine diphosphate (ADP) phosphorylation, and this result suggested that PQ appeared to uncouple the oxidative phosphorylation process (Thakar and Hassan, 1988). High concentrations of PQ appeared to disrupt the mitochondrial electron chain transfer resulting in a reduction of metabolic functions (Molck and Friis, 1997). The role of the respiratory chain in PQ toxicity was verified with yeast (Blaszczynski et al., 1985).

3. Conclusion

Mitochondria are considered to be the major source of reactive oxygen species in cells and according to this hypothesis, PQ through oxidation and reduction processes is able to participate in the redox system in mitochondria. The potential ability of PQ to accept electrons from complex (I, II, III, and IV) leads to rapid reaction with molecular oxygen to yield superoxide anion, which in turn causes the formation of more toxic reactive oxygen species, e.g., superoxide anion, that is often taken as the main toxicant (Farrington et al., 1973).

Lipid peroxidation due to PQ has been implicated in a number of deleterious effects such as increased membrane rigidity, osmotic fragility, decreased mitochondrial components, reduced mitochondrial survival and lipid fluidity (Aydin et al., 2004).

Therefore, it is necessary to combine various treatments based on more than one hypothesis. We suggest a possible mechanism of mitochondrial PQ toxicity, alternative electron acceptors, according to this hypothesis, PQ based on suitable oxidation and reduction potential is able to participate in the redox system in mitochondria. Knowledge of the mechanism by which a toxin such as PQ acts on some biological process provides the ultimate basis for rational treatment of intoxication.

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