Preventive Effect of Taurine against Acute Paraquat Intoxication in Beagles

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Abstract—The continuous infusion of taurine markedly protected against paraquat (PQ)-induced oliguria in beagles. Pharmacokinetic studies revealed that taurine infusion increased the blood concentration of PQ and reduced the net content in the kidney and, to a lesser extent, in the lung. The excretion of PQ into the urine was unaltered. The infusion of glycine did not have such effects.

Paraquat (PQ: 1,1'-dimethyl-4,4'-bipyridium chloride), a widely used herbicide, produces superoxide anions or other forms of reactive oxygen which stimulate lipid peroxidation, resulting in damage to biological membranes (1). On the other hand, taurine, an ubiquitous sulfur amino acid, has been shown to possess antioxidant effects (2, 3) and/or to suppress lipid peroxidation (4). Based on findings that taurine is present in high concentrations in tissues which have a considerable potential for producing oxidants and that taurine reacts with toxic oxidized chlorine to produce stable monochlorotaurine, Wright et al. (5) suggested that taurine may act as a general detoxifier. In the present study, we investigated whether taurine is able to protect against acute intoxication of PQ in beagles.

Female adult beagles (9–14 kg) were used, because the kinetic study on PQ suggested that the dog is a good model for studying the effects of PQ-induced renal failure in man (6). All substances were dissolved in distilled water. The pHs of 0.9% saline, 3.0% glycine (40 mmol/dl), 2.5% taurine and 5.0% taurine (40 mmol/dl) were 5.40–5.50, 5.80–6.10, 5.60–5.80 and 5.33–5.50, respectively. Each solution was infused into the radial vein at a rate of 0.85 ml/kg/min for 2 hr, one min prior to the injection of PQ (20 mg/kg, s.c.).

During the 2 hr infusion, the general condition of dogs injected with PQ was continuously observed and compared between animals given taurine, glycine and saline. During the time, urine was collected in a metabolic cage for determination of urinary volume and PQ excretion into the urine. Blood samples were taken into heparinized tubes at 1 and 2 hr following the PQ injection for measurement of PQ concentration. The dogs were anesthetized with pentobarbital (26 mg/kg, s.c.) and exsanguinated at 2 hr after the PQ injection for determination of PQ concentration in tissues and histological examinations. In the kidney, a transitional portion of the cortex and medulla was taken, since this part contains the glomerulus and tubules (7). For the lung, an inferior part was taken as PQ largely accumulated in this portion (8).

The PQ concentration was measured by the method of Knepil (9), which was slightly modified. Although this method was developed for plasma PQ analysis, it was found to be also useful for determination of tissue and urinary concentrations of PQ (10).
Briefly, after adding 10 volumes of distilled water to tubes containing tissues cut by a blade into small pieces, the tissues were homogenized using a Polytron (set 6) for 30 sec. An aliquot (2 ml) of homogenate was taken and added to a centrifuge tube containing 2 ml of solvent consisting of chloroform and ethanol (4:1, V/V) and 1.2 g of ammonium sulphate and well-mixed. The resulting homogenate was centrifuged at 1000 g for 10 min. The supernatant (1.4 ml) was then transferred to a tube, and 0.2 ml of alkaline dithionite solution was added and mixed. The absorbance of the solution was measured using a double beam spectrophotometer (Hitachi 150-20) at 396 nm. Standard solutions containing 0.3–3.0 mg PQ per liter were prepared by dissolving paraquat dichloride in tissue homogenates from an untreated beagle. The PQ added in a concentration of 0.5 μg/ml to a PQ-free plasma or tissue homogenate specimen from kidney and lung was recovered at the rate of 91.7±2.6 (n=6), 93.2±4.1 (n=4) and 89.3±3.5% (n=4), respectively. Urinary PQ concentration was measured similarly, and the recovery rate was found to be almost identical to that in plasma. In a preliminary experiment, spectrum analysis demonstrated no abnormal wave form in a mixed solution containing PQ and taurine, suggesting that they do not constitute a complex. Statistical analysis was conducted by analysis of variance.

All four beagles infused with 5.0% taurine and injected with 20 mg/kg of PQ showed a stable condition with tail up and flicking during the infusion, whereas all control dogs given 0.9% saline (n=5) or all animals given 3.0% glycine (n=4) were vomiting and the tail was down between the legs. The infusion of 2.5% taurine had also protective effects, to a lesser extent, against these symptoms. Urinary volume in beagles which had been infused with 5.0% taurine and injected with PQ was 4 times greater than that in animals given saline and the PQ injection (Fig. 1). The urinary volume in each taurine-treated animal was roughly equivalent to the infused volume, indicating that the fluid balance between the intake and output is well-maintained by 5.0% taurine. The effect of taurine was dose-dependent. On the other hand, the administration of a 3.0% glycine solution did not have a similar effect on urination.

Despite these beneficial effects of taurine, a significantly high level of PQ in the blood was obtained in the dogs infused with 5.0% taurine both at 1 and 2 hr following the administration of PQ (Table 1). The effect of taurine was also dose-dependent, while the effect of glycine did not reach a significant level. The actual concentration of PQ in tissues 2 hr after administration was variable in animals according to treatment. Since these values reflect the mixed PQ concentration in both tissue (T) and blood (B), the ratio (T/B) was taken as an indicator of the net tissue concentration. The gross concentration of PQ in the kidney in the 5.0% taurine-treated animals did not differ from that in the 0.9% saline-treated animals, although there was a tendency toward a decrease in the concentration. The ratio, on the other hand, was sharply reduced, com-
Table 1. Effect of taurine on paraquat concentration in blood, kidney and lung in beagles

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood 1 hr (µg/ml)</th>
<th>Blood 2 hr (µg/ml)</th>
<th>Kidney T/B ratio</th>
<th>Lung T/B ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PQ + 0.9% Saline</td>
<td>23.1 ± 1.4</td>
<td>11.2 ± 1.0</td>
<td>42.6 ± 9.2</td>
<td>13.6 ± 0.6</td>
</tr>
<tr>
<td>PQ + 3.0% Glycine</td>
<td>21.9 ± 2.7</td>
<td>14.9 ± 3.2</td>
<td>42.5 ± 8.1</td>
<td>19.5 ± 2.6</td>
</tr>
<tr>
<td>PQ + 2.5% Taurine</td>
<td>25.9 ± 1.6</td>
<td>16.2 ± 2.3</td>
<td>29.8 ± 2.4</td>
<td>21.8 ± 2.4</td>
</tr>
<tr>
<td>PQ + 5.0% Taurine</td>
<td>31.1 ± 3.2*</td>
<td>18.5 ± 1.8**</td>
<td>31.0 ± 1.8</td>
<td>16.4 ± 1.7</td>
</tr>
</tbody>
</table>

Each solution was infused into the radial vein (0.85 ml/kg/min), one min prior to the injection of PQ (20 mg/kg, s.c.). Venous blood was taken into heparinized tubes at 1 and 2 hr after the PQ injection. Two hours following the PQ injection, the dogs were anaesthetized with pentobarbital (26 mg/kg, s.c.) and exsanguinated. Data are expressed as means±S.E. PQ, paraquat; T, tissue concentration; B, blood concentration; n, number of animals. *P<0.05, **P<0.01.

pared with that in animals given saline or glycine, and the effect was dose-dependent (Table 1). In the lung, the gross concentration of PQ in the controls was about 3 times lower than that in the kidney. The gross accumulation of PQ in the lung appeared to be increased by glycine and taurine. However, there was no significant difference in the T/B ratio between animals given 5.0% taurine, or 3.0% glycine and 0.9% saline. It should be noted that the value of the ratio was below 1.0 in the animals treated with 5.0% taurine, indicating that the virtually accumulated PQ concentration in the lung was less than the level of circulating PQ. The total amount of excretion of PQ into urine was unchanged between animals infused for 2 hr with saline, glycine and taurine (Fig. 1).

Macroscopic examination showed that all the investigated organs including the kidney and lung in dogs treated with 5.0% taurine and sacrificed 2 hr after the injection of PQ (20 mg/kg, s.c.) were hyperemic, while those in animals with 0.9% saline plus PQ were ischemic. Histological studies revealed only mild cellular swelling of renal tubular epithelial cells, which is considered to be a reversible injury, in dogs treated with 5.0% taurine plus PQ, but not in control animals with 0.9% saline plus PQ. In the lung, only slight cellular swelling of alveolar epithelial cells was observed in both groups of animals.

In the present study, we demonstrated that beagles infused with taurine maintained a good general condition and normal urination in spite of a high concentration of PQ in the blood, whereas the condition of animals infused with saline or glycine deteriorated markedly as evidenced by vomiting and oliguria, despite lower levels of PQ concentration in the blood. The protective effect of taurine was observed in a dose-dependent manner and appears to be specific for this amino acid, since the administration of 3.0% glycine, the dose of which was equimolar to that of 5.0% taurine, did not have a beneficial effect. In addition, the effect of taurine on urination was probably due neither to an osmotic diuresis nor to a difference in the pH of the solutions, since the administration of a 3.0% glycine solution, the osmolarity of which was equivalent to that of a 5.0% taurine solution, did not have a similar effect, and since the pH of all solutions was almost within the same range. Therefore, it is most likely that the observed protective effect of taurine against acute PQ intoxication may be due to a primary action of this sulfur amino acid. These results suggest that during the 2 hr infusion of taurine, plasma volume and renal function were maintained normally, even in those cases with a high level of PQ in the blood. In view of the established concept of PQ intoxication (11), these data might be unusual in view of the fact that the magnitude of the toxicity and blood concentration are not positively correlated. This implies that the pharmacokinetics of PQ may be altered by taurine. In fact, the increased PQ level in the blood attributable to taurine accompanied a decrease in the net content of PQ in the kidney, as represented by the T/B
ratio, without affecting PQ excretion into the urine. These effects may be specific for this amino acid and are due neither to an osmotic action nor to a marginal difference in the pH of the solutions. Thus, the results suggest that taurine may inhibit the entrance of the toxic compound into the renal tissues. However, the fact that no significant difference in urinary excretion of PQ was obtained between saline- and taurine-treated animals also suggests that taurine administration may not alter the PQ clearance from the kidney at least during the 2 hr infusion. Concerning this possibility, further studies will be required.

In the lung, the gross contents were increased by 2.5% taurine and by 3.0% glycine. These contents, however, may seem to be values reflecting the high level of PQ in the blood. Indeed, the net content in taurine-treated beagles did not differ from that in controls or glycine-treated animals. Thus, the decreasing effect of taurine on the PQ content appeared not to be prominent in the lung. This may be possibly related to the fact that in this organ, the PQ accumulation is a slow process (12). Therefore, it may be too early to justify the effectiveness of taurine on the lung in the acute experiment. However, it should be emphasized that the T/B ratio of PQ was below 1.0 in the dogs given 5.0% taurine, suggesting that the energy dependent uptake of PQ into the lung tissue might also have been inhibited.

Mechanisms through which taurine inhibits the entrance of PQ into tissues remain to be elucidated. Since taurine has been demonstrated to have an antioxidant property (2, 3) and because taurine is known to possess a membrane-stabilizing effect (13), at least these two elements should be considered as a contributing factor to the protective effect. Pathological examinations revealed that no specifically abnormal findings existed either in the kidney or in the lung in both animal groups treated with saline or taurine. These data, therefore, suggest that initial oliguria may be attributable to hypovolemia rather than renal failure.

Finally, in PQ intoxication, many acutely-affected human patients suffer from vascular collapse and loss of fluid from vessels into the interstitial space, often leading to death from hypovolemia, with or without renal failure (14, 15). The present results suggest that continuous taurine infusion may be able to protect against hypovolemic shock. Therefore, we suggest that while taurine is being infused, a rapid elimination of PQ from the blood by hemoperfusion or hemofiltration could be effective in acute instances of PQ intoxication. Such a study using animals is currently in progress at our laboratory.

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