Protective Effects of Antioxidants on Paraquat-Induced Acute Renal Failure in Mice

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ABSTRACT — The protective effects of antioxidants against elevated blood urea nitrogen (BUN) were studied in newly developed acute renal failure in mice induced by paraquat. The administration of paraquat caused marked azotemia accompanied by a decreased glomerular filtration rate. In contrast, elevated BUN was significantly reduced by the preadministration of either dimethylthiourea, deferoxamine or α-tocopherol. These data suggest that acute renal failure induced by paraquat is mainly related to the hydroxyl radicals produced via the iron-catalyzed Haber-Weiss reaction.

Keywords: Acute renal failure, Reactive oxygen species, Paraquat

It has been considered that reactive oxygen species play an important role in the pathogenesis of renal injury. Recently, protective effects of various types of antioxidants against proteinuria have been reported in experimental nephritic animal models of immune-mediated (1), toxic (2, 3) and ischemic tissue injury (4).

It is widely accepted that paraquat, a bipyridylium herbicide, is easily reduced because of its low redox potential and can utilize molecular oxygen as a one electron acceptor, and consequently produces superoxide anion (O$_2^-$) and highly reactive hydroxyl radical (OH·) via the iron-catalyzed Haber-Weiss reaction (5).

This study was conducted to determine whether the administration of paraquat would cause renal failure in mice, and if so, what the protective effects of various antioxidants on the elevated blood urea nitrogen (BUN) would be.

Six week-old male ddY strain mice were obtained from the Shizuoka Laboratory Animal Center (Japan). A dose of 120 mg/kg of paraquat dichloride (methyl viologen) was injected intraperitoneally, and then seven mice were placed in a metabolic cage without food and water for 6 hours to collect urine. Control mice were injected with saline (10 ml/kg, i.p.). Three or 6 hours after the administration of paraquat, blood was collected from the inferior cava of the animals under ether anesthesia. The concentrations of urea nitrogen and creatinine were determined with commercial test kits (Wako Pure Chemical Industries, Ltd., Japan). Urinary protein concentration was determined by the Biuret reaction method. The concentrations of electrolytes (Na$^+$, K$^+$ and Cl$^-$) were measured by an automated electrolyte analyzer (PVA-α, A & T). Each clearance in paraquat-treated mouse was calculated with the serum concentrations of creatinine, urea nitrogen and electrolytes at 3 hours after paraquat administration.

The protective effects of various antioxidants against elevated BUN were studied in paraquat-treated mice. Either dimethylthiourea (DMTU, 500 mg/kg), deferoxamine mesylate (250 mg/kg), α-tocopherol (10 mg/kg), superoxide dismutase (SOD, 30 mg/kg) or catalase (10000 units/kg) was administered intraperitoneally 30 min before the administration of paraquat dichloride (120 mg/kg). Six hours after the administration of paraquat, blood was collected for measurement of BUN.

The drugs used were paraquat dichloride (Sigma), deferoxamine mesylate (Ciba-Geigy), dl-α-tocopherol (Sigma), N,N'-dimethylthiourea (Nacalai Tesque), bovine erythrocyte Cu,Zn-superoxide dismutase (Sigma) and catalase (Worthington Biochemicals). The activities of SOD and catalase were 3020 units/mg protein and 84150 units/mg protein, respectively. They were dissolved in saline, apart from dl-α-tocopherol, which was suspended in 0.5% tween 80 solution with saline.

Serum creatinine and BUN at 6 hours after the injection of paraquat significantly increased compared to the control mice (Table 1). In contrast, the excretion of creatinine and urea into the urine for 6 hours was reduced, so that calculated creatinine and urea clearances were reduced to about 70% in paraquat-treated mice.
Marked elevated excretion of electrolytes into the urine and electrolyte imbalance (hyponatremia, hypopotassemia and hypochloremia) were observed. Consequently, the calculated electrolyte clearances increased in the paraquat-treated mice. Paraquat-treated mice also showed polyuria and proteinuria.

In studies with antioxidants, elevated BUN caused by paraquat was significantly reduced by the preadministration of either DMTU, deferoxamine or α-tocopherol (Fig. 1). In contrast, mice treated with either SOD or catalase showed no significant reduction of BUN compared to mice treated with paraquat alone.

The administration of paraquat caused marked elevation of BUN accompanied by a decrease in creatinine clearance. Since creatinine clearance represents the glomerular filtration rate (GFR), the azotemia observed in a paraquat-treated mouse is considered nephrogenic in origin. The occurrence of proteinuria observed in a

### Table 1. Parameters representing renal functions in control and paraquat-treated mice

| Parameters                          | Control mouse | Paraquat-treated mouse |
|-------------------------------------|---------------|------------------------|
| Urine volume (ml/7 mice)            | 3.4           | 6.9                    |
| Serum creatinine (mg/dl/mouse)      | 0.30 ± 0.03   | 0.65 ± 0.06**          |
| Urinary creatinine (mg/dl/7 mice)   | 40.4          | 16.7                   |
| Creatinine clearance (µl/min/mouse) | 181.7         | 130.3                  |
| Blood urea nitrogen (mg/dl/mouse)   | 17.6 ± 1.0    | 57.0 ± 2.3**           |
| Urinary urea nitrogen (mg/dl/7 mice)| 1425.1        | 962.3                  |
| Urea clearance (µl/min/mouse)       | 109.2         | 81.8                   |
| Serum Na⁺ (mEq/l/mouse)             | 148.4 ± 0.4   | 141.0 ± 2.2*           |
| Urinary Na⁺ (mEq/l/7 mice)          | 122.3         | 286.0                  |
| Na⁺ clearance (µl/min/mouse)        | 1.11          | 5.47                   |
| Serum K⁺ (mEq/l/mouse)              | 6.93 ± 0.44   | 6.37 ± 0.36            |
| Urinary K⁺ (mEq/l/7 mice)           | 146.3         | 78.6                   |
| K⁺ clearance (µl/min/mouse)         | 28.5          | 40.5                   |
| Serum Cl⁻ (mEq/l/mouse)             | 113.2 ± 0.4   | 98.2 ± 3.2**           |
| Urinary Cl⁻ (mEq/l/7 mice)          | 137.9         | 265.8                  |
| Cl⁻ clearance (µl/min/mouse)        | 1.64          | 6.83                   |
| Urinary protein (mg/7 mice)         | 67.3          | 111.8                  |

The serum concentrations of creatinine, urea nitrogen and electrolytes are indicated as means ± S.E. of 7 mice obtained at 6 hours after the administration of saline or paraquat. Each clearance in a paraquat-treated mouse was calculated with the serum concentrations of creatinine, urea nitrogen and electrolytes at 3 hours following paraquat administration (data not shown). *P < 0.05, **P < 0.01, ***P < 0.001 (Student’s t-test), as compared with control mice.

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![Fig. 1. Effects of the preadministration (30 min before) of dimethylthiourea (DMTU), deferoxamine (DEF), α-tocopherol (VE), superoxide dismutase (SOD) and catalase (CAT) on elevated blood urea nitrogen (BUN) in paraquat (PQ)-injected mice. The left column shows BUN for intact mice injected with saline alone. Each column indicates BUN (mean ± S.E. of 7 mice) at 6 hours after paraquat administration. *P < 0.05, **P < 0.01, ***P < 0.001 (Student’s t-test), as compared with control mice (open column): *P < 0.05, **P < 0.01 (Student’s t-test), as compared with mouse treated with saline plus paraquat (solid column).](image-url)
Paraquat-treated mouse suggests the destruction of the glomerular basement membrane by reactive oxygen species generated by paraquat. Marked elevation of urinary protein has also been reported in nephritic animal models induced by anti-glomerular basement membrane antibody (1), gentamicin (2), puromycin aminonucleoside (3) and adriamycin (6), which are all capable of generating reactive oxygen species. The low levels of electrolytes in the serum are considered due to the large excretion of electrolytes into the urine. Polyuria and the apparent increased electrolytes clearance suggest failure in the ability of tubular reabsorption. Paraquat is known to be filtered at the glomerulus and actively secreted by tubular cells (5). In addition, it has been reported that the kidney has the highest concentration of paraquat in organs within 4 hours after the systemic injection of paraquat in rats (7). Therefore, it is considered that both glomerular and tubular cells are damaged in a paraquat-treated mouse.

The protective effects of various antioxidants against elevated BUN were investigated to clarify the reactive oxygen species involved in the pathogenesis of paraquat-induced acute renal failure since BUN represents renal function. Mice pretreated with DMTU, a potent hydroxyl radical scavenger, showed a significantly reduced BUN compared to mice treated with paraquat alone. The ability of DMTU to scavenge hydroxyl radicals has been reported in in vitro studies (8). In addition, the protective effects of DMTU against proteinuria have been reported in experimental nephritic animal models whose etiology is related to reactive oxygen species (1–3). The hydroxyl radical, known to be highly reactive and toxic, thus quite likely appears to be an important mediator of the acute renal failure induced by paraquat.

The protective effect of deferoxamine, an iron chelator, is also considered as evidence for the involvement of hydroxyl radicals in the acute renal failure induced by paraquat since iron catalyzes the Haber-Weiss reaction in which hydroxyl radicals are produced. Paraquat toxicity has also been shown to be enhanced by the administration of iron and reduced by deferoxamine in mice (9).

It thus appears reasonable to consider α-tocopherol, a lipid peroxidative chain reaction inhibitor, prevents the elevation of BUN in paraquat-treated mice, since reactive oxygen species are considered to injure membrane structures by lipid peroxidation.

Partial reduction in BUN was observed in mice treated with SOD (which converts O2− to H2O2 and O2) or catalase (which converts H2O2 to O2 and H2O). The partial protective effects of SOD and catalase may be explained as follows. First, the circulating half-life times of these agents are short in vivo. Secondly, these agents could not scavenge intracellularly generated reactive oxygen species owing to their high molecular weights. Thirdly, endogenous SOD and/or catalase might increase following the administration of paraquat since it has been reported that paraquat increases the activity of Cu,Zn-SOD in rat kidney tissue (10).

In conclusion, the present data indicate that the acute renal failure induced by paraquat is related primarily to hydroxyl radicals produced via the iron-catalyzed Haber-Weiss reaction.

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