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Paraquat toxicity

by

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Abbreviations
DQ: diquat
GSH: glutathione
GSSG: glutathione disulfide
HPLC: high-performance liquid chromatography
IS: internal standard
PQ: paraquat
RIA: radioimmumoassay
SOD: superoxide dismutase

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Abstract

Paraquat (1,1'-dimethyl- 4, 4'- bipyridinium) undergoes a single electron reduction to form the reduced radical with NADPH serving as an electron donor for the reduction. Reduced paraquat is rapidly reoxidized by molecular oxygen with formation of oxidized paraquat and superoxide radicals. The formation of free radicals and the change of NADPH concentrations evoke paraquat toxicity. A simple method for simultaneous determination of paraquat in biological samples is using high-performance liquid chromatography (HPLC). A radioimmunoassay (RIA) is a more sensitive method for the identification and quantification of paraquat.

Introduction

All fatalities of paraquat poisoning have been due to accidental or suicidal intake. The ingestion of toxic paraquat is followed by an initial phase of gastrointestinal disturbances due to the irritant action of the chemical, followed by a second intermediate phase of transientory hepato-renal changes, subsequently to this, a third phase of delayed and lethal lung effects, as shown in Figure 1. Also, in cases with huge doses of paraquat, toxic effects in liver, kidney and lungs appear together with the initial gastrointestinal disturbances. In cases with moderately toxic doses, poisoning is limited to the first and second phase [1].

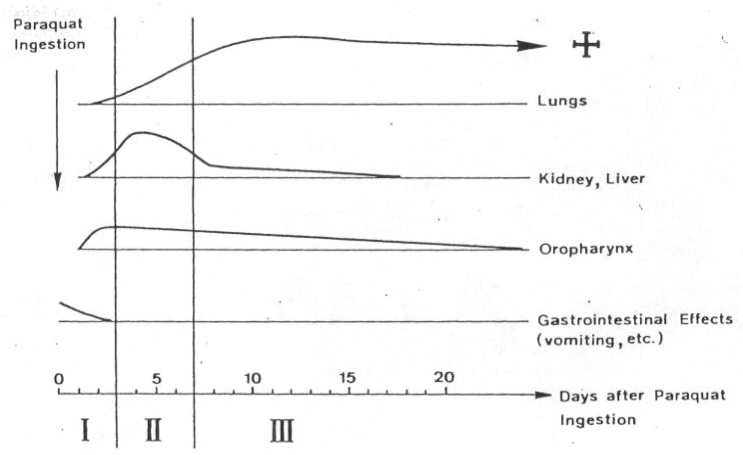
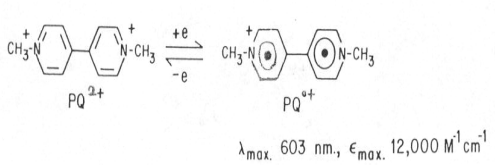


Figure1. The triphasic clinical courses of paraquat intoxication [1].

Paraquat is extremely soluble in water and paraquat salts are strong electrolytes. The free radical of paraquat is blue and the formation of such colored reduced species is of great value for the chemical detection [1]. The purpose of this report is to describe major aspects of paraquat electron transfer reactions, the mechanism of toxicity and the principle of its detection.

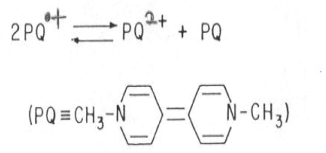
Electron transfer reactions

Cation radicals of paraquat are formed from neutral molecules by one-electron oxidation process. Paraquat dichloride (PQ^{2+}) (1,1'-dimethyl-4,4'-bipyridylium dichloride) have herbicidal properties which depend on the reversible one electron reduction of PQ^{2+} to form cation radicals $PQ^{\bullet+}$, as shown in the reaction 1 [2].

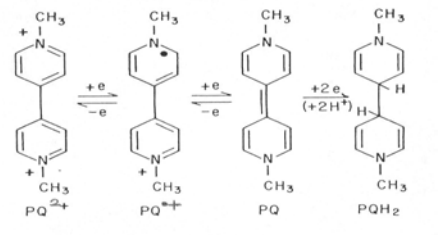


(1)

For this paraquat system, the value of *k* is 6 x 10^6 $M^{-1}s^{-1}$, so the cation radicals are thermodynamically preferred. Paraquat is useful oxidant in aqueous solution and its redox potentials ($E^{0'}$ = -446 mV) exhibits useful herbicidal properties. The positions of equilibrium for paraquat lie to the left hand side of the reaction 2. Also, reduction of paraquat does not stop at the one- or two-electron stage but can proceed further to give a tetrahydro product corresponding to overall four-electron reduction, as shows in the reaction 3 [2].

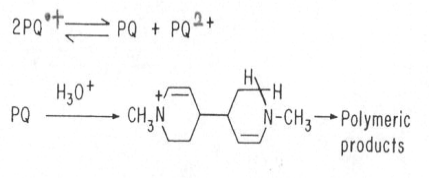


(2)



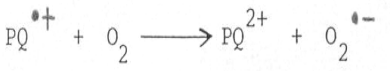
(3)

The cation radical of paraquat is rapidly destroyed by protic acids in water, so there is no free PQ^{2+} present. Although the position of equilibrium in the equation lies in favor of $PQ^{\bullet+}$, protic acid will rapidly shift the position by protonation of the reactive enamine PQ, as shows in reaction 4 [2].



(4)

Paraquat function as efficient herbicides in a catalytic manner requiring the present of oxygen and of chloroplast photo-activation. Reaction of $PQ^{\bullet+}$ with oxygen in water is rapid and PQ^{+} react with both O_2 and O_2^- (-0.59V and +1.12V respectively), as shown in reaction 5 and 6 [2].



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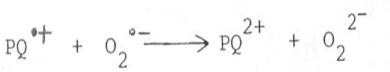

(6)

Increasing the organic contents of the solvent increases the thermodynamic stability of $PQ^{\bullet+}$ relative to PQ^{2+} with a consequent decrease in the reactivity of $PQ^{\bullet+}$ towards oxidizing agents such as O_2 [2]. Studies on the mechanism of the herbicidal activity of paraquat provided insights into the mechanism of mammalian toxicity. Paraquat is able of undergoing a single electron reduction to a blue-colored form with a redox potential of – 446 mV. In the presence of oxygen, reduced paraquat is immediately reoxidized by molecular oxygen to the colorless parent compound [2].

Mechanism of toxicity

The proposed mechanism of *in vivo* paraquat can be discussed in four categories. First, paraquat can undergo cyclic oxidation-reduction in the presence of oxygen with subsequent generation of superoxide free radicals ($O_2^{\bullet-}$). Paraquat reduction can be catalyzed by microsomal NADPH-cytochrome c reductase. Superoxide free radicals may dismutate to singlet oxygen or be detoxified by superoxide dismutase (SOD) and catalase to water and oxygen. Singlet oxygen can also attack the polyunsaturated lipids of cell membranes to form lipid hydroperoxydes.

Second, lipid free radicals can form from lipid hydroperoxides and react with membrane polyunsaturated lipids. The formation of more lipid free radicals continues the chain reaction process of lipid peroxidation.

Third, lipid hydroperoxides may be enzymatically detoxified to stable lipid alcohols by GSH peroxidase utilizing reduces glutathione (GSH) as substrate. The activity of GSH peroxidase in

reducing lipid hydroperoxides has an essential requirement for selenium and vitamin E which is a free radicals scavenger. Thus, this reaction can interrupt the free radical chain reaction.

Fourth, paraquat induce an increase in the activity of glucose-6-phosphate dehydrogenase, an enzyme of the pentose pathway which may provide the NADPH for reduction of oxidized glutathione (GSSG) and for the subsequent function of GSH peroxidase in reducing lipid hydroperoxides [1]. Therefore, paraquat evokes its toxicity by leading to the formation of free radicals and the change of NADPH concentrations.

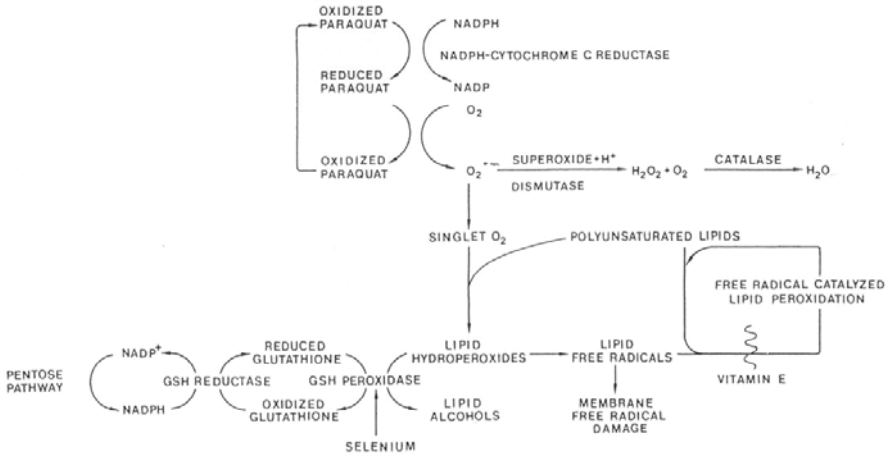

Figure 2. Mechanism of paraquat toxicity in mammals [1].

Since lung tissue is capable of reducing paraquat, in the *in vitro* studies of the paraquat mechanism, anaerobic incubation of paraquat with mouse lung microsomes and NADPH resulted in the formation of the blue-colored paraquat radical, as shown in Figure 3. This result confirmed the ability of lung tissue to reduce paraquat *in vitro* [2].

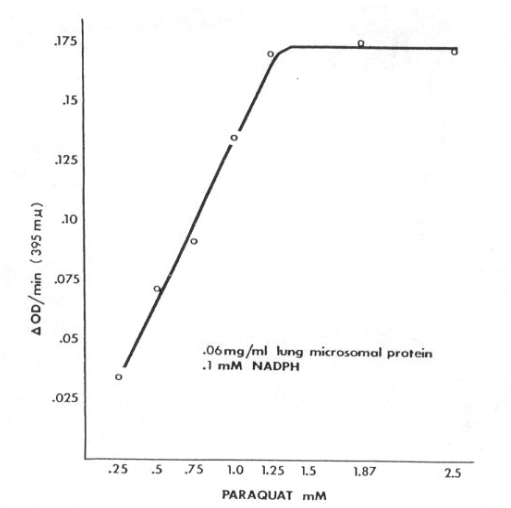

Figure 3. Anareobic reduction of paraquat in the presence of mouse lung microsomes and NADPH [2].

Detection

The simple method for determination of paraquat in human biological material is using high-performance liquid chromatography. Paraquat is separated on the octadecylsilica column with a mobile phase of potassium bromide in methanol solution containing triethylamine. Ultraviolet wavelength of 256 nm for paraquat can be selected by HPLC. Figure 3 shows chromatograms obtained from a plasma sampling containing paraquat. Also, paraquat (PQ), diquat (DQ) and internal standard (IS) can be separated clearly [3].

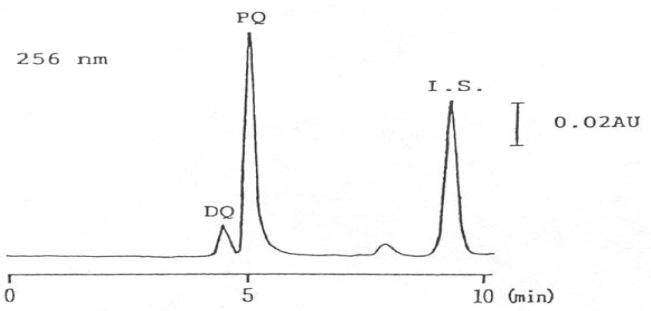

Figure 3. Chromatograms of an extract from blood containing PQ, with detection of 256 nm [3].

Radioimmunoassay (RIA) is another method used to examine the paraquat concentrations in biological samples. This quantitative determination of paraquat in plasma, urine, and bronchoalveolar lavage fluid uses the synthesis of a iodinated paraquat tracer- 1-(N-methyl-4,4'-bipyridinium)-4-(p-hydroxyphenyl)butane salt (MBPB).The structure of synthesized MBPB is verified by proton NMR spectrum, as shown in Figure 4 [4].

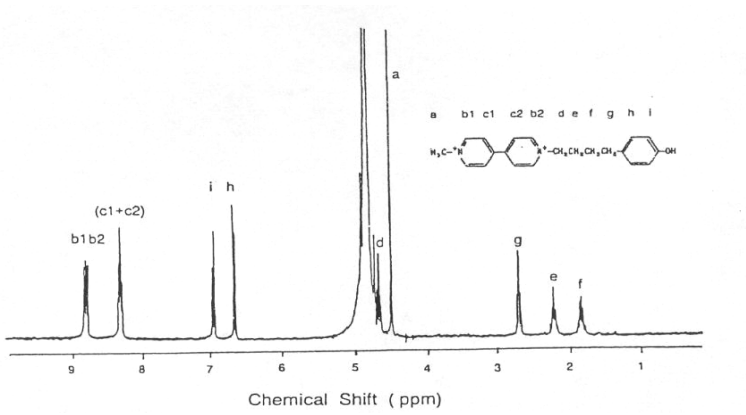

Figure 4. The 300 MHz proton NMR spectrum of MBPB [4].

Tracers is produced in the presence of MBPB and [^{125}I]sodium iodide. After removal of unreacted [^{125}I]sodium iodide by ion-exchange chromatography, the iodinated derivative was assayed for immumological integrity by ELISA. Finally, antibody-bound paraquat in which ^{125}I activity can be obtained in samples. Generally, the working range of this assay was 0.46 – 165 ng/ml [4].

Summary

Paraquat is not metabolized in the human and the chemical undergoes redox cycling during which the free radical form is continuously produced. Toxicity result as a consequence by free radical action or diminished concentrations of cellular NADPH. Quantitative determination of paraquat in biological samples can be examined by HPLC or RIA.

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