This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

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Paraquat, toxicity and mechanism

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For 77:222, Spring 2005

28 Feb 2005

Abbreviations:

GPx, glutathione peroxidase.
GSH, glutathione
GSSG, glutathione disulfide.
GR, glutathione reductase
H_2O_2, hydrogen peroxide.
$HO^{\bullet}$, hydroxyl radical.
HOCl, Hypochlorous acid
MPO, myoloperoxidase.
NADPH, nicotinamide adenine dinucleotide phosphate.
$NO^{\bullet}$, Nitric oxide
$NO_2^{\bullet}$, nitrogen dioxide .
$O_2^{\bullet-}$, superoxide.
$^1O_2$, singlet oxygen
$ONOO^-$, Peroxynitrite.
PQ^{2+}, paraquat.
$PQ^{\bullet+}$, paraquat radical.

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Abstract

Paraquat (1,1`-dimethyl-4,4`-bipyridinium dichloride) has been used for many years in many countries as a broad-spectrum herbicide. It is extremely toxic, causing multiple organ failure in humans. It preferentially damages the lungs, kidneys and liver, and may result in death. Paraquat is reduced by PQ^{2+} diaphorases or reducing agents. In aerobic condition, the reduced paraquat radical is reoxidized by oxygen, with formation of $O_2^{\bullet-}$, which in turn may be metabolized to other reactive oxygen species, including the highly reactive $HO^{\bullet}$, $ONOO^-$, or $^1O_2$. Moreover, paraquat redox cycling leads not only to the generation of $O_2^{\bullet-}$, but also to a potential depletion of intracellular NADPH. The inability to maintain physiological levels of NADPH may cause cell damage. This review will focus on the physical and chemical properties of paraquat, its toxicity and the mechanism underlying it.

Introduction

Paraquat (PQ^{2+}) is one of the most widely used herbicides. It is used to control broad-leaved weeds and grasses, being less effective on deep-rooted plants such as dandelions. Paraquat does not harm mature bark, and is thus widely used for weed control in fruit orchards and plantation crops [A]. Paraquat is highly toxic to animals and has serious and irreversible delayed effects if ingested. The lungs selectively accumulate PQ^{2+}, and therefore contain higher concentrations than other tissues, which leads to fibrosis. Liver damage occurs and renal failure may follow as the kidneys remove absorbed PQ^{2+} [1]. Paraquat has also been used as a model factor inducing oxidative stress both *in vivo* and *in vitro* [2]. Although a definitive mechanism of toxicity of PQ^{2+} has not been delineated, it was proposed that a cyclic single electron reduction/oxidation of the PQ^{2+} molecule is a critical mechanistic event. Two consequences related to its toxicity are generation of reactive oxygen species and depletion of intracellular NADPH [3]. This review will focus on the physical and chemical properties of paraquat, its toxicity and the mechanism underlying it.

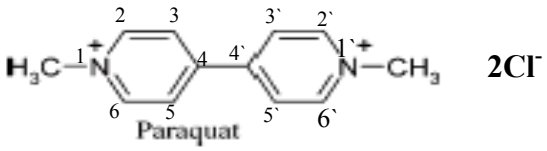


Figure. 1. **Structure of herbicide paraquat dichloride.**
The basic bipyridyl consists of two pyridine rings linked together. N is counted as atom number-one in each ring [4].

[A] http://www.pan-uk.org/pestnews/actives/paraquat.htm Accessed on 26/02/05.

The chemical and physical properties of paraquat

The herbicide paraquat is a quaternary nitrogen compound, whose basic chemical mucleus is bipyridyl consisting of two pyridine rings joined together. Their number-4 carbon atoms join the two pyridine rings. Each nitrogen atom has a methyl group (**Figure 1**) [4]. In its usually oxidized form, paraquat is ionized with two positive charges. So paraquat is usually manufactured as a salt with chloride ion (**Figure 1**).

Like other bipyridyl salts, paraquat is non-volatile both in the solid and in solvated state. Paraquat dichloride is extremely water-soluble and completely insoluble in non-polar organic solvents [5]. Paraquat is very stable in acid or neutral solutions. In alkaline solution paraquat decomposes to various complex colored degradation products [6]. The free radical forms of $PQ^{\bullet+}$ are water-soluble and have a characteristic intensive color (**Reaction 1**). At 400 nm, the extinction coefficient for $PQ^{\bullet+}$ is 4.6×10^4 $M^{-1}cm^{-1}$, which allows the chemical detection of paraquat by spectrophotometry [9]. Paraquat has a redox potential of –446 mV [6], which allows the single electron reduction. **Table 1** summarizes some of the physical and chemical properties of paraquat.

$$\underset{\text{(Colorless)}}{PQ^{2+}} \xrightarrow{+e} \underset{\text{(Blue)}}{PQ^{\bullet+}} \xrightarrow{+e} \underset{\text{(Yellow, } H_2O\text{-insoluble)}}{PQH_2} \qquad (1)$$

Toxicology of paraquat

The extent of poisoning caused by paraquat depends on the amount, route, and duration of exposure and the person's condition of health at the time of the exposure. Paraquat

Table 1. Physical and chemical properties of paraquat [5].

Physical state	White (pure), yellow (technical), solid
Molecular weight	186.2 (Cation) 257.2 (dichloride)
Specific gravity	.24-1.26 20/20
Boiling point:	175 - 180°C [B]
Decomposition	300°C
Decompose	in ultraviolet light[C]
Vapor pressure	Nonvolatile
Density (g cm-3):	1.25 g/cm^3
Solubility at 20°C	Acetone—very slight Carbon disulfide—almost zero Dimethylformamide--slight Ethanol—extremely low Kerosene—almost zero Water—high soluble
Corrosiveness	Very to metals
Storage stability	Indefinitely long in original container
Incompatibilities	Alkyl sulfonate or alkyl aryl sulfonate Wetting agents, hydrolyzed by alkali Incompatible with strong oxidizing agents.

causes direct damage when it comes into contact with the lining of the mouth, stomach, or intestines[D]. The lethal ingestion dose of paraquat in humans is 35 mg/kg[E]. After paraquat enters the body, tissue distribution is ubiquitous with an apparent volume of distribution ranging from 1.2 to 1.6 l/kg [7]. In the research of Houze *et al*, the toxicokinetics of PQ^{2+} was studied in 18 cases of acute human poisoning using a specific radioimmunoassay. Plasma PQ^{2+} concentration exhibited a mean distribution half-life of 5 h and a mean elimination half-life of 84 h. Death related to pulmonary fibrosis occurred late and was associated with the elimination phase [7].

[B] http://ptcl.chem.ox.ac.uk/MSDS/PA/paraquat.html. Accessed on 27/02/05.
[C] http://www.inchem.org/documents/jmpr/jmpmono/v070pr19.htm. Accessed on 27/02/05.
[D] http://www.bt.cdc.gov/agent/paraquat/basics/facts.asp. Accessed on 26/02/05.
[E] http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/paraquat-ext.html. National Library of Medicine. Hazardous Substances Databank. Paraquat. 1992. Accessed on 09/03/05.

1. Immediate signs and symptoms of paraquat exposure

Ingestion of large amounts of paraquat can be extremely toxic. Symptoms such as pulmonary edema, lung scarring, liver failure, kidney failure, confusion, coma, seizures, injury to the heart, fast heart rate, muscle weakness, respiratory failure may happen within a few hours, possibly leading to death. Ingestion of small to medium amounts of paraquat may lead to adverse health effects like liver failure, kidney failure, heart failure and lung scarring after several days to weeks.

2.The long-term health effects

If a person survives the toxic effects of paraquat poisoning, long-term lung damage (scarring) is highly likely. Other long-term effects may also occur, including kidney failure, heart failure, and esophageal strictures[D].

Mechanism of paraquat toxicity

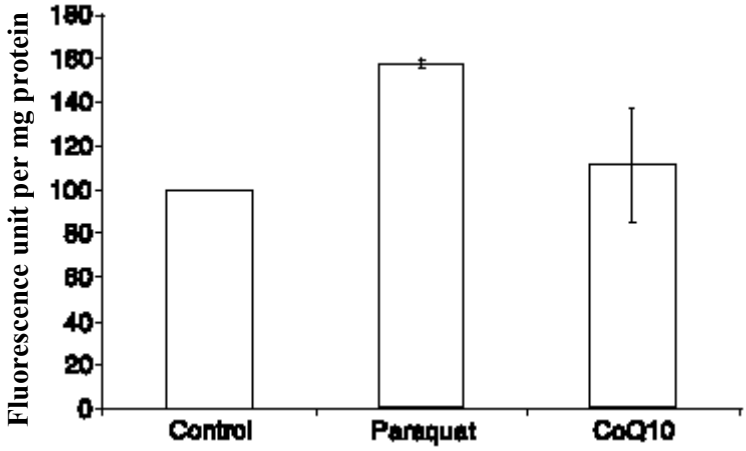
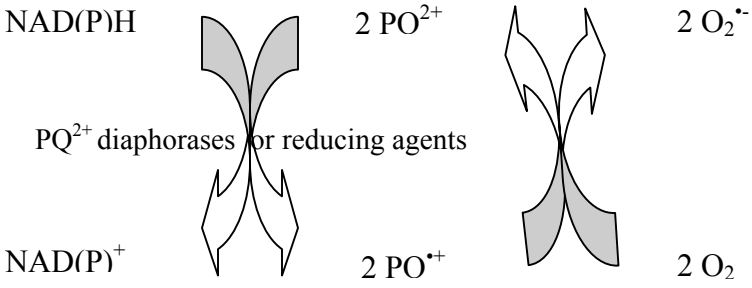


Figure. 2. Generation of reactive oxygen species. DCFDA was used to measure total cell ROS generation. After 48 h of paraquat treatment, human neuroblastoma SHSY-5Y cells showed a considerable increase in ROS production compared to control cells or cells pretreated with CoQ_{10}. Results were calculated per microgram

[D] http://www.bt.cdc.gov/agent/paraquat/basics/facts.asp. Accessed on 26/02/05.

Produce Superoxide

Although a definitive mechanism of toxicity of paraquat has not been delineated, a cyclic single electron reduction/oxidation of the parent molecule is a critical mechanistic event [2]. Paraquat can induce oxidative stress and the following neuronal cell death (**Figure 2**) [7]. One-electron reduction of PQ^{2+} can be achieved by chemical reducing agents such as ascorbic acid (**Reaction 1**) [6, 13] or PQ^{2+}diaphorase [16]. PQ^{2+}diaphorase are usually oxidoreductase enzymes that contain flavins and use either NADH or NADPH as electron donors. A common cellular diaphorase that can redox cycle with PQ^{2+} is cytochrome P450 reductase (**Reaction 2**) [16]. So the oxidation of 1 mol of NADPH produces 2 mol of $O_2^{\bullet-}$.



(2)

$PQ^{\bullet+}$ reacts very quickly with O_2 to give $O_2^{\bullet-}$ (**Reaction 3**), with $k_2 = 7.7 \times 10^8\ M^{-1}\ s^{-1}$ [8].

$$PQ^{\bullet+} + O_2 \xrightarrow{k_2} PQ^{2+} + O_2^{\bullet-} \qquad (3)$$

After transfection of cDNA for human CuZn superoxide dismutase to NIH/3T3 cells, it becomes resistant to paraquat, which strongly supports the fact that formation of $O_2^{\bullet-}$ is a necessary part of its cytotoxic effects [10].

Produce Hydroxyl radical

Superoxide may be dismutated to H_2O_2. In the presence of Fe^{2+}, highly reactive and toxic radicals such as $HO^{\bullet}$ can be formed (**Reaction 4 & 5**).

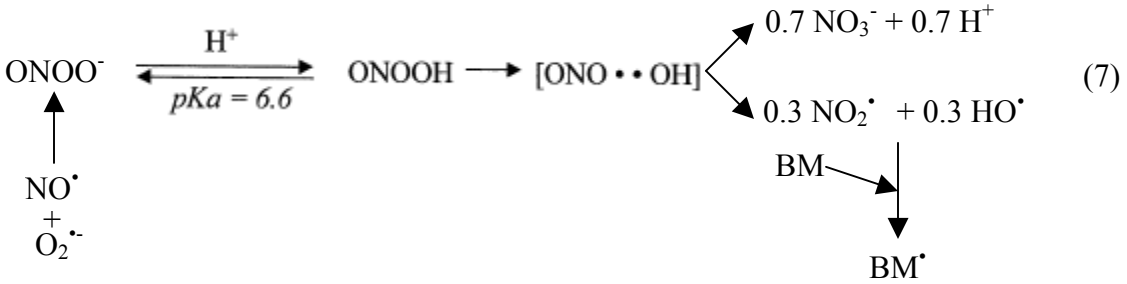
$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 \qquad (4)$$

$$H_2O_2 + Fe^{2+} \longrightarrow HO^{\bullet} + OH^- + Fe^{3+} \qquad (5)$$

$$PQ^{\bullet+} + H_2O_2 \longrightarrow PQ^{2+} + OH^- + HO^{\bullet} \qquad (6)$$

In the research done by Winternourn, $PQ^{\bullet+}$ can react with H_2O_2 to produce $HO^{\bullet}$ directly in the absence of metal catalyst (**Reaction 6**) [13]. The reaction is fast and able to compete with the reaction of with O_2. So production of $HO^{\bullet}$ from $PQ^{\bullet+}$ and H_2O_2 may therefore be of major significance in paraquat toxicity.

Produce $ONOO^-$ and then $HO^{\bullet}$



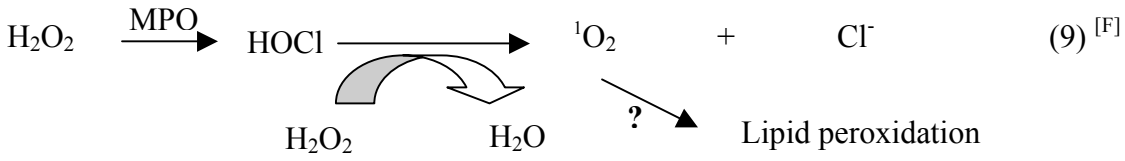
(7)

Nitric oxide ($NO^{\bullet}$) has been implicated in PQ^{2+} induced lung injury. It is thought to be due to its rapid reaction with $PQ^{\bullet+}$-formed $O_2^{\bullet-}$ to produce the strong oxidant peroxynitrite (**Reaction 7**) [14]. Peroxynitrite is a very reactive species, the "bent-form" of which has an E°` of +2100 mV, which is nearly as oxidizing as $HO^{\bullet}$ (+ 2310 mV). Peroxynitrite is

stable at alkaline pH but upon protonation (pKa = 6.6) it decomposes rapidly (k = 0.17 s^{-1} at pH 7.4 and k = 1.1 s^{-1} at pH 5.4, 25°C) to yield 70% nitrate and 30% hydroxyl radical and nitrogen dioxide ($NO_2^{\bullet}$). Hydroxyl radical is capable of reacting with all biological macromolecules (lipids, proteins, nucleic acids and carbohydrates) to the corresponding radicals (**Reaction 7**) [15].

Produce Singlet Oxygen

Hara *et al* studied the PQ^{2+}-stimulated NADPH-dependent lipid peroxidation in mouse brain and pulmonary microsomes. They found that the lipid peroxidation was inhibited by SOD and singlet oxygen quenchers, but not by catalase or hydroxyl radical scavengers. These findings suggest that activated oxygen species, especially superoxide and singlet oxygen, may play a major role in the stimulation of microsomal lipid peroxidation by paraquat in both brain and lung [11]. In the absence of metal catalyst, hypochlorous acid (HOCl) can be generated enzymaticaly by myoloperoxidase (MPO) from H_2O_2. Reaction of HOCl with H_2O_2 yields $^1O_2$ (**Reaction 9**)[F].



The result of Hara *et al* is weird, however, it has been reported that singlet oxygen ($^1O_2$) can lead to lipid peroxidation [17]. Peroxidation of Linolenate has been reported by a photochemical source of singlet oxygen [18], however the mechanism is unclear.

[F] http://www.rndsystems.com/asp/g_sitebuilder.asp?bodyId=222. Accessed on 10/03/05.

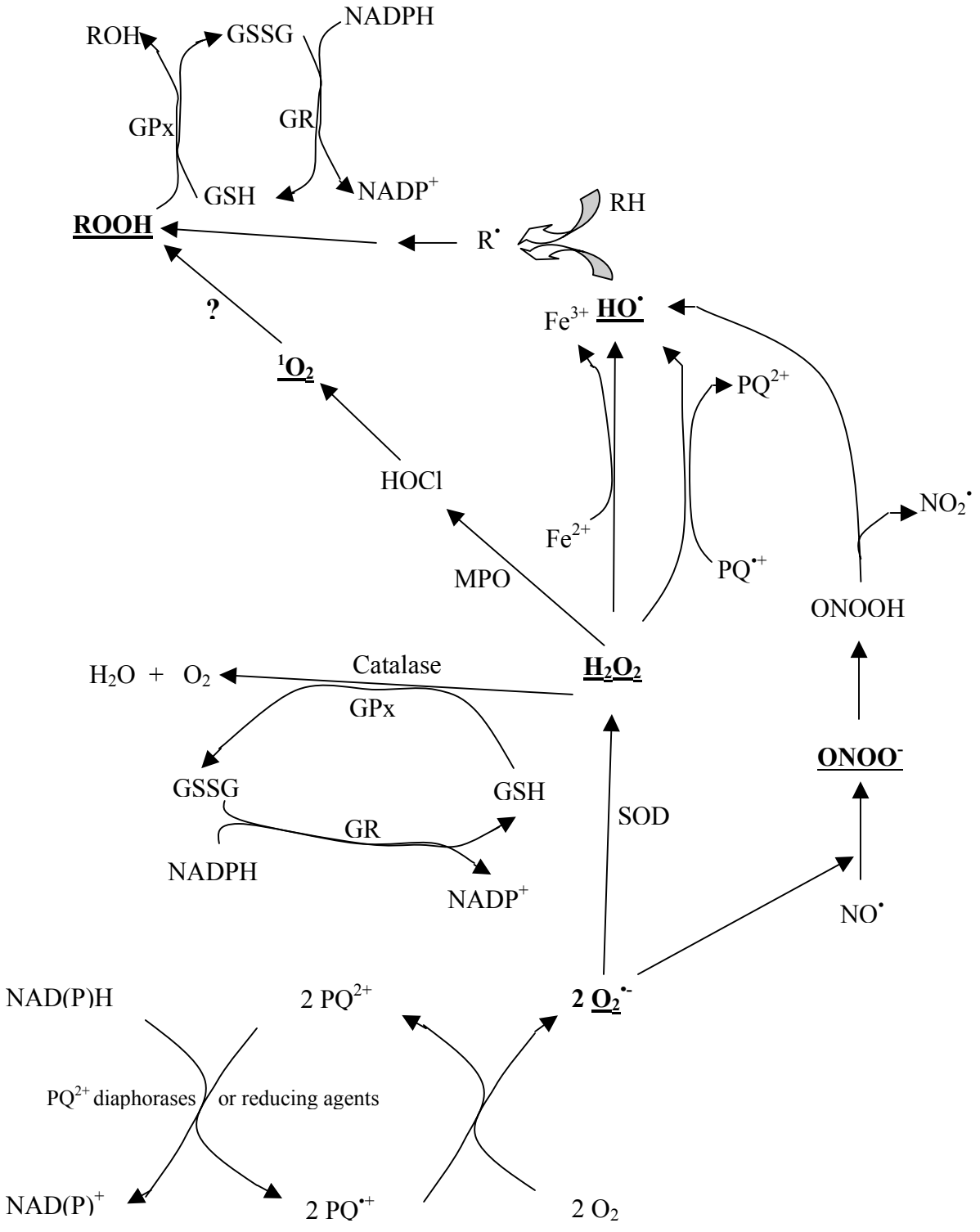


Figure 3. Proposed mechanism of action for the in vivo toxicity of paraquat. Important intermediate species or products are shown in bold and underlined.

Depletion of NADPH

Several enzymes are present in cells that can catabolize the $O_2^{\bullet-}$ and reduce lipid peroxidation. Superoxide is converted to H_2O_2 and O_2 by SOD, and H_2O_2 is further inactivated to H_2O and O_2 by catalase or glutathione peroxidase (GPx). The reduction of lipid peroxides by GPx requires glutathione (GSH). Because the reduction of glutathione disulfide (GSSG) is coupled with the oxidation of NADPH through glutathione reductase (GR), the availability of sufficient NADPH is a critical factor for the detoxification of paraquat. So the redox cycling of paraquat and the detoxification of $O_2^{\bullet-}$, H_2O_2 and lipid peroxidation consume NADPH. The inability to maintain physiological levels of NADPH may cause cell damage (**Figure 3**). Cells become more susceptible to free radical attack and peroxidation of vital cellular constituents.

Summary

Current reports suggest that the redox cycling, single-electron reduction/oxidation of the parent molecule is the critical event underlying the toxicity of paraquat. Following production of reactive oxygen species and lipid production lead to loss of NADPH can cause cell death.

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