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ATTENUATION OF PARAQUAT TOXICITY IN MICE

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ABSTRACT

Paraquat (PQ) is a widely used herbicide. However, a large number of cases of accidental or suicidal poisoning from PQ has been reported. Membrane damage induced by lipid peroxidation, inactivation of protein or damage to DNA by radical formation have been suggested as toxicity mechanisms of PQ. In the present work, the effects of atropine, propranolol, procainamide and dipyridamole on PQ-induced intoxication have been studied.

Groups of male albino mice were used under standard conditions. All the drugs were injected intraperitoneally in different doses. The results indicated that administration of PQ (40 mg/kg, i.p.) increased the death rate of animals (77%) during 3 days, whereas a dose of 20 mg/kg of PQ only decreased the lung tissue total protein and glutathione (GSH) content. This poison also produced serious histopathologic changes in lung tissue. Administration of propranolol (10 and 20 mg/kg), procainamide (20 and 40 mg/kg), dipyridamole (30 and 60 mg/kg) and atropine (5 and 10 mg/kg) decreased the PQ (40 mg/kg)-induced mortality rate in the pre- or post-treatment regimens. These drugs were also effective in reversing the PQ-induced alteration in the lung tissue protein and GSH content, however the pathological findings attenuated in the treated animals. Although the exact mechanism of these drugs against paraquat-toxicity in mice is still unknown, it appears that some of the drugs used may be effective in reversing PQ-induced poisoning in mice and possibly their effects are related to the inhibition of membrane lipid peroxidation via different mechanisms.

Keywords: Paraquat, atropine, propranolol, procainamide, dipyridamole, mice.

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INTRODUCTION

Paraquat (1,1-dimethyl-4-4-bipyridylium chloride, PQ) is a widely used herbicide, known to cause toxicity in humans and animals.[9,17,19,26] However, a large number of cases of accidental or suicidal poisoning from PQ has been reported in several studies.[8,20,26] It has been demonstrated that PQ undergoes a one electron reduction by the flavoenzyme NADPH cytochrome P450 reductase. Thereby, a free radical is formed which can react rapidly with molecular oxygen to produce the superoxide anion radical with the regeneration of PQ intoxication.[12,13,16,21,30] It has been suggested that membrane damage induced by lipid peroxidation, inactivation of protein or damage to DNA may subsequently lead to cell death.[23,24]

Death is usually due to progressive respiratory failure, although PQ is a multi-system poison capable of causing

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Table I. Effect of pretreatment of propranolol, procainamide, dipyridamole and atropine on paraquat (PQ)-induced lethality.

Drug	Dosage mg/kg	% Mortality (day)		
		1	2	3
Saline	10 mL/kg	44	66	77
Propranolol	10	11**	22**	55
Propranolol	20	0.0**	11**	66
Procainamide	20	33	44	55
Procainamide	40	11**	11**	33*
Dipyridamole	30	11**	33*	55
Dipyridamole	60	22*	44*	66
Atropine	5	44	55	100
Atropine	10	11**	33*	55*

Groups of 9 mice were treated with saline or drugs 1 hr before PQ (40 mg, i.p.) administration. The mortality rate was determined at 1, 2 and 3 days after paraquat administration.

* $p<0.05$, ** $p<0.01$, significantly different from saline treated animals.

Table II. Effect of post-treatment of propranolol, procainamide, dipyridamole and atropine on paraquat (PQ)-induced lethality.

Drug	Dosage mg/kg	% Mortality (day)		
		1	2	3
Saline	10 mL/kg	44	66	77
Propranolol	20	33	44	88
Procainamide	40	11**	33*	77
Dipyridamole	60	11**	66	66
Atropine	10	22	55	66

Groups of 9 mice were treated with saline or drugs 1 hr before PQ (40 mg, i.p.) administration. The mortality rate was determined at 1, 2 and 3 days after paraquat administration.

* $p<0.05$, ** $p<0.01$, significantly different from saline treated animals.

injury to the kidneys, liver, heart, adrenals and central nervous system.[2,5,8,20]

It has been shown that corticosteroids in high concentration reduce the increased lung vascular permeability in some models of acute lung injury[13] and antioxidants such as superoxide dismutase, ascorbic acid and vitamin E prevent lipid peroxidation, but not death in mice.[3,4,11,19,22,28]

Other agents including dimethyl thiourea,[9] metal chelators[1,30] and various adsorbents[27,28] have also been investigated, but they were only partially effective. It is generally agreed that lipid membrane damage is a critical event in PQ toxicity. There is evidence that perturbation in calcium homeostasis of the cell plays an important role in cell death.[10] In the present work, the effects of some possible protective drugs that are able to change intracellular calcium concentrations and have membrane stability properties have been investigated. The influence of propranolol, procainamide, dipyridamole and atropine on the PQ-induced lethality rate, changes in lung glutathione (GSH), and total protein content in mice have been studied.

MATERIALS AND METHODS

Male albino mice (20-25g) were housed under standard conditions of food, water, constant temperature (22±1°C) and a 12 hr light/12 hr dark cycle. Food and water were continuously available. Each animal was only used once.

Drugs

The chemicals used were paraquat dichloride (1-1-dimethyl-4-4-bipyridylium dichloride, PQ) (Sigma), propranolol HCl (ICI), procainamide HCl (Sigma), dipyridamole (Sigma), and atropine sulfate (Merck).

All other reagents were purchased from Sigma Chemical

Table III. Effect of multiple dose propranolol, procainamide, dipyridamole and atropine on paraquat (PQ)-induced lethality.

Drug	Dosage		% Mortality (day)		
	1 hr	24 hrs	1	2	3
Saline	10	10	44	66	77
Propranolol	20	10	11**	22*	66
Procainamide	20	40	11**	22*	33*
Dipyridamole	60	30	22*	33*	33*
Atropine	10	5	22*	55	77

Groups of 9 mice were treated with saline or drugs 1 hr prior and 24 hrs after PQ (40 mg, i.p.) administration. The mortality rate was determined at 1, 2 and 3 days after paraquat administration.
* $p<0.05$, ** $p<0.01$, significantly different from saline treated animals.

Table IV. Effect of propranolol, procainamide, dipyridamole and atropine on paraquat-induced changes in lung tissue protein, GSH concentration and lung weight/body weight ratio (LW/BW).

Drug	Dosage (mg/kg)	Protein (μg/mg)	GSH conc. (nmol/mg)	LW/BW ratio
Saline	10 mL/kg	3.5 ± 0.6	2.09 ± 0.26	0.82 ± 0.12
Paraquat	20	2.0 ± 0.2††	0.23 ± 0.18††	0.88 ± 0.08†
Propranolol	20	2.1 ± 0.3	0.73 ± 0.05*	0.80 ± 0.14
Procainamide	40	2.2 ± 0.2	0.89 ± 0.02*	0.78 ± 0.13
Dipyridamole	60	2.6 ± 0.3*	0.73 ± 0.03*	0.86 ± 0.08
Atropine	10	2.7 ± 0.6*	0.79 ± 0.06*	0.99 ± 0.08

The animals were pretreated by saline or drugs 1 hr before paraquat (20 mg/kg, i.p.) administration. LW/BW ratio, lung tissue protein and GSH determinations were done 48 hr after paraquat injection. Each point is the mean ± SEM of 9 mice.
* $p<0.05$, significantly different from paraquat-treated animals.
† $p<0.05$, †† $p<0.01$, significantly different from control saline-treated animals.

Company. The drugs were dissolved in physiological saline solution and given in a volume of 10 mL/kg. All the drugs were injected intraperitoneally (i.p.). Groups of 9 mice were chosen randomly. The mice were treated with different doses of paraquat (10, 20, and 40 mg/kg i.p.) or saline. The chosen toxic dose of PQ for mortality studies caused a death rate of 77% within 3 days. The toxic dose of 20 mg/kg of PQ, that had not caused mortality within 2 days (44% up to 3 days) was chosen for total lung protein and glutathione (GSH) determination. The doses of the drugs were chosen from previous studies.[30,33-35]

Mortality studies

For evaluation of death rate, animals were divided into 3 groups:

The first group of animals received different doses of propranolol (20 and 10 mg/kg), procainamide (20 and 40 mg/kg), dipyridamole (30 and 60 mg/kg) and atropine (5 and 10 mg/kg) 1 hr prior to PQ (40 mg/kg) administration.

The second group received propranolol (20 mg/kg), procainamide (40 mg/kg), dipyridamole (60 mg/kg) and atropine (10 mg/kg) 1 hr after PQ (40 mg/kg) administration.

The third group of animals were injected 1 hr before and 24 hrs after PQ (40 mg/kg) administration with propranolol (20 and 10 mg/kg), procainamide (40 and 20 mg/kg), dipyridamole (60 and 30 mg/kg) and atropine (10 and 5 mg/kg).

The mortality rate of the animals was observed 1, 2 and 3 days after PQ injection.

Determination of total lung protein and glutathione (GSH)

The effects of the drugs on PQ-induced alteration in the lung weight/body weight ratio, protein and GSH content of lung tissues were measured as follows.

Animals were treated with either saline (10 mL/kg, i.p.) or propranolol (20 mg/kg), procainamide (40 mg/kg), dipyridamole (60 mg/kg) and atropine (10 mg/kg) 1 hr before (pretreatment) or 1 hr after (post-treatment) PQ (20 mg/kg, i.p.) administration.

For determination of lung tissue protein and GSH, lungs were removed from the animals immediately after

decapitation (48 hrs after PQ administration) and were rinsed with ice-cold saline to remove excess blood.

All subsequent steps were carried out at 0-4°C. 100 mg of lung sample was homogenized with an electrical homogenizer in a sufficient volume of cold, normal saline. The homogenates were centrifuged at 9000 g for 10 min. Protein determinations were made by the method of Lowry et al.[15] using bovine serum albumin (BSA) as the standard, and GSH in pulmonary homogenates was determined as described by Suntres et al.[30]

Histopathologic examinations

The histopathologic examinations were based on methods of Shibamoto and Kobayashi.[24] Lung samples were collected randomly from different groups of animals that received PQ or PQ in addition to the drugs for histopathology. The tissues were fixed in 10% formalin, and processed by the histopathological technique. The tissue sections were stained with hematoxylin and eosin. The slides were examined by light microscopy.

Statistical analysis

Mortality rate data were analyzed by Fisher's exact probability test. ANOVA followed by Newmans - Keuls test were used to evaluate differences between control and treated groups in other experiments.

RESULTS

Mortality studies

Intraperitoneal administration of PQ, 40 mg/kg, but not 20 mg/kg, caused the mortality of animals within three days after injection. The mortality rate during this period was 77%.

Drug pretreatment

Intraperitoneal pretreatment of animals with propranolol (10 and 20 mg/kg), procainamide (20 and 40 mg/kg), dipyridamole (30 and 60 mg/kg) or atropine (5 and 10 mg/kg) 1 hr prior to PQ injection decreased the mortality rate during the 1st and 2nd day, but not the 3rd day after PQ administration (Table I). Higher doses of the drugs were more effective and were chosen for the following studies.

Drug post-treatment

Treatment of the animals with propranolol (20 mg/kg) and procainamide (40 mg/kg) 1 hr after PQ (40 mg/kg) administration decreased the mortality rate during the 1st and 2nd day, but not the 3rd day after PQ injection. Administration of dipyridamole (60 mg/kg) and atropine (10 mg/kg) only decreased the mortality rate of mice on the 1st day.

Mice received two injections of propranolol (20 and 10 mg/kg), procainamide (40 and 20 mg/kg), dipyridamole (60 and 30 mg/kg) or atropine (10 and 5 mg/kg) 1 hr and 24 hrs after PQ (40 mg/kg) injection, respectively. Results indicated that procainamide and dipyridamole decreased the mortality rate induced by PQ during the 3 days after PQ injection (Table I).

Propranolol decreased the PQ-induced death rate on the 1st and 2nd day of PQ administration, but atropine elicited the response only during the 1st day (Table II).

Effect of drugs on lung tissue protein and GSH contents

Pretreatment of the animals with PQ (20 mg/kg) decreased lung tissue protein and GSH concentration as compared with the saline control group. Pretreatment of mice with propranolol (20 mg/kg), procainamide (40 mg/kg), dipyridamole (60 mg/kg) or atropine (10 mg/kg) 1 hr before PQ (20 mg/kg) administration increased the concentration of lung tissue protein and GSH as compared with the PQ-administered group.

PQ by itself increased the ratio of lung weight/body weight as compared with saline controls. However, the drugs used did not elicit any response in this respect (Table III).

Effect of drugs on lung histopathology

Microscopic examination of the lung tissue collected from the animals revealed that PQ at a dose of 40 mg/kg and 20 mg/kg produced alveolar edema and hemorrhage. Moreover, infiltrations of lymphocytes, plasma cells and macrophages were observed. This was more pronounced on the 2nd day and with the high dose of PQ.

The above pathological findings were less serious in the animals that were treated with the different regimens of the drugs.

DISCUSSION

Intraperitoneal administration of the higher dose of PQ caused death of the animals (77%) and produced significant pathological changes in the lung tissue. Injection of the lower dose of PQ decreased the total protein and GSH content of mice lung tissue.

The obtained results are in agreement with other studies.[2,5,8,13,23] Several mechanisms have been proposed to explain the PQ responses in animals. One hypothesis suggests that the toxicity may be due to the peroxidative destruction of cell membrane lipids caused by reactive oxygen derivatives formed during the redox cycling of the parent PQ.[1,21] Membrane damage induced by lipid peroxidation, inactivation of protein or damage to DNA may subsequently lead to the disturbance of calcium homeostasis and cell death.[10,21,23] Different protocols for preventing or decreasing PQ-induced toxicity were proposed, although the effective

agents(s) have not yet been introduced.[3,4,11,19,22,28]

In the present study, pretreatment with propranolol and procainamide attenuated PQ toxicity in mice. However, administration of dipyridamole and atropine also decreased PQ toxicity, but their effect was not as significant.

Calcium plays an important role in cellular function, and perturbation in calcium homeostasis is believed to play an important role in necrotic cell death.[6,10] Propranolol, procainamide and dipyridamole, with different mechanisms, are able to change the intracellular calcium concentration.[18] There may be a possibility that propranolol and procainamide, via stabilization of the membrane, prevent the disturbance of calcium homeostasis and thereby decrease PQ toxicity.

It is also suggested that propranolol competes with PQ for cellular binding sites,[7] therefore propranolol might increase PQ's urinary excretion if less PQ binds to the tissues. Hence, by using this mechanism PQ toxicity may be decreased.

It has been shown that PQ in pulmonary endothelial cells inhibits acetylcholinesterase activity[29] and may induce its toxic effect indirectly through this phenomenon.[2,28] Therefore, it is possible that atropine antagonizes the cholinergic hyperactivity produced by PQ. According to this, the effect of atropine on PQ-induced mortality is related to the anticholinergic properties of the drug.

Propranolol, procainamide, dipyridamole and atropine inhibit the PQ-decreased GSH and protein content in lung tissue. The depletion of the cell from GSH induced by the different chemical agents favors lipid peroxidation and cell damage.[6] There is evidence to suggest that PQ induces its toxic effects in the lung at least partly by depleting glutathione compounds. Our results may indicate that the drugs employed protect against PQ-induced oxidation of GSH, which has an important role in detoxification of lipid peroxides.[28]

The results of the present study showed that PQ-induced changes in the lung weight/body weight ratio have not been altered by the drugs. It may be postulated that the mechanisms of effectiveness of the drugs on the mortality rate, lung GSH and protein contents are not responsible for changes in the lung and body weight.

In the present work, the PQ-induced histopathological changes have not been examined precisely. However, the findings were in agreement with those of other investigators.[13,14] The protective effects of the drugs on PQ-induced morphological changes in the lung correspond well with mortality and biochemical changes seen in mice. However, more work may be required to prove the exact mechanism(s) involved. The protective responses of the drugs employed may be valuable in practice in the future.

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