

Stress Signaling in Paraquat-Induced Target Organ Toxicity

Shuyi Wang¹, Wei Guo^{1,2}, and Jun Ren¹

¹Center for Cardiovascular Research and Alternative Medicine, and ²Department of Animal Sciences, University of Wyoming, Laramie, WY 82071, USA

Correspondence: wguo3@uwyo.edu (W.G.)

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ABSTRACT | Paraquat is a rather toxic pro-oxidant herbicide, prompting multi-organ failure, including the heart, brain, and lung injuries, although the precise underlying mechanism(s) remains poorly understood. Up-to-date, a number of signaling machineries have been postulated for paraquat toxicity, such as accumulation of free radical species and development of oxidative stress. Paraquat is believed to serve as a potent ROS generator, resulting in detrimental biological effects through oxidative stress injury and mitochondrial dysfunction. In this mini-review we will recapitulate some aspects of paraquat toxicity in main body organs, including the lungs, brain, and heart. Cellular mechanisms behind paraquat toxicity will be discussed with a focus on oxidative stress, mitochondrial injury, and autophagy. Special attention will be given to the direct stress signaling and pro-inflammatory signaling cascades triggered by paraquat exposure in the herbicide-induced organ damage.

KEYWORDS | Antioxidants; Cardiac function; Inflammation; Mitochondrial injury; Oxidative stress; Paraquat; Reactive oxygen species; Stress signaling; Target organ toxicity

ABBREVIATIONS | CVD, Cardiovascular disease; ERK, extracellular signal-regulated kinase; JNK, c-Jun-N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; NQO2, dihydronicotinamide riboside (NRH):quinone oxidoreductase 2; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha; XO, xanthine oxidase

CONTENTS

1. Introduction
2. Oxidative Stress Mechanism in Paraquat-Induced Toxicity
 - 2.1. Paraquat and ROS Generation
 - 2.2. Paraquat and Oxidative Imbalance
 - 2.3. Paraquat and Mitochondrial Injury and Stress Signaling
3. Paraquat and Target Organ Injury
 - 3.1. Paraquat and Brain Injury: ROS and Autophagy
 - 3.2. Paraquat and Lung Injury: ROS and Mitochondrial Injury
 - 3.3. Paraquat and Liver Injury

3.4. Paraquat and Cardiovascular Injury

4. Perspectives

1. INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride) is perhaps one of the most widely employed herbicides for weed control in agriculture [1, 2]. Intentional or accidental ingestion of paraquat has been reported to be a rather serious public health threat, with an estimated annual incidence of over 2000 toxic ingestions and a high mortality rate of 60%–70% due to multiple organ injuries in the lungs, kidneys, liver, and heart [3, 4]. On the other hand, chronic paraquat exposure is deemed a major risk factor for the onset and development of a number of neurodegenerative diseases in humans, such as Parkinson's disease [5]. Inhalation of paraquat, especially in confined spaces like a greenhouse, can prompt fatal pulmonary disease which usually leads to respiratory insufficiency, *en route* to respiration failure and death [6]. Furthermore, high dose of paraquat administration has been associated with devastating organ failure in many other organ systems beyond the major paraquat target organs, namely, the heart, lungs, liver, and kidneys [7]. A plethora of cardiogenic events, such as arrhythmias, heart failure, and cardiac arrest, develop, occasionally leading to sudden death in individuals with severe paraquat intoxication [8]. Not surprisingly, myocardial injury constitutes an important secondary response to severe paraquat poisoning [9].

2. OXIDATIVE STRESS MECHANISM IN PARAQUAT-INDUCED TOXICITY

Paraquat is a non-selective, albeit highly effective, contact herbicide [2]. The herbicide is widely known as a redox cycling agent, belonging to the chemical class of bipyridyl (also known as bipyridylum) quaternary ammonium herbicide, featured by two covalently linked methylpyridine rings [1, 5]. The commonly accepted herbicidal mechanism of paraquat is related to its interference with the intracellular electron transfer systems in chloroplasts during photosynthesis. As a result, paraquat is capable of inhibiting the process of NADP⁺ reduction while accepting one electron from NADPH-cytochrome P450

reductase. This disruption process is mainly responsible for the production of reactive oxygen species (ROS) upon paraquat exposure, including superoxide anion (O₂^{•-}), singlet oxygen, as well as peroxy and hydroxyl radicals [4]. These ROS interact with the unsaturated lipids of biological membranes thus resulting in the destruction of plant organelles, inevitably leading to cell death [5, 6].

2.1. Paraquat and ROS Generation

Paraquat has been widely employed to trigger oxidative stress given its unique potency to generate O₂^{•-} in nearly all experimental systems ranging from isolated mitochondria and mammalian cells to whole organisms, including *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and rodents [4, 10]. Through a carrier-mediated and membrane potential-dependent process, paraquat is taken up across the mitochondrial inner membrane to mitochondrial matrix where the herbicide acts as a redox cycling compound to interact with oxygen molecule to form O₂^{•-}. Besides exerting direct toxic effects, O₂^{•-} can also serve as a source for the production of secondary pro-oxidants, such as hydrogen peroxide (H₂O₂) through dismutation reaction, and hydroxyl radical (OH[•]) by interacting with reduced transition metals like Fe²⁺. Hydroxyl radical is one of the most potent oxidants ranking only second to fluorine in nature. Several lines of evidence have favored the involvement of ROS in the direct cytotoxicity of paraquat exposure [4].

2.2. Paraquat and Oxidative Imbalance

In the aerobically living cells, ROS are incessantly produced to participate in a number of biological reactions. Typically, various endogenous antioxidant defense systems are present to be responsible for scavenging intracellular ROS produced by pathological stimuli. Among various antioxidant enzymes known to date, superoxide dismutase (SOD) and catalase may constitute the primary enzymatic defense system to neutralize or interact with excessive ROS and transform them into less harmful products [11, 12]. Recent advance in redox signaling biology has

revealed the importance of pro- and anti-oxidant fine balance in the homeostasis of biological systems and bodily function [12]. Production of ROS may be mediated by a number of pro-oxidant enzymes, such as NADPH oxidase, xanthine oxidase (XO), and uncoupled endothelial nitric oxide synthase (eNOS). On the other hand, glutathione peroxidase (GPx), heme oxygenase (HO), and paraoxonase (PON) along with the main enzymes—SOD and catalase, round up the cascade of potent antioxidant defense. Mitochondrial respiration, mitochondrial electron transport, mitochondrial enzymatic reactions, as well as inflammatory response are also involved in balancing the generation and degradation of ROS [12].

The machinery of paraquat toxicity is complex involving ROS generation exceeding the capacity of ROS scavenging, leading to an imbalance between the formation and degradation of ROS. NADPH oxidase is reported to participate in the process of ROS generation triggered by paraquat [13, 14]. Upon paraquat challenge, several enzyme complexes, including XO and NADPH oxidase, are activated to generate excessive ROS [13, 14]. Accumulating results have shown a rapid increase in the mixed disulfide level followed by a drop in NADPH level in the paraquat-induced lung injury [14, 15]. In line with the above notion, ROS production prompted by paraquat exposure was counteracted by treatment with NADPH oxidase inhibitors, including apocynin and diphenyleneiodonium [16]. Taken together, these results suggest that paraquat may promote oxidation of NADPH and formation of ROS from NADPH oxidase, which may contribute, at least in part, to paraquat-induced oxidative injury [4, 13, 17].

2.3. Paraquat and Mitochondrial Injury and Stress Signaling

Although low and transient levels of ROS can be removed effectively, excessive ROS presence that is beyond the cellular clearance capacity usually triggers oxidative stress through peroxidation of membrane polyunsaturated fatty acids. Free radical scavengers can protect cells from paraquat-induced apoptosis, suggesting that free radicals elicited by paraquat exposure may serve as a potential mechanism in the onset and development of cell death. Increasing evidence has also depicted that mitochondrial generation of ROS along with mitochondrial dysfunction plays a role in the pathophysiological

processes of various chronic diseases, including aging, cancer, neurodegenerative and inflammatory disorders, and diabetes mellitus [18, 19].

Oxidative stress from either environmental or intracellular process is capable of interfering with mitochondrial integrity and function. In particular, frequent auto-oxidation may take place for the electron transport chain within mitochondria, leading to ROS production. With paraquat challenge, hallmarks of morphological alterations develop with mitochondrial swelling being the first ultrastructural change, followed by mitochondrial degeneration. It has been shown that human peroxiredoxin 5 located in mitochondria may protect against paraquat toxicity more effectively compared with that located in the cytosol [20]. This is supported by the observations that RNA interference (siRNA) knock-down of the mitochondria-specific SOD (MnSOD) prompted cells to be more vulnerable to paraquat toxicity and that heterozygous MnSOD mice exhibited hypersensitivity to paraquat toxicity [21]. Moreover, *Tetrahymena* is perhaps the most paraquat-resistant organism in nature. Although mitochondrial injury is present in *Tetrahymena* following paraquat exposure, a unique process, namely mitophagy, may be turned on to remove the damaged mitochondria. Such mitophagy process is believed to contribute to the good survival of *Tetrahymena* upon paraquat challenge. Intriguingly, *Tetrahymena* cells contain much more mitochondria than many other kinds of cells (nearly 1,440 mitochondria per cell, constituting 15% of the total cell volume) to present a much bigger reservoir of mitochondria in order to compensate for the loss of mitochondria [22]. The role of mitochondria in paraquat toxicity gets further substantiated from studies on the striatal mitochondria, where paraquat exposure suppresses complexes I and IV while promoting H_2O_2 production, along with cardiolipin oxidation and depletion. These findings indicate that paraquat prompts striatal mitochondrial oxidative injury through both redox reaction and disruption of the mitochondrial electron transport chain [23].

Besides directly mediating paraquat cytotoxicity, paraquat-induced ROS also act as second messengers to turn on a number of intracellular stress signaling molecules, including mitogen-activated protein kinase (MAPK) signaling cascades. MAPKs have been demonstrated to be involved in cellular pro-inflammatory responses. MAPKs are constituted of extracellular signal-regulated kinases 1/2 (ERKs), c-

Jun-N-terminal kinases (JNKs), and p38 MAPKs. JNK and p38 MAPKs are main members of the complex superfamily of MAP serine/threonine protein kinases and are collectively known as stress-activated kinases. These stress signaling kinases are turned on in response to a wide variety of exogenous and endogenous stress-inducing stimuli, including ultraviolet (UV) lights, toxins, ROS, oxidative stress, hyperglycemia, and pro-inflammatory cytokines [24]. Among MAPK signaling cascades, ERKs are mainly responsible for growth and differentiation, while JNKs and p38 are responsible for the formation of potent pro-inflammatory cytokines and regulation of apoptosis [24].

JNKs are believed to be involved in paraquat-induced oxidative stress to promote apoptosis, the effect that can be suppressed by antioxidants [25]. More recent evidence depicted that heat shock protein 70 (Hsp70) suppressed paraquat-induced neurodegeneration by inhibiting JNK activation and apoptosis in the *Drosophila* model of Parkinson's disease [26]. p38 MAPKs, on the other hand, are also activated in response to paraquat challenge to coordinate with various other cellular stress responses to the herbicide's exposure [27, 28]. Paraquat exposure may induce lung inflammation through p38 regulation, which further facilitates the production of cytokines like interleukin-1 beta (IL-1 β) and tumor necrosis factor alpha (TNF- α) in rodents, and these effects can be nullified by p38 inhibitors [27, 29, 30]. Paraquat exposure can inhibit apoptosis and prolong survival of neutrophils through ROS accumulation and thus disrupt neutrophil homeostasis, *en route* to acute lung injury. It has been suggested that paraquat-induced inhibition of neutrophil apoptosis may be attributed to p38 activation [28]. These results favored a role for p38 MAPKs in triggering pro-inflammatory reaction in response to paraquat-elicited oxidative stress.

ERKs are also implicated in paraquat-induced survival and apoptotic regulation. Inhibitors for ERK1/2 have been shown to partially attenuate paraquat-induced ROS production and cell death [16]. It is noteworthy that the three main stress signaling pathways may display disparate responses to paraquat under various experimental settings. In a study using bovine pre-implantation embryo cells, a cellular model vulnerable to oxidative stress, paraquat challenge suppressed ERK activity, but increased p38 activity. Moreover, melatonin protected bovine em-

bryos against paraquat-induced damage in a p38-dependent manner, and the protection was not related to either ERK or JNK signaling cascade [31].

Another major intracellular target of paraquat-induced oxidative stress is nuclear factor-kappa B (NF- κ B), which is turned on by a variety of exogenous and endogenous stimuli, including elevated free fatty acids, ROS, TNF- α and other pro-inflammatory cytokines, MAP kinase signaling, and UV irradiation. NF- κ B is believed to play an essential role in mediating immunological and inflammatory responses as well as apoptosis in response to paraquat challenge. Alterations in NF- κ B signaling have been reported in response to paraquat exposure [18]. In a recent study, paraquat-induced lung injury was found to be associated with activation of NF- κ B. Interestingly, induction of autophagy using rapamycin effectively inhibited paraquat-induced acute lung injury. These effects appeared to be associated with elevated ROS production [18].

Several natural products, such as the pigment betanin, were reported to counteract paraquat-induced oxidative stress and inflammation. A recent study reported that treatment with betanin alleviated paraquat-induced acute kidney injury, which was evidenced by histological improvement and lowered serum and urine markers for kidney injury. Notably, betanin antagonized paraquat-induced inflammation, as indicated by reduced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase, blunted activation of NF- κ B, and diminished lysosomal protease activities. These findings suggest a unique role for NF- κ B and subsequently, oxidative stress and inflammation, in paraquat-induced renal injury and the renal protection by betanin via suppressing the above responses [32]. Taken together, these data suggest that NF- κ B, JNK, ERK, and p38 MAPK pathways are potential stress-signaling systems contributing to paraquat-induced organ injuries.

3. PARAQUAT AND TARGET ORGAN INJURY

The clinical features of paraquat intoxication include irritating or burning sensation in the mouth, throat, and gut, resulting in nausea, vomiting, abdominal pain, and diarrhea [7, 33]. Ingestion and skin contact are two most frequent routes of paraquat exposure in humans and animals. Direct contact with paraquat

through the skin may cause local burning and dermatitis [34, 35]; splash in the eyes can induce irritating and burning sensation, and can lead to serious corneal damage [36]. Once ingested, paraquat is hardly transformed and degraded in the body and remains in the original form until excretion from the kidneys [7]. As described below, exposure to paraquat can cause injury in multiple organs.

3.1. Paraquat and Brain Injury: ROS and Autophagy

Parkinson's disease is a common neurodegenerative disorder with a main feature of low dopamine due to the progressive loss and depletion of the dopaminergic neurons in the substantia nigra [37]. Chronic exposure to paraquat has been suggested to promote the pathogenesis of Parkinson's disease since the herbicide shares a similar chemical structure with the known human dopaminergic neurotoxicant 1-methyl-4-phenylpyridinium ion (MPP⁺) [37]. The toxic effect of paraquat on the nigrostriatal system is likely due to its pro-oxidant activity and the subsequent accumulation of ROS. Paraquat may damage the dopaminergic cell bodies in the substantia nigra and striatal nerve terminals by inhibiting mitochondrial oxidative phosphorylation and dopamine release from the synaptic terminals [37, 38]. Besides oxidative stress injury, recent evidence also depicted a rather unique role for dysregulation of autophagic clearance mechanism in the pathogenesis of Parkinson's disease.

Autophagy is a lysosome-dependent pathway, normally responsible for degradation of damaged organelles and protein to ensure cell homeostasis. It remains at a low basal level under normal conditions and may be triggered in response to starvation or various metabolic stresses [39]. The autophagy machinery has been found to be compromised in paraquat-induced Parkinson's disease [37]. In particular, paraquat exposure can dampen basal autophagy levels and promote protein aggregate formation in the brain [40]. Garcia-Garcia and colleagues have found that paraquat impairs basal autophagy-related protein 5 (ATG5)-dependent autophagy and promotes accumulation of α -synuclein in the intoxicated dopaminergic neurons [41]. Although the precise mechanism of action remains largely unknown at this time, an elevation in mitochondrial ROS level is believed to contribute to paraquat-induced dysregulation of au-

tophagy and neuronal homeostasis. Mitochondrial ROS generated by paraquat can inhibit basal level of autophagy both in vitro and in vivo, and treatment of neurons with autophagy inducers, such as rapamycin, can effectively protect the neurons against paraquat toxicity both in vitro and in vivo [42]. The role of oxidative stress in paraquat-induced toxicity received further support from studies involving pharmacological inhibition of oxidative stress. For example, it has been reported that pharmacological inhibition or short harpin RNA (shRNA) knock-down of dihydronicotinamide riboside (NRH):quinone oxidoreductase 2 (NQO2) effectively obliterates paraquat-induced oxidative neurotoxicity in vivo, indicating a role for NQO2 in paraquat-induced neuronal ROS generation. These results suggested that ROS produced by NQO2-mediated redox activation of paraquat might be capable of blocking the normal level of autophagy, resulting in neurotoxicity [42, 43].

3.2. Paraquat and Lung Injury: ROS and Mitochondrial Injury

The lung is one of the primary targets for paraquat toxicity, whose injury is responsible for high mortality of paraquat poisoning. Paraquat has been found to be selectively accumulated in the pulmonary system through an energy-dependent transporting mechanism in Clara cells and type I/II alveolar epithelial cells, leading to lung injury, which is manifested mainly as pulmonary edema, hypoxia, acute respiratory distress syndrome (ARDS), respiratory failure, and pulmonary fibrosis. It is shown that survivors of paraquat poisoning may develop a long-term restrictive pulmonary defect that may unfavorably impact the individual's health condition [1, 3, 37, 44].

Substantial clinical and experimental evidence has depicted a bi-phasic process in paraquat-induced lung injury based on their distinctive morphological characteristics. During the early destructive phase, the type I and type II alveolar epithelial cells suffer from damage, which is followed by a proliferative phase, manifested as pulmonary edema, infiltration of immune cells, and inflammation. Based on the mechanistic data from previous studies, a single electron reduction/oxidation seems to be a critical event in paraquat-induced lung injury. Such a one-electron reduction/oxidation process may generate highly reactive ROS, including O₂^{•-}, H₂O₂, and hydroxyl radicals, in response to paraquat challenge. These

paraquat-elicited ROS are capable of oxidizing a number of reducing equivalents essential to cell survival (e.g., NADPH, reduced glutathione) [45].

3.3. Paraquat and Liver Injury

Paraquat ingestion can result in toxic hepatitis and hepatic dysfunction in humans although the pathogenesis is still not clear [46, 47]. In laboratory settings, a single injection of paraquat can cause acute hepatic damage in both mice and rats, which is evidenced by histological changes and increased liver enzymes, such as aspartate transaminase (AST) and alanine transaminase (ALT). The hepatic injury is accompanied by elevated ROS levels and mitochondrial permeability transition pore opening, suggesting that oxidative stress and mitochondrial injury are involved [48, 49]. Consistently, the antioxidant betanin is shown to protect against paraquat-induced liver injury [50].

3.4. Paraquat and Cardiovascular Injury

Cardiovascular diseases (CVDs) are the most detrimental disorders worldwide as they represent the leading cause of mortality and create a major burden to the health care [51]. There are many factors that contribute to the risk of CVDs. Some of them can be prevented simply by adopting a healthy lifestyle, such as turning to a healthy diet, smoking cessation, and regular exercise; others can be limited by a proper control of the primary diseases, such as diabetes mellitus, hypertension, and hyperlipidemia. However, for certain factors, like environmental pollutants, we often neither hardly notice nor pay any attention to them even when we are severely exposed to them, and we currently lack effective and practical ways to get rid of or avoid them. In this context, environmental pollution has been increasingly recognized as a significant risk factor for human CVDs.

A specific type of cardiomyopathy, known as “oxidative cardiomyopathy”, characterized by cardiac hypertrophy and myocardial contractile dysfunction, has been shown to be associated with environmental toxicants, including paraquat. In experimental settings, an overt increase in pulmonary vascular permeability occurs shortly after paraquat challenge. This is in line with the clinical observations of pulmonary dysfunction and fibrosis following paraquat intoxication, which would inevitably affect the cardi-

ac function. On the other hand, paraquat may also directly cause cardiac injury. Indeed, *in vivo* studies have shown that paraquat can cause significant contractile dysfunction in both rats and mice, as evidenced by decreased fractional shortening and cardiac remodeling (increased left ventricular end-systolic diameter and end-diastolic diameter). Histopathological studies also showed that edema, congestion, and hemorrhage occurred in the myocardium after administration of paraquat. These *in vivo* observations are consistent with the *in vitro* findings showing decreased peak shortening and maximal velocity of shortening/lengthening along with a prolonged duration of lengthening. The above results indicated that paraquat could compromise myocardial and cardiomyocyte contractile function. Furthermore, acute paraquat challenge can also interrupt intracellular Ca^{2+} homeostasis, which is evidenced by the depressed peak and electrically stimulated release of intracellular Ca^{2+} levels as well as a prolonged intracellular Ca^{2+} clearance time [52–55].

Oxidative balance is critical in maintaining the normal cardiac structure and function. Oxidative stress has been shown to be involved in the pathogenesis of various CVDs [56]. Under pathological conditions, the reserve of antioxidants may become insufficient, resulting in ROS accumulation and oxidative stress, leading to geometric and functional defects of myocardium. In this context, paraquat has been shown to overtly compromise myocardial contractile function in cardiopulmonary failure, and treatment with the antioxidant vitamin E can protect ventricular myocytes from paraquat toxicity, consolidating the ROS theory in this herbicide-induced cardiac toxicity [57]. This notion is in line with the documented detrimental effects of ROS in the development of cardiomyocyte apoptosis, cardiac hypertrophy, myocardial infarction-induced remodeling, pressure overload, and cardiac aging [58, 59].

Paraquat toxicity often results in death due to irreversible circulatory shock during both acute and sub-acute intoxication phases. These unfavorable hemodynamic effects may also contribute to paraquat-induced “oxidative cardiomyopathy” although the direct myogenic effect seems to play a more significant role. Several mechanisms have been postulated for oxidative stress-induced myopathic changes, including mitochondrial injury, impaired Ca^{2+} handling, oxidation and defect of key contractile proteins, and accumulation of toxic metabolites, such as reactive

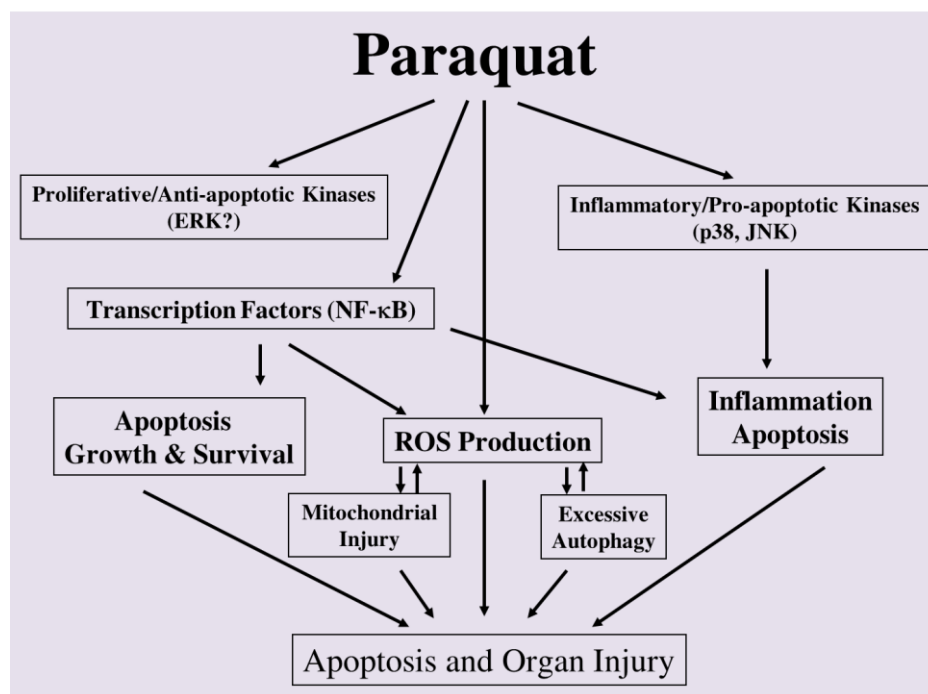


FIGURE 1. Schematic diagram depicting proposed mechanisms responsible for paraquat-induced cell death and organ injury. Either direct toxicity through oxidative stress or indirect route through ROS accumulation and activation of pro-inflammatory stress signals/cytokines can contribute to paraquat-induced toxicity and organ injury. As depicted, there is also a crosstalk between ROS production and either mitochondrial injury or excessive autophagy. On the one hand, ROS cause mitochondrial injury and excessive autophagy. On the other hand, both mitochondrial injury and excessive autophagy also lead to increased ROS production.

aldehydes derived from ROS-mediated peroxidation of polyunsaturated fatty acids [57].

4. PERSPECTIVES

Toxicity of paraquat has long been an issue due to the lack of an effective antidote and published guidelines of treatment. Paraquat exposure may cause neurodegeneration (e.g., parkinsonism) and lung injury through oxidative stress-induced abnormal autophagy and mitochondrial injury [2, 4]. ROS produced by paraquat also directly promote a unique oxidative cardiomyopathy featured by cardiac remodeling and contractile dysfunction. The mechanism of paraquat-induced target organ toxicity probably lies in two aspects: (1) ROS accumulation may serve as an ultimate insult to directly damage mitochondrial protein

and DNA as well as to oxidize membrane lipids; and (2) ROS may act as second messengers to transduce oxidative stress to downstream proteins and trigger pro-inflammatory responses through stress signaling pathways (Figure 1). A better understanding of the mechanism of paraquat-induced target organ toxicity is warranted to guide the workers and farmers for the proper handling and application of this toxic herbicide. A thorough understanding of paraquat toxic machinery would also contribute to the eventual development of an effective antidotal therapy for the management of paraquat poisoning.

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