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.


INTRODUCTION

Paraquat (1,1-dimethyl-4,4-bipyridyl chloride, PQ) is a widely used herbicide, known to cause toxicity in humans and animals. However, a large number of cases of accidental or suicidal poisoning from PQ has been reported in several studies. 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. It has been suggested that membrane damage induced by lipid peroxidation, inactivation of protein or damage to DNA may subsequently lead to cell death.

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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage mg/kg</th>
<th>% Mortality (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Saline</td>
<td>10 mL/kg</td>
<td>44</td>
</tr>
<tr>
<td>Propranolol</td>
<td>10</td>
<td>11**</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.0**</td>
</tr>
<tr>
<td>Procainamide</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>Procainamide</td>
<td>40</td>
<td>11**</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>30</td>
<td>11**</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>60</td>
<td>22*</td>
</tr>
<tr>
<td>Atropine</td>
<td>5</td>
<td>44</td>
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<tr>
<td>Atropine</td>
<td>10</td>
<td>11**</td>
</tr>
</tbody>
</table>

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.

<table>
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<tr>
<th>Drug</th>
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<td>60</td>
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</tr>
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<td>10</td>
<td>22</td>
</tr>
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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 injury13 and antioxidants such as superoxide dismutase, ascorbic acid and vitamin E prevent lipid peroxidation, but not death in mice.3,4,11,19,28

Other agents including dimethyl thiourea,9 metal chelators13,30 and various adsorbents7,28 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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
<th>1 hr</th>
<th>24 hrs</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>10 mL/kg</td>
<td>3.5 ± 0.6</td>
<td>2.09 ± 0.26</td>
<td>0.82 ± 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraquat</td>
<td>20 mg/kg</td>
<td>2.0 ± 0.2††</td>
<td>0.23 ± 0.18††</td>
<td>0.88 ± 0.08†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>20 mg/kg</td>
<td>2.1 ± 0.3</td>
<td>0.73 ± 0.05*</td>
<td>0.80 ± 0.14</td>
<td></td>
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</tr>
<tr>
<td>Procainamide</td>
<td>40 mg/kg</td>
<td>2.2 ± 0.2</td>
<td>0.89 ± 0.02*</td>
<td>0.78 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>60 mg/kg</td>
<td>2.6 ± 0.3*</td>
<td>0.73 ± 0.03*</td>
<td>0.86 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atropine</td>
<td>10 mg/kg</td>
<td>2.7 ± 0.6*</td>
<td>0.79 ± 0.06*</td>
<td>0.99 ± 0.08</td>
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</tbody>
</table>

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 (40 mg/kg, i.p.) administration.

For determination of lung tissue protein and GSH, lungs were removed from the animals immediately after PQ administration.
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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 
Determined protein concentrations were made by the method of 
KOBAYASHI.24 Lung samples were collected randomly from different groups of animals that received PQ 
and 2nd day, but not the 3rd day after PQ injection.

Different protocols for preventing or decreasing 
PQ-induced toxicity were proposed, although the effective 
1st day.

10 mg/kg) only decreased the mortality rate of mice on the 
administration (fable I). Higher doses of the drugs were 
lower dose of PQ decreased the total protein and GSH 
20 mg/kg), procainamide (40 and 20 mg/kg), dipyridamole (60 
mutual inactivation of protein or damage to DNA may subsequently 
death.0,21,23 Different protocols for preventing or decreasing 
membrane lipids caused by reactive oxygen derivatives 
lead to the disturbance of calcium homeostasis and 
alveolar edema and hemorrhage. Moreover, infiltrations of lymphocytes, plasma cells and 
from the animals revealed that PQ at a dose of 40 mg/kg and 
20 mg/kg produced alveolar edema and hemorrhage. The obtained results are in agreement with other 
Effect of drugs on lung histopathology 
Microscopic examination of the lung tissue collected 
and 2nd day of PQ administration, but atropine elicited 
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 
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 
Effect of drugs on lung tissue protein and GSH contents 
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.10,21,22 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 activity29 and may induce its toxic effect indirectly through this phenomenon.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.13,34 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.

ACKNOWLEDGEMENT

The authors wish to thank Dr. M. R. Zarrindast and Dr.

F. Ebrahimii for their editorial comments and Mrs. M. Haghbayan for typing this manuscript.

REFERENCES


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