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Review Article

Mechanism of Cytotoxicity of Paraquat

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Abstract

Acute paraquat poisoning seems to be very complex because many possible mechanisms of paraquat cytotoxicity have been reported. Some may not be the cause of paraquat poisoning but the result or an accompanying phenomenon of paraquat action. The mechanism critical for cell damage is still unknown. Paraquat poisoning is probably a combination of several paraquat actions. Arguing which mechanism is more critical may not be important, and these clarified mechanisms should be connected and utilized in the development of treatment for paraquat poisoning. Many people still die of pulmonary fibrosis after paraquat exposure. The next target of study will be to verify the mechanism of pulmonary fibrosis by paraquat on the basis of the outcome of studies such as this review.

Key words: paraquat, cytotoxicity, free radicals, oxidative stress, lipid peroxidation

Introduction

1,1'-dimethyl-4,4'-bipyridium dichloride (paraquat) is a herbicide used all over the world, and it causes fatal damage to multiple organs, especially pulmonary fibrosis. In Japan, the number of deaths by paraquat has decreased since 1986, and the mixture of paraquat and diquat is becoming the main cause of accidental death instead of paraquat alone. However, the mechanism of paraquat poisoning, especially pulmonary fibrosis, is still unknown, and there is no effective treatment. Therefore many people still suffer paraquat poisoning from accidental and suicidal ingestion. Curing acute poisoning and the prevention of chronic poisoning can be achieved by clarifying the mechanism of paraquat poisoning. It is currently controversial whether the site of paraquat radical formation is in the microsome fraction or mitochondria, and whether the formed free radicals are the direct cause of citotoxicity or trigger the following damaging process. In this review, we focus on these main arguments and discuss the treatment for paraquat poisoning.

1. Paraquat radical formation site

1.1. Microsome fraction origin theory

The mechanism of paraquat cytotoxicity was first explained from the viewpoint of paraquat radical formation and lipid peroxidation via microsomal NADPH-cytochrome *c* reductase (1). Since then, many researchers have supported this microsome origin theory. Paraquat is reduced by NADPH-cytochrome *c* reductase, and the reduced paraquat is reoxidized by cytochrome P-450 in the presence of tertiary amine N-oxides (2). The interaction of paraquat with NADPH-cytochrome P-450 reductase and ferric complexes resulted in an increase in radical oxygen generation (3). Paraquat stimulated hydrogen peroxide production and the rate of superoxide production in mouse liver microsomes (4). Hydroxyl free radicals were also formed during paraquat biotransformation in incubation mixtures containing liver microsomes and NADPH generating systems (5).

1.2. Mitochondria origin theory

Ultrastructural changes in pulmonary alveolar epithelial cells were described in paraquat-treated rats, and it was proposed that paraquat primary affected type II cells, with lesions first occurring in mitochondria (6). Since this discovery, mitochondria have been focused on as the site of paraquat radical formation and its target. Now there are several hypotheses in the mitochondria origin theory.

1.2.1. NADH-quinone oxidoreductase of the outer membrane

Paraquat was reduced anaerobically by intact mitochondria in the presence of NADH, but not NADPH. Oxygen radicals were produced during NADH oxidation by the mitochondrial outer membrane (7), and the existence of an NADH-dependent paraquat reduction system in rat liver mitochondria was demonstrated (8). The outer membrane fractions catalyzed rotenone-insensitive NADH oxidation by paraquat. It was concluded that mitochondrial NADH-quinone oxidoreductase of the outer membrane was responsible for paraquat cytotoxicity. However, in this report, the NADH-paraquat reduction activity was not inhibited by anti-NADH-cytochrome b_5 reductase antibody but was inhibited by

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p-hydroxymercuribenzonate, the inhibitor of NADH-cytochrome b_5 reductase. Moreover the effect of the other NADH dependent paraquat reduction enzymes in microsomes could not be ignored in this experiment because the outer membrane fractions were contaminated with a few microsomes. Therefore this experimental design should be reconsidered to reach a conclusion.

1.2.2. NADH-ubiquinone oxidoreductase of the inner membrane

The effect of paraquat on mitochondrial oxidative phosphorylation was demonstrated. Paraquat stimulated basal oxygen consumption without influencing the oxygen utilization associated with ADP phosphorylation, and this result suggested that paraquat appeared to uncouple the oxidative phosphorylation process(9). The toxic mechanism and metabolic effects of paraquat were observed using isolated renal proximal tubules from rabbits. High concentrations of paraquat appeared to disrupt the mitochondrial electron chain transfer resulting in a reduction of metabolic functions (10). The role of the respiratory chain in paraquat toxicity was verified with yeast (11). In this report, antimycin A and nitrofluorfen, inhibitors of ubiquinol-cytochrome *c* reductase (complex III) in the electron transfer chain of mitochondria caused a resistance to paraquat. On the other hand, paraquat enhanced NADH-dependent lipid peroxidation in bovine heart submitochondrial particles (SMP) in the presence of ADP-Fe^{3+} chelate (12), and paraquat induced the production of superoxide with NADH-dependent respiration in bovine heart SMP (13). These results suggested that NADH-ubiquinone oxidoreductase (complex I) might be related to superoxide production by paraquat.

We first verified NADH oxidation in a reaction assay of complex I in bovine liver mitochondria, in which paraquat was an electron acceptor, and observed paraquat radical formation as the turning of the reaction solution to blue (14). The site around the 30 kD subunit of complex I was expected to be the radical formation site (15). From the fact that paraquat is highly water soluble and has difficulty entering the mitochondrial inner membrane, some researchers doubt whether complex I catalyzes the NADH-paraquat reaction in vivo (7, 8). This 30 kD subunit is, however, transmembranous protein, therefore there is a possibility that complex I can catalyze the NADH-paraquat reaction using NADH in matrix and paraquat in the cavity between the outer and inner membranes. Indeed, the effect of acute paraquat exposure on complex I was demonstrated in vivo using rats (16, 17).

The first one-electron reduction steps of paraquat and diquat were compared using microsomal and mitochondrial fractions of rat liver, lung and kidney, and both fractions reduced each herbicide effectively (18). Which fraction is the first lesion to produce the paraquat radical is unknown, but it seems to be clear that free radicals are formed in both mitochondria and microsome fractions.

2. Role of free radicals formed by paraquat on cell injury

2.1. Damage to membrane lipids or proteins

Lipid peroxidation was increased by paraquat in rat lung SMP, and suppressed by superoxide dismutase (SOD) (19). Linoleic acid hydroperoxide formed during a free radical attack was an important source of biomembrane damage (20). Paraquat produced selective peroxidation of membrane phosphatidylserine that preceded apoptosis in myeloid cells (21). On the other hand, oxygen radicals are known to form protein aggregation and fragmentation (22, 23), and peroxidized lipids are also thought to cause protein damage (24). Among complex I subunits, 30, 42 and 75 kD proteins, which are transmembranous and iron-sulfur proteins, are decreased by the paraquat radical (15). Paraquat produced oxygen radicals which are thought to damage proteins by two mechanisms, direct damage and indirect damage via lipid peroxide.

2.2. Excitotoxicity

Excitotoxicity may occur in peripheral organs, such as the lungs. It was suggested that N-methyl-D-aspartate (NMDA) receptors exist in the lungs, and excessive activation of these receptors might provoke acute edematous lung injury, and this injury could be modulated by blocking one of three critical steps: NMDA receptor binding, inhibition of nitric oxide (NO) synthesis, or activation of poly(ADP-ribose) polymerase (25). This study group continuously reported that NMDA receptor blocker dizocilpine maleate attenuated oxidant injury induced by paraquat, and they concluded that excitotoxicity might be a key factor in oxidant tissue injury (26). This hypothesis is very unique, but quite few reports have studied it.

2.3. NO-related activity

In acute oxidant injury induced by paraquat in isolated guinea pig lungs, NO synthesis was markedly stimulated (27). This report concluded that NO might play a critical role in the production of lung tissue injury due to paraquat, and it might be a pathogenetic factor in other forms of oxidant tissue injury. On the other hand, another report asked whether NO reacts with superoxide generated by paraquat to produce the toxin peroxynitrite (28). In this study, paraquat-induced endothelial cell toxicity was attenuated by inhibitors of NO synthase that prevent NADPH oxidation, and it was concluded that paraquat used NO synthase as an electron source to generate superoxide and, in the process, decreased the generation of NO. The role of NO in paraquat-induced cell injury has been controversial. NO may be both beneficial and deleterious, depending on the concentrations produced (29).

2.4. ADP-ribosylation

There are several reports about the participation of paraquat in protein modification, in which its physiological role is still unclear. The effects of oxidative stress by paraquat on DNA damage and associated reactions, increased poly-(ADP)-ribose polymerase activity and decreased NAD and ATP contents, were demonstrated in the primary cultures of porcine aortic endothelial cells (30). The effect of free radical generating agents on the mono-(ADP) ribosylation of rat liver cytosolic proteins was also studied (31). This post-translational modification was activated by lipid peroxidant agents via the activation of cytoplasmatic mono-(ADP)-ribosyltransferases. The modification of proteins caused by free radicals may result in cell damage.

2.5. Apoptosis

It was reported that paraquat caused S-phase arrest of rat liver and lung cells in vivo (32), and the effect of paraquat on DNA was noted. Many researchers have reported apoptosis by paraquat in relation to neuronal cell death, especially the etiology of Parkinson's disease (33, 34, 35). It was documented that selective phospholipid peroxidation occurred after paraquat exposure, and was specifically associated with apoptosis in mouse myeloid cells (36).

A549 cells, a human lung epithelial cell line, incubated with sublethal doses of paraquat for up to 24 h showed no apoptotic features but, their following incubation in paraquat-free medium resulted in the time-dependent appearance of apoptosis (37). In this experiment, the antioxidants, ascorbic acid and N-acetyl-cysteine, proved effective in reducing paraquat-induced apoptosis. Oxidative stress by paraquat may contribute to apoptotic cell death (38). Hyperoxia, however, inhibited oxidant-induced apoptosis in lung epithelial cells (39). Although the effect of oxygen concentration on apoptosis is unclear, the apoptosis of alveolar epithelial cells by paraquat may lead to pulmonary fibrosis (40).

2.6. Injury to mitochondrial DNA

Several respiratory chain polypeptides are encoded by the mitochondrial genome which is sensitive to reactive oxygen species because mitochondria has only a limited arsenal of DNA repair processes. Mitochondrial DNA mutations cause defects in the respiratory chain function. Many researchers are interested in the effect of free radicals on mitochondrial DNA relating to aging and mitochondrial disease (41, 42). However, there have been no reports on mitochondrial DNA damage by paraquat. The effect of the paraquat radical on mitochondrial DNA in the lungs should be demonstrated to study the mechanism of pulmonary fibrosis by paraquat or possible chronic paraquat poisoning.

3. Other actions of paraquat leading to cell injury

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

It was reported that oxidative damage to mitochondria was mediated by Ca^{2+}-dependent inner-membrane permeability transition and that the deleterious effect of free radicals on mitochondria was triggered by the cyclosporin A-sensitive and Ca^{2+}-dependent membrane transition (43). Another report also suggested that paraquat caused opening of the cyclosporin A-sensitive, Ca^{2+}-dependent permeability transition pore synergistically with NO (44). In this study, paraquat was thought to induce an increase in the permeability of the inner mitochondrial membrane leading to membrane depolarization, uncoupling and swelling.

3.2. NADPH exhaustion

Paraquat enhanced the oxidation of NADPH, but not NADH by the cell supernatant of alveolar macrophages (45). NADPH depletion occurred after dosing with paraquat, but not diquat, coinciding with the development of lung damage, and it was concluded that an abrupt decrease of the NADPH level was a critical biochemical event in the development of alveolar epithelial cell damage following paraquat administration (46). Mitochondria may form NADH from NAD at the expense of reduced paraquat if the reduced paraquat is electrochemically regenerated (47). These results suggest that the depletion of NADPH, but not NADH, by paraquat may lead to alveolar epithelial cell damage.

3.3. Cytoskeletal damage

The direct effect of paraquat on actin dynamics in solution was as follows. Actin selectively bound paraquat, and paraquat induced the formation of actin supramolecular structures in depolymerizing medium (48). However, paraquat selectively disrupted the balance between energy supply and demand of the neurons either by interacting with mitochondrial respiration or glycolysis in contrast with compounds like delayed neurotoxic organophosphates which exert a selective direct effect on cytoskeleton elements (49). Therefore, whether paraquat damages the cytoskeleton directly or secondarily is now controversial.

3.4. Inhibition of manganese-dependent SOD

It was reported that paraquat 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 paraquat (50). On the other hand, CuZnSOD-deficient cells were more sensitive to oxygen toxicity than were MnSOD-deficient cells (51). CuZnSOD is located in the cytosol and MnSOD in the mitochondria. In the same study, paraquat caused free radical-induced damage in both the mitochondria and cytoplasm, however, SOD compartmentalized in the cytosol could not compensate for the loss of SOD in the mitochondria. Further study is needed to solve this puzzle.

4. The significance of lipid peroxidation in the cytotoxicity of paraquat

4.1. Lipid peroxides as mediators of paraquat cytotoxicity

The significance of lipid peroxidation in the cytotoxicity of paraquat is now controversial. Many researchers have studied it from the viewpoint of oxidative stress theory concerning lipid peroxidation, and have evaluated the severity of paraquat poisoning by monitoring lipid peroxide. The peroxidation of lipids in biological membranes is thought to be a destructive phenomenon that can be elicited in various ways (52), and patients have been investigated for evidence of lipid peroxidation after ingesting paraquat (53). Paraquat induced time- and dose-dependent lactate dehydrogenase release, lipid peroxidation, and cell death, and lipid peroxidation was a good indicator of cell death (54). Based on this theory, the establishment of effective treatment has been attempted. SOD administration ameliorated the 50% mortality of rats (55), and hMnSOD modulated paraquat-mediated toxicity in mammalian cells (56). Several inhibitors have been reported to effectively inhibit in vivo lipid peroxidation in rat tissues induced by paraquat (57). Another report suggested that paraquat uncoupled oxidative phosphorylation by inducing lipid peroxidation and had an inhibitory action on the redox chain and ATP synthase activity (58).

4.2. Lipid peroxides as a result of paraquat action

Several researchers doubt that lipid peroxidation is a process of paraquat toxicity. The effects of oxidative stress on adult male houseflies were examined by paraquat administration, which stimulated the activity of catalase but did not affect the activities of SOD or glutathione reductase (59). The mortality rate of paraquat poisoned mice treated with SOD was higher than that of SOD untreated (60). The pulmonary macrophage was activated by paraquat (61), and lipid peroxidation occurred following phagocytic activities of the macrophage rather than through toxic pulmonary cell injury (62). These results suggest that paraquat toxicity does not result from lipid peroxidation.

The excretion of free malondialdehyde (MDA), an index of lipid peroxidation, in the urine of rats was examined after oral paraquat administration. The concentration of free MDA decreased following the intake of paraquat. The total amount of free MDA increased temporarily, but then decreased significantly

to below normal values (63). We observed that the changes of lipid peroxides in the lungs and blood of rats had two phases, and that after the first peak of lipid peroxidation, complex I activities decreased over time (16). Peroxidized lipids are thought to cause protein damage and cytotoxicity (24). Lipid peroxides are, however, unstable in a living body, therefore the severity of cell damage may not parallel the amount of lipid peroxides.

Conclusion

It is still under discussion where the paraquat radical is formed first, but in any case, it is clear that free radicals are formed in both the mitochondria and microsome fractions. Based on the cell damage mechanisms by paraquat which have been clarified so far, the principal objective is to develop treatment for paraquat poisoning. At present, various treatment methods for acute paraquat poisoning are being considered (64–67). As this review shows, the action of paraquat on the living body is not straightforward. Therefore, it is necessary to combine various treatments based on more than one hypothesis. In particular, a cure for pulmonary fibrosis will become an important problem in the future (68–71). Whether pulmonary fibrosis can be prevented by reducing paraquat toxicity in the acute phase or another switching mechanism must be studied - studying the mechanism of paraquat cytotoxicity still has many problems.

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