

Diagnostic and Therapeutic Approach for Acute Paraquat Intoxication

Hyo-wook Gil,¹ Jung-Rak Hong,²
Si-Hyong Jang,³ and Sae-Yong Hong¹

¹Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Cheonan, Korea;

²Department of Internal Medicine, Metropolitan Hospital Center, New York, USA; ³Department of Pathology, Soonchunhyang University Cheonan Hospital, Cheonan, Korea

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Address for Correspondence:

Sae-Yong Hong, MD

Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, 8 Soonchunhyang 6-gil, Dongnam-gu, Cheonan 330-721, Korea

Tel: +82.41-570-3682, Fax: +82.41-574-5762

E-mail: syhong@sch.ac.kr

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Paraquat (PQ) has known negative human health effects, but continues to be commonly used worldwide as a herbicide. Our clinical data shows that the main prognostic factor is the time required to achieve a negative urine dithionite test. Patient survival is a 100% when the area affected by ground glass opacity is < 20% of the total lung volume on high-resolution computed tomography imaging 7 days post-PQ ingestion. The incidence of acute kidney injury is approximately 50%. The average serum creatinine level reaches its peak around 5 days post-ingestion, and usually normalizes within 3 weeks. We obtain two connecting lines from the highest PQ level for the survivors and the lowest PQ level among the non-survivors at a given time. Patients with a PQ level between these two lines are considered treatable. The following treatment modalities are recommended to preserve kidney function: 1) extracorporeal elimination, 2) intravenous antioxidant administration, 3) diuresis with a fluid, and 4) cytotoxic drugs. In conclusion, this review provides a general overview on the diagnostic procedure and treatment modality of acute PQ intoxication, while focusing on our clinical experience.

Keywords: Paraquat; Reactive Oxygen Species; Antioxidants; Hemoperfusion

INTRODUCTION

Paraquat (N, N'-dimethyl-4, 4'-bipyridinium dichloride; PQ) is one of the most widely used herbicides in the world (1), despite its ban in the European Union since 2007 for not meeting health standards (2). PQ intoxication is a serious public health problem, with an estimated annual incidence of 2000 toxic ingestions associated with a mortality rate of 60%-70%, in some Asian countries (3-7). PQ is a widely used suicide agent in developing countries due to its widespread availability, low toxic dose, and relatively low cost.

Several methods have been studied for modifying the toxicity of PQ (8-12) over the past 40 yr, but none have proven to be effective to date. Therefore, the clinical outcome of PQ intoxication is usually determined by the degree of exposure (13, 14).

Intentional ingestion of pesticide is a common way of committing suicide in Korea. Our pesticide intoxication institute therefore experiences a high incidence of acute PQ intoxication, with more than 10,000 cases during the past 30 yr.

In this article, we describe our clinical experience and propose a diagnostic and therapeutic approach for patients with acute PQ intoxication.

MECHANISM OF PARAQUAT (PQ) POISONING

Mechanisms of critical toxicity in the human body

Several pathways are identified as causative factors of critical toxicity of PQ in the human body. Firstly, the lungs are vulnerable to PQ intoxication (Fig. 1). PQ concentration within the lungs increases continuously during the first several hours post-PQ ingestion despite decreasing plasma PQ levels. Many scientists attribute this phenomenon to high affinity of PQ to alveolar cells. Injury to pneumocytes is initiated by nicotinamide adenine dinucleotide (NADH)-dependent reduction of PQ to monocation radicals (PQ^{•+}). Spontaneous reaction with molecular oxygen yields the superoxide radical (O₂^{•-}) and reversibly forms PQ^{•+}, which can be reduced again. PQ intoxication thus frequently causes death due to respiratory failure (15, 16).

Secondly, reactive oxygen species (ROS) mediated cell signal transduction causes inflammation (17, 18). Consequently, the initial pathologic finding is characterized by inflammatory cell infiltration (Fig. 2). Many physicians thus attempt anti-inflammatory drugs as a treatment modality in patients with acute PQ intoxication. This topic is further addressed in the following paragraphs. Thirdly, mitochondria damage by PQ. Finally, PQ releases free form iron from ferritin, aggravating ROS production (19-22).

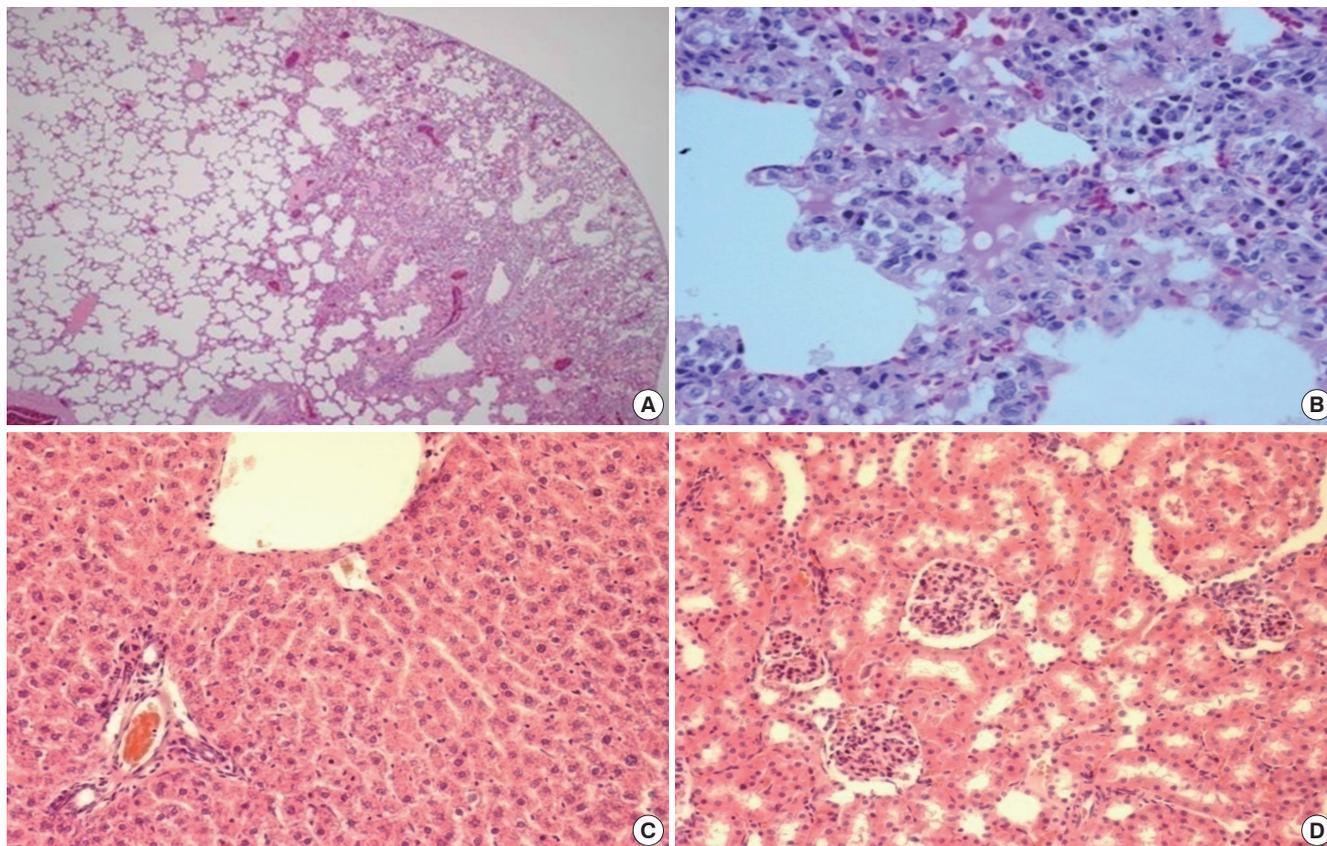


Fig. 1. Lung is a vulnerable organ in acute paraquat intoxication. Lethal dose of paraquat (25 mg/kg) was injected intraperitoneally in mice and 3 days later, the mice were sacrificed. The lung, liver and kidney were obtained and examined by light microscopy. (A) The lung parenchyma show interstitial widening and inflammatory infiltrates predominantly in the sub-pleural area (H&E, $\times 40$). (B) High power examination of the lung reveals dense infiltration of lymphoplasmic cells (H&E, $\times 400$). The liver (C) and kidney (D) are histologically unremarkable (H&E, $\times 100$).

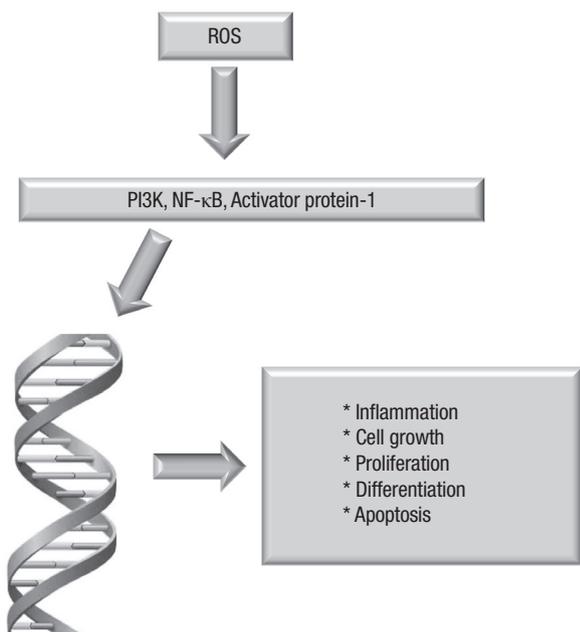


Fig. 2. ROS mediated signal transduction in inflammation. Free radicals stimulate protein complexes, such as PI3K, NF- κ B, and Activator protein-1 which control transcription of DNA, involved in cellular functions such as cell growth, proliferation, and differentiation.

CLINICAL MANIFESTATIONS

Clinical characteristics of acute PQ intoxication

The clinical features of acute PQ intoxication are characterized by high mortality, rapid progression, and frequent injury to the lung and kidney. Acute PQ intoxication is usually asymptomatic in the early stage. Irritation and numbness of the tongue and oral mucosa are generally the only complaints noted in most cases, within the first few days. Table 1 summarizes the main symptoms reported according to severity of PQ intoxication.

Shortness of breath, an increased respiratory rate (tachypnea), lethargy, and confusion appear within 3 to 4 days post-PQ ingestion, as the lung injury (consolidation on imaging) progresses in cases of moderate to severe intoxication.

Clinical features of acute kidney injury in patients with acute PQ

The incidence of acute kidney injury (AKI) secondary to PQ intoxication is approximately 50% (23-26). Non-oliguric renal failure is observed in > 50% of AKI patients, especially when a large volume of fluid is administered in early management. Average serum creatinine level reaches its peak around the 5th

Table 1. Symptoms and signs of acute PQ intoxication, according to the severity of intoxication

Severity of intoxication	Ingested amount (mL)	Symptoms	Signs
Mild	< 10	No specific symptom	No specific sign
Moderate	10-40	Sore tongue	Tachypnea
		Shortness of breath	Tachycardia
		Agitation	Increased serum creatinine
		Abdominal discomfort Head lightness	Oral mucosa necrosis
Severe	> 40	Sore tongue	Tachypnea
		Shortness of breath	Tachycardia
		Hiccup*	Increased serum creatinine
		Agitation	Oral mucosa necrosis
		Confusion†	Jaundice

*Hiccups generally occur when the ingestion amount is > 100 mL and far ominous symptoms; †Confusion is usually accompanied by death within a few days.

day post-ingestion, and is usually normalized within 3 weeks. There is no report on permanent loss of renal function after PQ intoxication; however, the mortality risk is significantly higher in patients with renal failure.

Prognostic markers for PQ intoxication

Lee et al. (27) identified the following significant prognostic factors for PQ intoxication based on the initial blood and urine samples obtained in the emergency room and the patient demographics. Age, the amount of PQ ingested, plasma PQ concentration, leukocyte count, blood urea nitrogen, serum creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase, and amylase were significant prognostic factors.

DIAGNOSIS

Detection and interpretation of PQ in a body fluid

The detection of PQ in a body fluid (e.g., serum, plasma, urine) is necessary and sufficient to confirm PQ intoxication. PQ levels are to be interpreted with reference to the time lag after PQ ingestion in keeping with the toxicokinetics of PQ. The kinetics of PQ distribution are best described by a 3-compartment model: 1) plasma, 2) a rapid uptake and removal compartment such as the kidney, and 3) a slow uptake compartment, reaching a maximum at approximately 4-5 hr, such as the lung (28). The plasma level peaks early, usually within 1 hr post-PQ ingestion, followed by a rapid and steep decline due to its rapid redistribution from the circulation to other compartments. During this period, plasma concentration shows substantial variation with slight changes at different time intervals after ingestion.

Interpretation of PQ concentration in urine

Seok et al. (29) described the diagnostic potential of a dithionite urine test, an indicator of the plasma PQ level, to determine the severity of PQ intoxication. The principle behind the dithionite test is that the absorbance of PQ changes as a result of the blue

color produced on reacting with dithionite. This chemical reaction is facilitated in an alkaline environment. Therefore, the first step of the dithionite test is addition of dithionite to a fresh urine sample in a colorless container followed by alkalization with a weak alkalizing agent such as sodium bicarbonate. The lowest detection level of PQ by high-performance liquid chromatography (HPLC) is 0.01 µg/mL, and by the dithionite PQ detection test, approximately 1 µg/mL. Despite its lower sensitivity, the dithionite test is a useful bedside screening tool for PQ intoxication because of its convenience and reproducibility.

A Foley catheter should be placed and the bladder should be fully emptied on immediate arrival of a PQ intoxicated patient, at the emergency room. This urine sample represents urine production over the previous several hours. Dithionite urine test results from the first sample represent the average blood PQ levels during the previous several hours. A second urine sample is collected after the first urine collection. The result of the dithionite test from the second urine sample represents the current blood PQ level. An observation of higher levels in the first urine sample than the second urine sample can be interpreted as decreasing serum PQ levels from the initial levels prior to patient hospitalization.

Sequential dithionite urine tests are conducted every 3-4 hr after the second dithionite urine test, until the results are negative. The time to achieve a negative urine dithionite test is a reliable marker for predicting mortality and/or essential organ failure (29). The sensitivity and specificity for mortality are 71.4% and 75.0%, respectively with a cut-off value of 34.5 hr for the time to negative conversion of the urine dithionite test. The incidence of acute kidney injury and respiratory failure with a time > 34.5 hr is 100% and 85.0%, respectively.

Evaluation of PQ-induced lung injury by imaging

A simple chest radiograph has poor sensitivity and specificity for evaluating PQ-induced lung injury. We recommend high-resolution computed tomography (HRCT) of the lungs on day 7 post-PQ ingestion. We find HRCT as a best modality in fully evaluating the extent of acute PQ lung injury (Fig. 3) (30-32).

The initial pathology of the lung is an inflammation of the alveoli, *i.e.* alveolitis, which presents as ground glass opacity (GGO) signal on HRCT imaging (30). The predilection site of GGO is the sub-pleural area. A GGO area > 50% of the total lung volume is usually fatal, but all surviving patients have a GGO area < 20% of the total lung volume on HRCT imaging at 7 days post-PQ ingestion (33).

When a patient hesitantly ingests a large amount of undiluted PQ (> 100 mL of 20% PQ) with multiple sips, there is a high chance of developing esophageal rupture and pneumomediastinum, with fatal consequences. GGO lesions progress to fibrosis after 2-3 weeks. Fibrosis is a progressive process, usually stopped within one month (34).

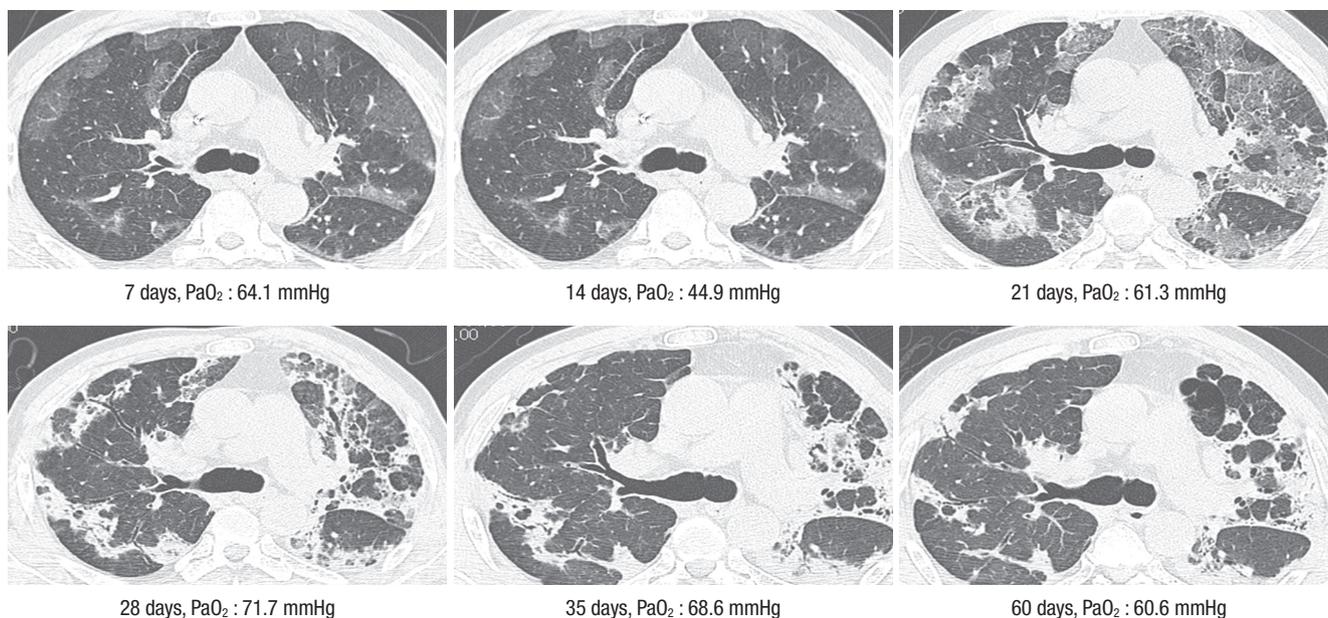


Fig. 3. Sequential measures on HRCT of the lung. A forty-three old woman intentionally ingested on paraquat in a suicide attempt. She presented to the hospital within 3 hr of ingestion. Serum paraquat level was 2.63 $\mu\text{g/mL}$. Note that the area of the lung lesion was not expanded in the sequential HRCT. The process of lung fibrosis completes by one month.

Evaluation of PQ-induced lung injury by arterial blood gas analysis (aBGA)

Tachypnea with low PaCO_2 indicates the progression of hypoxia. PaO_2 decreases progressively as the PQ induced lung injury proceeds. Notably, in cases of $\text{PaO}_2 < 60$ mmHg, there is a significant rise in mortality. Pulmonary fibrosis develops in the later stage of lung injury. A pulmonary function test after PQ induced lung injury can demonstrate the restriction of lung volume, but the diffusion capacity of the remnant lung remains intact (34).

TREATMENT

Treatment strategy of acute PQ intoxication

The guidelines for developing a new treatment modality for PQ intoxication are derived from well-designed and controlled clinical trials. However, because of the high mortality rate (80%-90%) and ethical problems, it is hard to perform a well controlled clinical trial with PQ. Based on our clinical experience and previous reports (35), we propose the following as a baseline to develop an effective treatment strategy:

- 1) There are some treatable sub-groups in which PQ lung injury progression can be halted by intensive treatment modalities,
- 2) Early initiation of treatment is the most important factor,
- and 3) Renal protection is the cornerstone of early treatment.

Identification of treatable cases of acute PQ intoxication

Proudfoot et al. (36) have reported that patients with plasma PQ levels $< 2.0, 0.6, 0.3, 0.16,$ and 0.1 $\mu\text{g/mL}$ at 4, 6, 10, 16, and

24 hr post-PQ ingestion, respectively, are most likely to survive. However, even with these predictive guidelines, it is difficult to determine which patients will survive in a clinical setting, because some patients with low PQ levels nevertheless die.

When the plasma PQ levels of 375 patients are plotted against the time lag since PQ ingestion, the highest PQ level of survivors for a given time is 3.44 $\mu\text{g/mL}$ at 2 hr, and then gradually decreases to 2.64 $\mu\text{g/mL}$ at 3 hr, 1.75 $\mu\text{g/mL}$ at 4 hr, 1.31 $\mu\text{g/mL}$ at 5 hr, 1.2 $\mu\text{g/mL}$ at 8 hr, and 0.16 $\mu\text{g/mL}$ at 24 hr from an earlier report (37). For non-survivors, the lowest PQ level is 0.92 $\mu\text{g/mL}$ at 2 hr, and then decreased to 0.54 $\mu\text{g/mL}$ at 4 hr, 0.12 $\mu\text{g/mL}$ at 5 hr, 0.02 $\mu\text{g/mL}$ at 12 hr, and 0.01 $\mu\text{g/mL}$ at 24 hr (Fig. 4) (37). We can obtain two connecting lines, connecting the highest PQ level for the survivors and the lowest PQ level among the non-survivors each at a given time. Patients whose PQ levels fall between these two lines at a given time (gray zone in Fig. 2) are indicated for intensive therapy. These are so-called "treatable patients".

Extracorporeal elimination

The best modality on extra-corporeal elimination of PQ is hemoperfusion (HP). The reduction rate of plasma PQ levels by HP is higher than hemodialysis (HD) (9).

The efficacy of HP in clearing serum PQ depends on the function of the HP cartridge, blood flow, and plasma levels. The reduction rate of PQ by HP is 67%-83% in 3 hr, which shows high efficacy throughout the HP in all subjects, according to a report by Hong et al. (9). In light of an almost constant reduction rate and a fixed value for blood flow, the main factor contributing to

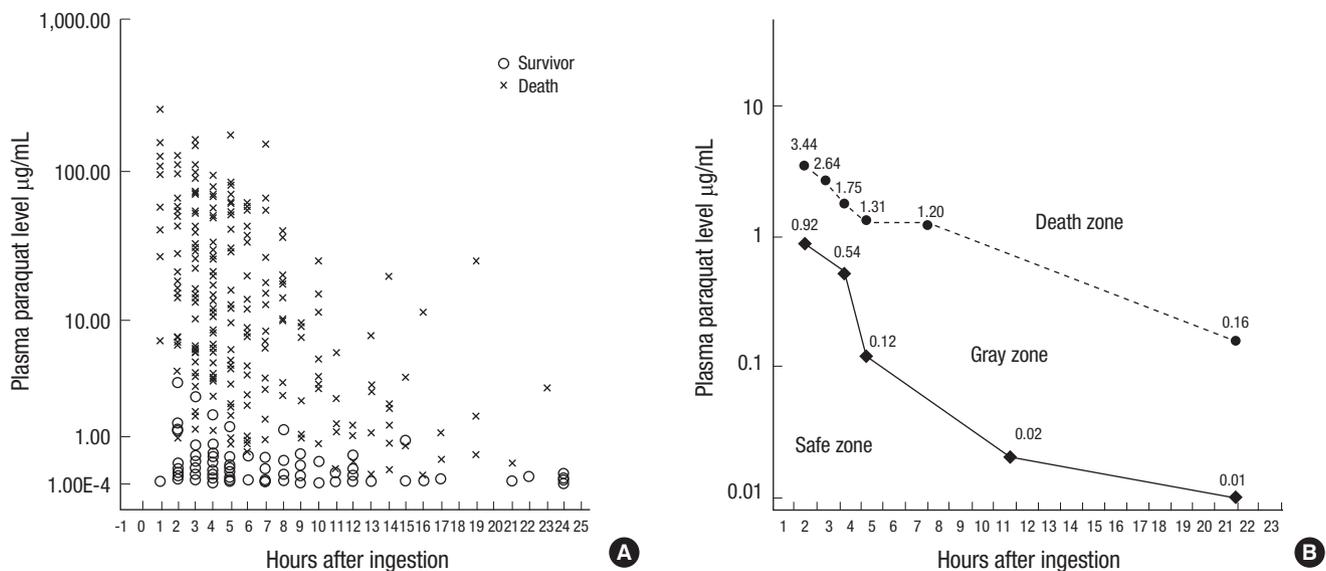


Fig. 4. Identification of cases with acute PQ intoxication those are treatable. The upper and lower limit of paraquat levels among survivors and non-survivors among 375 patients with acute paraquat intoxication. These levels can be divided into a dead zone (above the upper line, no survivors), gray zone (between the two lines; uncertain final outcome), and a safe zone (below the lower line, no deaths).

the efficacy of HP for PQ elimination appears to be the PQ plasma level. A linear correlation exists between the amount of PQ eliminated and the plasma PQ levels, suggestive that the initiation of HP as early as the PQ level reaches its peak level, would be the most effective way to eliminate PQ from the body.

The high incidence of AKI after PQ intoxication is challenging to renal elimination of PQ. The kidney can remove more PQ than HP, and is very effective when renal function is normal. However, once renal function declines, so does the ability to clear PQ. Therefore, the efficacy of the kidneys and HP in elimination of serum PQ varies depending on a number of factors. The kidney is very effective in clearing PQ but is vulnerable to PQ injury. Deterioration of renal function is commonly observed at the early stage of intoxication especially when the PQ level is high. Therefore, renal protection is critical during the early treatment of PQ intoxication.

The elimination of PQ during HP is also limited by blood flow dynamics, which can be regulated by using a jugular venous catheter at a rate of 200-300 mL/min in adults. Keeping in mind both the large PQ distribution volume of 1.0-1.5 L/kg body weight and the very rapid progression of ROS injury on PQ intoxication, PQ elimination must be carried out as soon as possible with high blood flow. Early HP is a required lifesaving treatment in PQ intoxicated patients, especially in cases of early kidney failure or high PQ plasma levels. The clinical effect of HP is below the theoretical expectation; it is unfortunate that the HP clearance is inferior compared to the renal clearance and the volume of distribution of PQ is large.

Background for antioxidant therapy

There are neither true pharmacological antagonists, nor chelat-

ing agents against PQ to date. However since PQ induces its toxic effects via oxidative stress-mediated mechanisms, innovations in management of PQ poisoning are directed towards antioxidant administration.

Depletion of glutathione (GSH) enhances PQ toxicity as observed by Bus et al. (38). Hagen et al. (39) and Brown et al. (40) show that the addition of GSH to type-II alveolar cells protects against PQ toxicity. Furthermore, the evidence that GSH peroxidase plays a key role in protecting animals against PQ toxicity is demonstrated on transgenic mice in which deletion of this enzyme enhances toxicity while addition of it affords some protection (41).

GSH provides the reducing equivalent for the reduction of hydrogen peroxide to H₂O, and is oxidized to its disulfide (GSSG). Most of the GSSG is immediately reduced back to GSH through a GSH reductase with the cofactor NADH.

N-acetyl-L-cysteine (NAC) is a precursor of GSH. Hoffer et al. (42) incubated NAC with type-II alveolar cells, and showed an enhanced GSH content and prevention of PQ induced cytotoxicity.

Optimal dose of anti-oxidants

A tremendous amount of ROS is produced immediately after acute PQ intoxication (Fig. 5). Therefore, antioxidants should be administered intravenously. NAC (43, 44) and GSH (45) are commonly used antioxidants, which are safe even at high dosage. In vitro, NAC suppresses ROS in a dose-dependent manner; 10 µM NAC suppresses approximately 50% of the ROS produced by PQ (43). Owing to a lack of pharmacokinetic data, the optimal dose is unknown, but in our experience, 50-70 mg/kg of NAC can be safely administered intravenously.

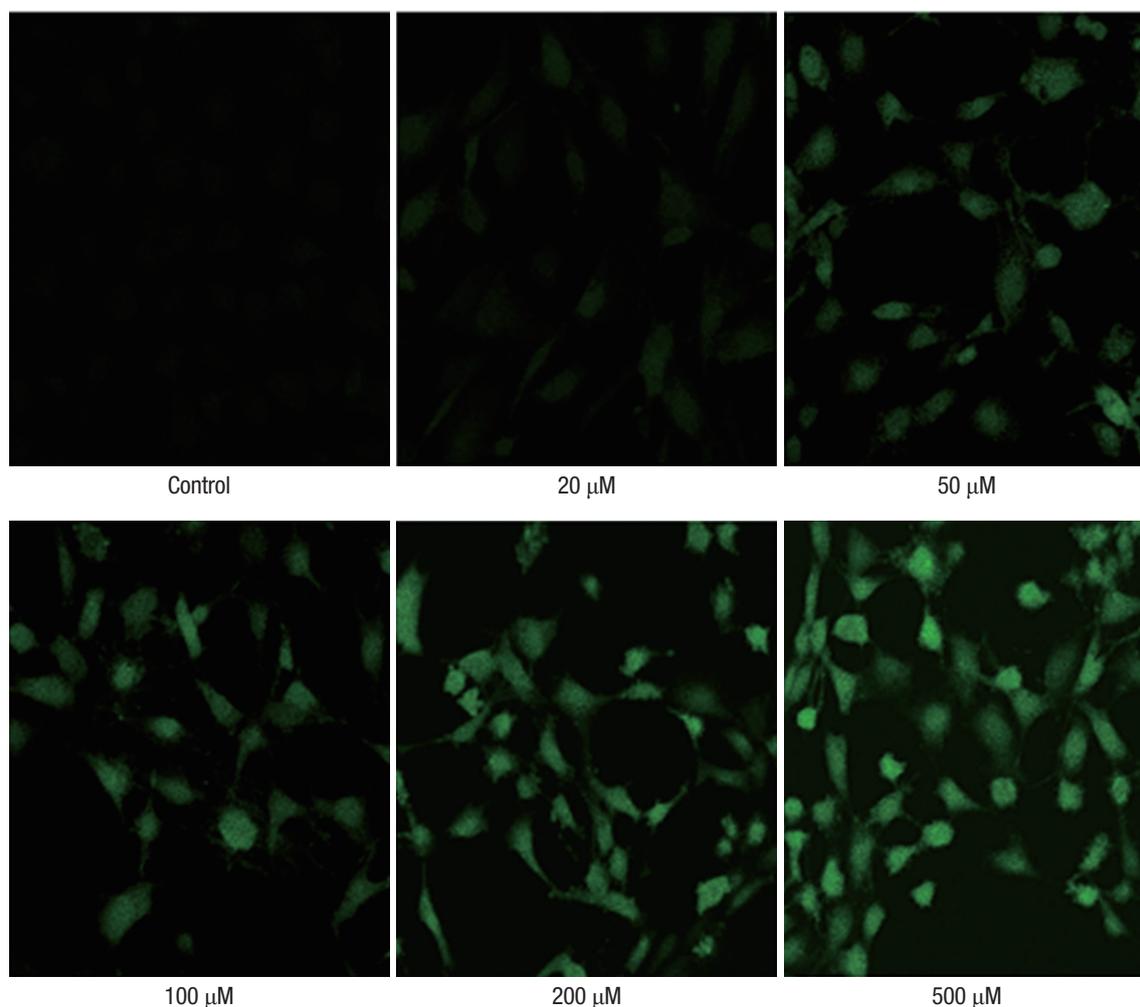


Fig. 5. Production of reactive oxygen species (ROS) by PQ treatment in a dose-dependent manner in Swiss 3T3. After 40 min incubation of the cells with 20-500 μM PQ, intracellular ROS generation was assessed with 2, 7-dichlorofluorescein and a laser scanning confocal microscope ($\times 400$).

The concentration required to suppress ROS levels by 50% is reported as 10 μM for cysteine and 50 μM for methionine. Based on metabolic kinetics analysis with the assumption that cysteine and methionine are metabolites of GSH, the expected concentrations of cysteine and methionine are estimated to be $> 20 \mu\text{M}$ and 50 μM , respectively, when GSH is administered at 50 mg/kg body weight (Fig. 6) (45).

Limitation of anti-oxidants in acute PQ intoxication

Enzymatic antioxidant defense, provided by superoxide dismutase, catalase, and glutathione peroxidase, is the main cellular mechanism, but there is no way to increase the activity of these enzymes thus far (Table 2). PQ intoxication leads to ROS generation not in the extra-cellular space but in intra-cellular space, though we can observe some of the free radicals in the circulation; hydroxyl radical is initially formed in the intracellular environment but crosses the plasma membrane and diffuse out from the cell. Therefore, antioxidants that can enter the cells through the plasma membrane are ideal. It is unfortunate that

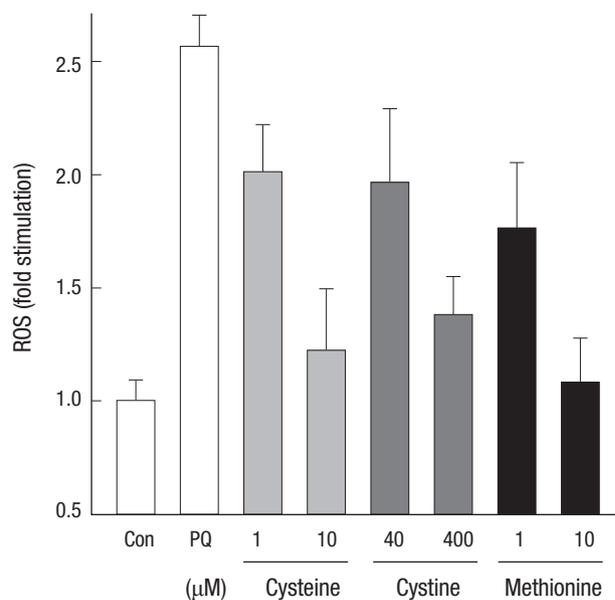


Fig. 6. Antioxidant effects of thiol-containing amino acid. Note that only 10 μM of both cysteine and methionine is enough to suppress ROS formation in PQ poisoning.

Table 2. Cellular antioxidant strategies

Type of action	Molecules
ROS scavenging	Glutathione Vitamin C & E N-acetylcysteine SOD
Prevention of RO generation	Glutathione peroxidase/reductase Control of iron Catalase
Free radical repair	Glutathione N-acetylcysteine Metallothionein

most of the antioxidants, such as protein thiol, ascorbic acid, and α -tocopherol have low plasma membrane permeability. The process, known as the redox cycle, is sustained by the extensive supply of electrons and oxygen in the lungs (46). Antioxidants exert their antioxidant effect with free radicals, forming a complex with 1:1 stoichiometry. Therefore theoretically, optimal dose of anti-oxidants should be administered without cessation, until the redox cycle is interrupted. However, it is impossible to identify the optimal dose of antioxidant at the patients' bed side and it is difficult to administer high dose of antioxidants for several days without cessation.

Back ground of cytotoxic drug use

The rationale for cytotoxic drug use in treatment of PQ intoxication is that PQ induces inflammatory cell infiltration in the lung (8, 47-49). Cyclophosphamide is recommended for a short period on the initial 3-4 days. This is because the cardinal pathologic changes are completed within the first few days of intoxication, and prolonged use of these drugs may be complicated by opportunistic infection. However, until now, cyclophosphamide has inconsistent treatment effects for PQ intoxication; some reports show a positive effect (8, 50), while others do a negative effect (48). We believe that cyclophosphamide administration is more or less effective in the suppression of the inflammatory cell infiltration in the PQ lung. However, to achieve this effect, early administration of cyclophosphamide, at least 2-3 hr after PQ exposure, is important because cyclophosphamide is not effective once cells have already infiltrated (from our animal experiment, data not published). In reality, most patients arrive several hours post-PQ ingestion, probably, leading to a limited clinical effect of cyclophosphamide.

Back ground of steroid therapy

Prednisone, a synthetic corticosteroid is particularly effective as an immunosuppressant drug and is used to treat certain inflammatory diseases. It has likewise become a treatment regimen for patients with acute PQ intoxication. In addition to this general anti-inflammatory effect, our recent in vitro experiment shows that methylprednisolone attenuates PQ-induced cell cy-

totoxicity via induction of efflux transporter, P-glycoprotein expression (51). However, as with cyclophosphamide, there is no current consensus on the effect of steroid therapy in PQ intoxication.

Iron chelating therapy

Iron chelating therapy is useful against the over-accumulation of iron and is expected to reduce oxidative stress, resulting from the Fenton reaction and Haber-Weiss reaction (52, 53).

The primary use of chelating has been via iron overload transfusion (54). However, coupled with advances in tissue iron quantitation (55, 56), there is tremendous promise of an individually tailored approach to chelating, and subsequent reduction in morbidity and mortality. With this background, appropriate iron chelating would be an effective treatment modality in patients with acute PQ intoxication. However, currently there is no randomized controlled study to prove the effect of iron chelating therapy in acute PQ intoxication.

PROGNOSIS

Proposed treatment modality and clinical outcome

As mentioned above, treatment of PQ intoxication is based on 3 main tenets: some patients are treatable; early treatment initiation is necessary; renal protection is the cornerstone of early treatment. We recommend the following treatment modality: Extracorporeal elimination (HP), Intravenous antioxidant administration (NAC, GSH), Diuresis with fluid, and Short-term cytotoxic drug treatment (steroid pulse therapy, cyclophosphamide).

We find the following clinical outcomes on using this treatment modality. The survival rate varies according to the ingestion amount: < 10 mL, almost 100%; 10-20 mL, 80%-90%; 20-40 mL, 50%-60%; 40-50 mL, < 10%; > 60 mL, < 1%. The cause of death also varies in accordance with the amount of PQ ingested. Individuals in the 'sudden death' group (death within the first 24 hr) ingest > 100 mL, and cardiac arrest is the most frequent cause of death. Individuals in the 'rapid progress' group (death within 7 days) ingest 50-100 mL, and the most common cause of death is respiratory failure, with or without acute renal failure. Finally, individuals in the 'slow progress' group (death within 2-4 weeks) ingests 15-40 mL, and death occurs in all due to respiratory failure.

A patient is defined as a 'survivor' if he or she survives > 3 months after PQ ingestion with stable vital signs and stable lung function. It is extremely rare for a patient to expire 3 months later, secondary to PQ related problems.

CONCLUSION

In conclusion, treatment of PQ intoxication is not well codified.

To date, most of the studies are prevalently performed in vitro or in animal models. However, based on our rich clinical experience, we believe a treatable group exists on PQ intoxication. Further studies are required to focus on improved treatment efficiency and on an expanded range of treatment groups.

DISCLOSURE

The authors declare no conflicts of interest, and the authors alone are responsible for the content and writing of the article.

ORCID

Hyo-wook Gil <http://orcid.org/0000-0003-2550-2739>

Sae-Yong Hong <http://orcid.org/0000-0003-1119-4456>

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