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LUNG TOXICITY OF PARAQUAT IN THE RAT

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In a rat model of paraquat-induced lung injury, pulmonary alveolar lavage fluid metabolic parameters were assessed to establish damage, and the use of surfactant was employed as a protective agent. Three groups of adult male Sprague-Dawley rats received intraperitoneal injection of paraquat (35 mg/kg body weight) in 1 ml saline, or received 1 ml saline, or no material. On d 3, 7, 14, and 21 after injection, pressure–volume curves and pulmonary alveolar lavage fluids were obtained. On d 3 paraquat significantly increased the lung wet/dry weight ratio and protein content but lowered phosphatidylcholine levels. There were no marked changes at other time points in the parameters examined. The pressure–volume curves initially moved downward and to the right on d 3 and 7 and then returned to control levels in the paraquat-treated rats. Immediate intratracheal administration of Survanta after paraquat injection (70 mg/kg body weight) tended to increase the survival rate on d 1 compared to rats without Survanta administration. Our results suggest that administration of exogenous surfactant may play a role in the treatment of patients poisoned with paraquat.

Paraquat dichloride (1,1′-dimethyl-4,4′-bipyridilium dichloride; methyl viologen) is an effective and a widely used herbicide. The intentional and accidental ingestion of the commercial liquid formulations of paraquat has caused a large number of human fatalities (Hart, 1987). Eight hundred and ninety-two cases of paraquat intoxication were reported to the Poison Control Center in Taiwan from July 1985 to December 1993, and the mortality rate was 54.4% (Department of Health Executive Yuan, ROC, 1995). Paraquat produces toxicity in humans, and the lung is the primary target organ, probably due to an active uptake system (Rose et al., 1974; Forman et al., 1982). The toxic effects of paraquat on the lung result in pulmonary edema, hypoxia, respiratory failure, and pulmonary fibrosis. Death is attributed to extensive pulmonary injury (Winchester, 1990). Respiratory distress in the early stages of paraquat poisoning may be produced by impairment of the surfactant system. Manktelow (1967) showed that paraquat produced changes in the lung comparable to the lesions

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observed in respiratory distress syndrome. However, other studies revealed inconsistent results in alterations of lung phospholipids (Fletcher & Wyatt, 1970; Malmquist et al., 1973; Fisher et al., 1973).

Ingestion of paraquat is associated with a high mortality rate, but this should not detract us from intensive management of such poisoned patients, since occasionally patients have fully recovered even after massive ingestion of herbicide with pulmonary involvement (Lheureux et al., 1995). The cytotoxicity produced by paraquat in the lung may be due to capacity to generate superoxide radicals (Krall et al., 1988) or to the formation of mixed disulfides (Keeling et al., 1982). oxygen therapy is not a good regimen to treat paraquat intoxication because the mortality is markedly increased by exposure to high concentrations of oxygen (Witschi et al., 1977). It is possible that surfactant replacement therapy might restore surfactant activity in paraquat-injured lung and improve the survival rate. Ambroxol, a surfactant inducer, given 2 d before intraperitoneal paraquat injection to rats decreased mortality rate (Pozzi et al., 1989). However, this regimen is not practical for clinical use.

The purpose of this study was twofold: (1) to determine the pulmonary inflammatory responses and measure the phospholipid levels of pulmonary surface-active material recovered in lavage fluid of rats treated with paraquat, and (2) to evaluate the possible therapeutic role of exogenous surfactant in experimental paraquat poisoning.

MATERIAL AND METHODS

Animal Preparation

Adult male Sprague-Dawley (SD) rats (approximate body weight 280–300 g) kept on a standard laboratory diet and water ad libitum were used in this study. The rats were randomly divided into three groups and studied concomitantly. One group was treated intraperitoneally (ip) with paraquat (35 mg/kg body weight, Sigma Chemical, St. Louis, MO) in 1 ml saline; the second group received 1 ml sterile saline ip; and no material was injected in the third group. On d 3, 7, 14, and 21 after injection, at least 5 rats in both the paraquat and the saline groups, as well as 6 untreated rats, were anesthetized by intraperitoneal injection of pentobarbital (50 mg/kg body weight). After the rat was exsanguinated via the abdominal aorta, the chest was opened.

Pressure–Volume Curve Measurements

After tracheostomy, the endotracheal tube was clamped for 3 min to allow absorption atelectasis to occur. A static deflation pressure–volume curve was begun by filling the collapsed lung with air to 25 cm H_2O pressure for 1 min and recording the maximal lung volume. The pressure was then progressively decreased and held for 30 s at 20, 15, 10, 5, and 0 cm H_2O, with lung volume measured at each pressure. The measured volumes

were corrected for air compression within the measurement system and expressed as milliliters per kilogram body weight. After the deflation pressure–volume curve measurements, the lungs were removed intact from the animal with the endotracheal tube in place, weighed, and lavaged.

Alveolar Lavage

Five aliquots of 5 ml saline at 4°C were used to fill the lungs. Each aliquot was flushed into and out of the airways three times. The recovered volumes, which were about 85% of the injected volumes, were saved as the pooled alveolar lavage. Aliquots of the alveolar lavage fluid from each rat were used to measure cell counts, total protein, and saturated phosphatidylcholine.

Lung Wet Weight/Dry Weight Ratio, Liver, Heart, and Kidney Weights

After alveolar lavage, all major cartilaginous airways were dissected free, and then the wet weight of the remaining lung tissue was determined. The lung was frozen at –70°C and then dried in an 80°C oven until its weight was stable on repeated determinations (2–3 d). The other organ weights of liver, heart, and kidneys were measured and the ratios of grams wet tissue to kilograms body weight were calculated.

Lavage Inflammatory Cells

Total cell counts were performed on uncentrifuged alveolar lavage fluid using a hemacytometer counting chamber (Reichert Scientific Instruments, Buffalo, NY). Differential cell counts were made on cytospin preparations (cytospin 3, Shandon Scientific Ltd., Cheshire, England) stained with Liu's stain (Tonyar, Diagnostics Inc., Taiwan) using standard morphologic criteria for macrophages, neutrophils, and lymphocytes (Baughman et al., 1986).

Lavage Total Protein

Total protein content in the lavage fluid was measured by using bovine serum albumin as a standard and was expressed as milligrams per kilogram body weight (Bradford, 1976).

Lavage Phospholipid

Lipid extracts from aliquots of the alveolar lavage were treated with osmium tetroxide, and saturated phosphatidylcholine was recovered by alumina column chromatography (Mason et al., 1976) and was quantified by phosphorus assay (Bartlett, 1959). The values were expressed as micromoles per kilogram body weight.

Effect of Exogenous Surfactant on the Survival of Paraquat-Treated Rats

All animals received a single dose of paraquat (70 mg/kg body weight) intraperitoneally. Following paraquat injection, Survanta (3 ml/kg body weight, Abbott Laboratories, North Chicago, IL) was given immediately through the intratracheal route (Weksler et al., 1994). A second group re-

ceived paraquat injection only. Following intratracheal surfactant instillation, the rats were monitored until awake for signs of distress. None of the animals showed evidence of loss of instilled surfactant material via cough reflex. All rats breathed room air following Survanta instillation. The mortality rate was calculated on d 10. Survanta is a natural bovine lung extract containing phospholipids, neutral lipids, fatty acids, and surfactant-associated proteins to which dipalmitoylphosphatidylcholine, palmitic acid, and tripalmitin are added to standardize the composition and mimic surface-tension-lowering properties of natural lung surfactant. The resulting composition provides 25 mg/ml phospholipids, 0.5–1.75 mg/ml triglycerides, 1.4–3.5 mg/ml free fatty acids, and less than 1 mg/ml protein.

Statistics

All data are presented as mean ± SEM. Analysis of variance (ANOVA) with Student–Newman–Keuls post hoc test was applied to determine the differences between saline-treated and paraquat-treated groups at each time point. Survival rate was evaluated with chi-square and Fisher's exact test. A p value $<.05$ was regarded as significant.

RESULTS

Mortality and Clinical Observations

All saline-treated rats survived to scheduled sacrifice. Nine of 36 paraquat-treated rats (35 mg/kg body weight) were dead before sacrifice. The timing and the number of death were as follows: <1 d, 1; 1–2 d, 1; 2–3 d, 4; 3–4 d, 2; 8–9 d, 1. Paraquat-treated rats consumed less food. There was hair loss and dryness. Some paraquat-treated rats also displayed hypokinesia, anorexia, and diarrhea.

Body Weights and Organ Weights

Effect of paraquat on body weights and organ weights are presented in Tables 1 and 2. The saline-treated rats gained body weight progressively, whereas body weight was significantly reduced at d 3 in paraquat-treated rats. However, from d 7 to 21 there was no marked change in body weight following paraquat treatment. The lung wet weight/dry weight ratio in the paraquat-treated rats was significantly increased on d 3 compared with saline-treated rats but returned to control levels at d 7 and remained there until d 21 (Table 2). The relative heart and liver weights were comparable among three groups. The relative kidney weight in the paraquat-treated rats was significantly greater than that in the saline-treated rats on d 3.

Pressure–Volume Curves

The pressure–volume curves in the saline-treated rats moved downward gradually over the 21 d (Figure 1A). Conversely, in the paraquat-treated rats the pressure–volume curves moved downward and to the right on d 3 and 7 and then returned toward the control levels on d 21 (Figure 1B).

TABLE 1. Effect of Paraquat on Body Weight and Lung Weight

Treatment	Day	*n*	Body weight: Initial (g)	Body weight: Change after paraquat	Wet lung weight (g)	Wet/dry weight ratio
None	0	6	299 ± 8	0 ± 0	1.32 ± 0.09	4.95 ± 0.30
Saline	3	5	306 ± 16	+10 ± 3	1.35 ± 0.09	5.06 ± 0.13
	7	5	292 ± 8	+43 ± 6	1.41 ± 0.05	5.55 ± 0.18
	14	5	287 ± 3	+71 ± 11	1.59 ± 0.23	5.27 ± 0.35
	21	5	281 ± 1	+140 ± 7	1.73 ± 0.11	5.42 ± 0.13
Paraquat	3	7	294 ± 4	–25 ± 15	1.97 ± 0.37	7.54 ± 1.09[b]
	7	6	288 ± 6	+3 ± 21	2.21 ± 0.48	6.15 ± 0.29
	14	7	299 ± 7	+53 ± 11	1.90 ± 0.12	6.00 ± 0.21
	21	7	286 ± 6	+105 ± 10[a]	1.89 ± 0.17	5.14 ± 0.16

Note. Adult male SD rats received a single ip injection of saline or paraquat (35 mg/kg body weight) and were sacrificed 3–21 d postdose. Data are presented as mean ± SEM.

[a]Significantly different from saline-treated rats on d 21 ($p < .05$).

[b]Significantly different from saline-treated rats on d 3 ($p < .05$).

Lavage Inflammatory Cells

The total cell counts in the pulmonary alveolar lavage fluid increased after paraquat injection and reached significant levels on d 14, then fell toward control values on d 21 (Table 3). There were no marked changes in the macrophage or white blood cell counts following paraquat treatment.

Lavage Protein

Total protein recovered in the pulmonary alveolar lavages was comparable at each time point in the control (no saline) and the saline-treated

TABLE 2. Relative Organ Weights Following Paraquat Treatment

Treatment	Day	*n*	Heart	Liver	Kidney
None	0	6	3.5 ± 0.1	45.4 ± 1.1	8.7 ± 0.4
Saline	3	5	3.5 ± 0.1	42.2 ± 1.3	8.0 ± 0.1
	7	5	3.5 ± 0.2	43.1 ± 2.4	8.3 ± 0.2
	14	5	3.6 ± 0.2	39.4 ± 1.7	7.6 ± 0.1
	21	5	3.3 ± 0.1	38.7 ± 1.6	7.3 ± 0.2
Paraquat	3	7	3.8 ± 0.2	41.7 ± 1.9	9.8 ± 0.4[a]
	7	6	3.7 ± 0.2	44.1 ± 0.7	9.2 ± 0.3
	14	7	3.1 ± 0.1	38.5 ± 1.2	8.0 ± 0.3
	21	7	3.1 ± 0.1	38.7 ± 1.1	7.7 ± 0.2

Note. Adult male SD rats received a single ip injection of saline or paraquat (35 mg/kg body weight) and were sacrificed 3–21 d postdose. Data are expressed as g wet tissue/kg body weight. Data are presented as mean ± SEM.

[a]Significantly different from saline-treated rats on d 3 ($p < .05$).

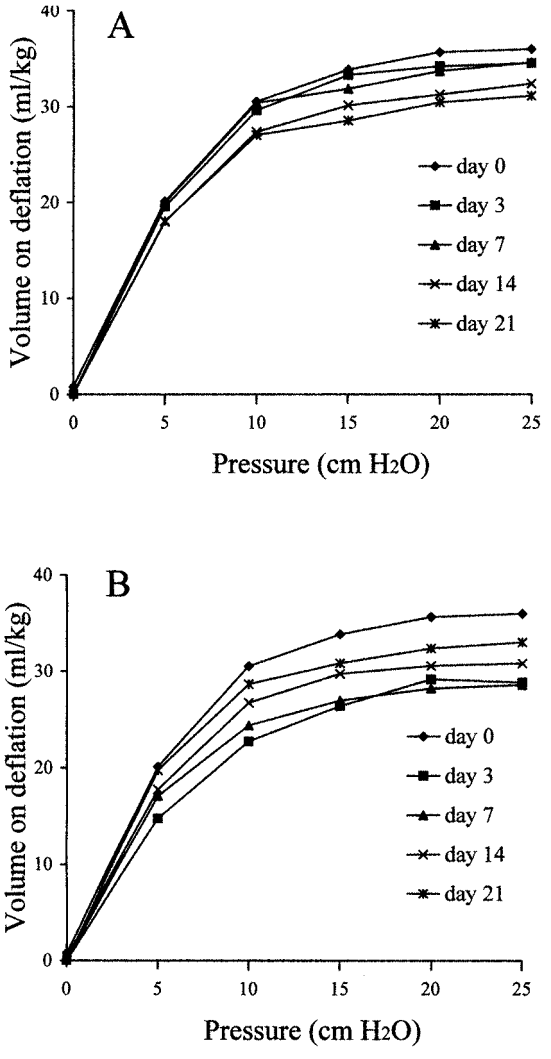

FIGURE 1. Deflation pressure–volume curves of the lungs in the saline and paraquat-treated rats. After static inflation of the lungs to 25 cm H_2O pressure, deflation volumes were measured and expressed as ml/kg body weight. (A) Lung volumes in saline-treated rats. (B) The pressure–volume curves in the paraquat-treated rats.

rats (Figure 2). However, total protein content in the paraquat-treated rats was significantly increased on d 3 and 7 compared with saline-treated rats and then returned to control levels on d 14.

Lavage of Saturated Phosphatidylcholine

Saturated phosphatidylcholine pool levels in the pulmonary alveolar lavage fluid were comparable in the control (no saline) and the saline-treated rats (Figure 3). In the paraquat-treated rats, saturated phosphatidyl-

TABLE 3. Cell Counts in Pulmonary Alveolar Lavage of Male SD Rats Treated with Paraquat

Treatment	Day	*n*	Total cells (10^4/ml)	Macrophages (10^3/ml)	Neutrophils (10^3/ml)	Lymphocytes (10^3/ml)	Monocytes (10^3/ml)
None	0	6	9.7 ± 1.9	51 ± 18	18 ± 14	26 ± 12	2 ± 1
Saline	3	5	13.0 ± 6.5	35 ± 6	37 ± 21	79 ± 52	6 ± 2
	7	5	13.0 ± 8.1	82 ± 38	44 ± 41	5 ± 2	0 ± 0
	14	5	8.5 ± 0.8	65 ± 5	10 ± 6	9 ± 4	1 ± 0
	21	5	8.1 ± 1.5	47 ± 7	18 ± 7	15 ± 6	0 ± 0
Paraquat	3	7	15.4 ± 3.8	72 ± 24	75 ± 28	7 ± 2	0 ± 0
	7	6	30.9 ± 8.2	147 ± 38	158 ± 98	5 ± 2	0 ± 0
	14	7	38.7 ± 9.6[a]	257 ± 81	117 ± 46	13 ± 5	0 ± 0
	21	7	10.7 ± 3.4	74 ± 82	18 ± 8	13 ± 6	1 ± 1

Note. Adult male SD rats received a single ip injection of saline or paraquat (35 mg/kg body weight) and were sacrificed 3–21 d postdose. Data are presented as mean ± SEM.

[a]Significantly different from saline-treated rats on day 14 ($p < .05$).

choline levels significantly decreased on d 3 and then returned to control after d 7.

Effect of Exogenous Surfactant on the Survival of Paraquat-Treated Rats

Rats (n = 10) treated with a higher dose of paraquat (70 mg/kg body weight) displayed 50% mortality by d 1 and 90% by d 2 (Figure 4). A 100% mortality was found by d 5 in this group. Coadministration of Survanta and paraquat resulted in significant enhancement in survival on d 1 and 2, with 1 rat still alive on d 10.

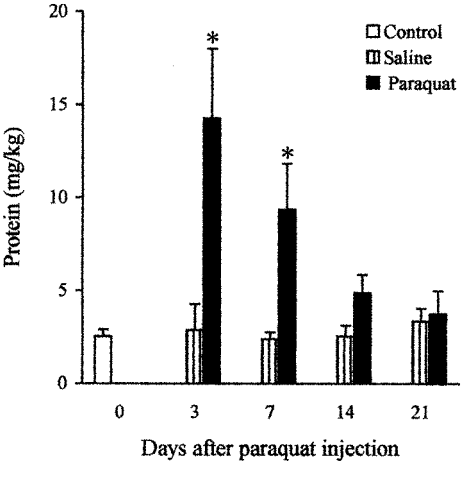

FIGURE 2. Influence of paraquat on pulmonary alveolar lavage protein. For experimental protocol, see Table 1. Data are mean ± SEM of five to seven rats. Asterisk indicates statistically significant difference from the respective saline-treated rats ($p < .05$).

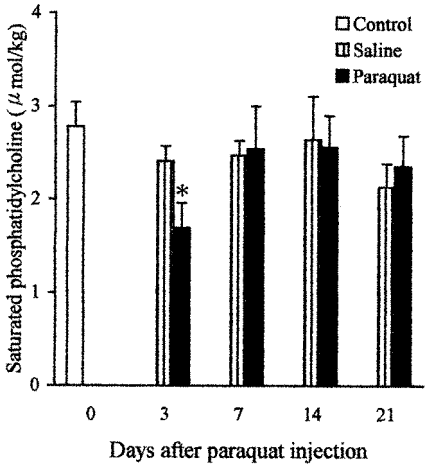

FIGURE 3. Effect of paraquat on saturated phosphatidylcholine in pulmonary alveolar lavage fluid. For experimental protocol, see Table 1. Asterisk indicates statistically significant difference from the respective saline-treated rats ($p < .05$).

DISCUSSION

Although it is evident that the surfactant system is one of the targets in paraquat toxicity (Haagsman, 1993) alterations in lung phospholipid were inconsistent in various studies. There were no significant changes in the content and composition of total lung lipids after paraquat poisoning reported by Fletcher and Wyatt (1970). In contrast, a significant decrease of phosphatidylcholine levels in lung homogenates and alveolar washes of rats was noted 24 h after a single subcutaneous injection of paraquat (Malmquist et al., 1973). Dipalmitoylphosphatidylcholine content in lung lavage fluid was reduced 3 d after a single intravenous dose of paraquat to rats (Fisher et al., 1973). Alveolar phospholipids were markedly reduced throughout the

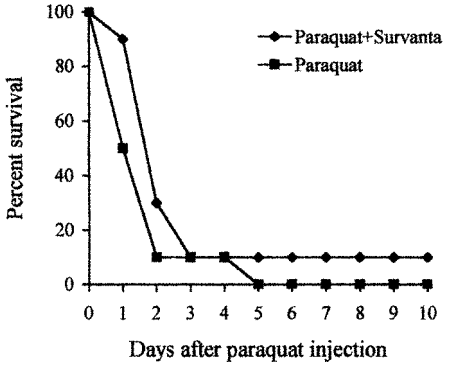

FIGURE 4. Effect of exogenous surfactant on the survival of paraquat-treated rats (70 mg/kg body weight). Each point represents 10 rats.

21-d investigation of alveolar phospholipid kinetics (Pozzi et al., 1989). In contrast to the latter study, our data show that phosphatidylcholine levels in alveolar lavage were significantly decreased on d 3 but then returned to control levels 7 d after paraquat injection. Differences in experimental protocols might account for the variation in the reported findings.

Pulmonary surfactant is critical for alveolar stability (Clements, 1962). Decreased surfactant activity results in reduced pulmonary compliance and shifting the pressure–volume curves to a lower volume (Gregory et al., 1994). It is of interest that in acute respiratory distress syndrome there is surfactant inactivation due to proteinaceous pulmonary accumulation (Holm et al., 1988). In this study, paraquat treatment increased total pulmonary alveolar protein content by about fivefold and fourfold on d 3 and 7, respectively. The increased protein content may be related to paraquat-induced pulmonary dysfunction as evidenced by decreased inflation capacity.

Despite evidence of endogenous surfactant dysfunction, the status of exogenous surfactant therapy in the treatment of paraquat intoxication remains uncertain. In this study, paraquat treatment significantly decreased pulmonary phosphatidylcholine levels. on the basis of this observation it was postulated that exogenous surfactant administration might restore the surfactant activity in paraquat-injured lung and improve the survival rate. In this study it was noted that exogenous surfactant therapy tended to increase survival of paraquat-treated rats on d 1. This is compatible with the turnover time of surfactant phosphatidylcholine in adult rats (Young et al., 1981). Because the optimal amount of surfactant to administer and the consequences of administration of surfactant were unknown, relatively lower quantities of surfactant than that used clinically to treat patients with acute respiratory distress syndrome were administered in this study (75 vs. 100 mg/kg body weight) (Gregory et al., 1994). This may account for the high mortality rates seen at 10 d following Survanta therapy.

The timing of exogenous surfactant administration is another important factor influencing the treatment response. Administration of exogenous surfactant at an early time point in saline lavage-induced lung injury resulted in more beneficial responsiveness as judged by morphological, physiological, and biochemical parameters (Ito et al., 1996). Based on the fact that maximal metabolic responses occurred within 3 d at one-half the paraquat dose used in the mortality study, a 24-h period was chosen to administer Survanta. This treatment effectively delayed mortality. It is conceivable that more Survanta treatments on d 2, 3, 4, etc. might have prolonged survival.

This study provides a preliminary view of the role that exogenous surfactant therapy may play in the treatment of patients poisoned with paraquat. Since only a single dose of Survanta was administered in the present experiment, the question remains of whether multiple doses could be more effective. It might be expected that repetitive surfactant therapy could extend survival time and allow a lung transplant to be performed in the paraquat-poisoned patients. Further studies are needed to clarify these pro-

tective effects. Despite the encouraging result, there are multiple factors requiring further investigation in the development of optimal surfactant treatment strategies. Such factors include the dosage and the ideal time for surfactant administration during the course of injury and the development of optimal exogenous surfactant preparations.

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