Recovery from paraquat pneumonitis

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ABSTRACT New Zealand white rabbits were evaluated for recovery from paraquat induced pneumonitis six weeks after the last exposure. The animals were exposed to a respirable aerosol of 100 ml distilled water or 250 mg paraquat in 100 ml distilled water. Blood gases, breathing frequency, and body weights were recorded before and at regular intervals after exposure. Groups included control and two paraquat exposures (separated by a five day interval). Morphometric and pathological measurements were made at death either three days or 42 days after the second exposure. The animals killed three days after the second exposure showed hypoxia, decreased breathing frequency, decreased body weight, increased A-aO₂ gradient, decreased per cent macrophages in bronchoalveolar lavage fluid, increased per cent neutrophils in bronchoalveolar lavage fluid, increased lung weight, and reduced lung volume compared with animals allowed to recover. None of these measures differed between control animals and animals allowed to recover, except that animals exposed to paraquat had significantly increased lung volume and lung weights. Pathological changes noted three days after two exposures were no longer found six weeks after exposure. It is concluded that rabbits exposed to paraquat aerosol develop a severe pneumonitis that resolves if exposure is stopped and recovery time is allowed. Physiological abnormalities remain, however.

Lung injury by oxidising agents such as a high concentration of oxygen or the herbicide paraquat may lead to cellular disruption and death from respiratory failure.^{1,2} These agents are thought to damage membranes by the production of oxygen free radicals,³⁻⁵ the damage ultimately leading to interstitial pneumonitis and fibrosis.^{6,7} Recovery from sublethal injury may occur more frequently than disease progression. This hypothesis was investigated in rabbits exposed to paraquat.

Crapo and Tierney showed that rats, guinea pigs, hamsters, and mice may develop tolerance to oxygen environments greater than 21%.⁸ He noted prolonged survival in animals exposed to 100% oxygen if the animals had one week's exposure to an 85% O₂ environment. Kimball *et al* confirmed these observations and found raised activities of superoxide dismutase, glutathione peroxidase, and glucose-6-phosphodiesterase (G-6-PD) in animals exposed to O₂.⁸ They reasoned that these enzyme

activities were induced to combat the increased numbers of oxygen radicals and allowed animals to develop oxygen tolerance. Fisher *et al* found that O₂ toxicity in rats was enhanced by paraquat pretreatment.⁷ Rhodes *et al* later reported that hypoxic environments were protective for mice exposed to paraquat.⁹ Most of the data reported on paraquat toxicity are concerned with acute changes.

We chose to examine the recovery of rabbits from paraquat injury. Previously we have noted severe subacute pneumonitis in rabbits exposed to paraquat aerosol.¹⁰ In the present report we give evidence that animals who survive acute pneumonitis have little pneumonitis six weeks after exposure, despite severe physiological and pathological derangements noted three days after the second of two exposures.

Methods

New Zealand white rabbits, about 6 months old and averaging 2 kg, were housed in separate cages in a

Received 27 February 1984
Accepted 30 April 1984

well ventilated room. The rabbits were weighed and breathing frequency was checked every other day throughout the experiments. The rabbits were isolated from other experimental animals to avoid cross contamination. Purina Rabbit Chow and water were provided freely.

We studied the following groups: controls exposed twice to double distilled water and experimental animals exposed to paraquat in double distilled water, both killed three days after the last exposure, and controls and animals exposed to paraquat allowed to recover for six weeks after the final exposure before being killed. Six weeks or 42 days was chosen arbitrarily as a time when some physiological measures had returned to control levels. Controls were exposed to inhalations of 100 ml of double distilled water from an ultrasonic nebuliser (DeVilbiss, Model 65), which generated an aerosol with a particle size in the range 2–5 μm , on two occasions five days apart, with each exposure lasting one hour. The paraquat group received 250 mg of paraquat dichloride in 100 ml double distilled water driven into the exposure chamber by an ultrasonic nebuliser; animals exposed to paraquat received two exposures lasting one hour each at the same interval in the closed, vented exposure chamber. The exposure chamber was a 140 l plexiglass glove box with a screen floor and a constant air flow of 5 l/min through the chamber. Expired CO_2 within the chamber was kept below 1% during exposures, ambient temperature was 25°C, and relative humidity was nearly 100%. The same chamber was used for all control and experimental exposures, with four to six animals per exposure. Seidenfeld *et al* have documented tissue deposition with the particle size and exposure system used for these studies.¹⁰

The acute and recovery experiments were run separately with appropriate controls. Complete data were available for 43 of the 50 acute experimental animals from three runs, 28 of the 32 chronic experimental animals from two runs, and a cumulated control group that included acute and chronic animals from 20 runs which used the same exposure interval. Only one death occurred in the control animals and this was due to pneumonia. One or two animals in the paraquat group of 15–16 experimental animals died each run. In most cases death was ascribed to severe pneumonitis.

All rabbits had arterial blood drawn from the central ear artery before exposure, two to three days after each exposure, and before being killed. Samples were iced and run within an hour after collection on an Instrument Laboratories 813 arterial blood gas analyser; PaO_2 , PaCO_2 , pH, HCO_3^- were determined and A-a O_2 values calculated. The analyser was calibrated just before running the samples.

At death, the trachea was isolated and cross clamped. The lungs and heart were removed through a parasternal incision; the heart, mediastinal fat and lymph nodes, and oesophagus were separated from the lungs and trachea. After weighing the lungs, a length of PE 330 (ID 2.92 mm–OD 3.73 mm) tubing was inserted in the trachea, tied in place above the carina, and a syringe attached.

The isolated lungs, with the trachea held uppermost throughout the procedure, were lavaged by the method of Myrvik *et al* with four 50 ml aliquots of heparinised (2 U/ml), modified 10% Hanks balanced salt solution without calcium or magnesium (Grand Island Biological Co).¹¹ The lavage medium was allowed to dwell passively for one minute before aspiration. The four aliquots from each specimen were pooled and centrifuged (Damon/IEC Division) for seven minutes at 1200–1500 rpm. The supernatant was decanted and the cell pellet resuspended in lavage medium and recentrifuged. This wash step was repeated twice. The cell pellet was resuspended in 20 ml lavage medium. A 20 μl sample of the washed cells was diluted in 10 ml physiological saline, two drops of a lysing agent (Scientific Products) were added to lyse any red blood cells present, and a cell count obtained with a Coulter Counter (model ZF, Coulter Electronics Co). A 200 μl aliquot of the washed cells was cytocentrifuged (Cytospin 2, Shandon Southern Instruments, Inc) and Wright stained; a differential cell count for macrophages, polymorphonuclear cells, and lymphocytes was determined on 500 cells.

Body weight and lung weight were recorded (initial body weight was noted before the exposures to paraquat). The lungs were fixed by endobronchial neutral buffered 10% formalin at 20 cm H_2O pressure for 24 hours, and the fixed volume (lung tissue volume plus residual air) determined by water displacement. Standardised and directed sections were taken from both lungs after the relative amount of gross disease was noted. Haemotoxylin and eosin stains of each section were reviewed blind.

This study used analysis of variance with repeated measures. The lavage and physiological data were analysed with parametric (F and *t* tests) and non-parametric statistical tests. Lung weight, lung volume, and the ratios of lung weight/body weight, lung volume/body weight, and lung weight/lung volume were examined by analysis of variance for differences between groups. Breathing frequencies, PaO_2 , PaCO_2 , $\Delta\text{A-aO}_2$, pH, and HCO_3^- were evaluated by 2-way analysis of variance (ANOVA) and by Student's *t* test.

Animal care was conducted within the guidelines of the National Research Council and an institutional review committee approved all procedures.

Physiological and pathological changes with injury and recovery

	<i>Paraquat exposed</i>		
	<i>Control</i>	<i>Acute</i>	<i>Recovery</i>
PaO ₂ (mm Hg)	71 ± 9 (n = 105)	60 ± 16 (n = 43) t = 3.84*	73 ± 7 (n = 28)
Body weight (kg)	2.464 ± 0.268 (n = 105)	2.280 ± 0.395 (n = 43) t = 6.5*	3.179 ± 0.275 (n = 28) t = -2.7, ‡ t = -11.3†
Breathing frequency (min ⁻¹)	140 ± 32 (n = 105)	117 ± 35 (n = 43) t = 4.93*	143 ± 30 (n = 28) t = -3.31‡
A-aO ₂ (mm Hg)	30 ± 7 (n = 105)	38 ± 14 (n = 43) t = -2.73*	30 ± 7 (n = 28) t = 2.69‡
% Macrophages	96 ± 6 (n = 17)	50 ± 25 (n = 18) t = 7.34*	97 ± 3 (n = 14) t = -6.84‡
% PMNs	2 ± 6 (n = 17)	39 ± 14 (n = 18) t = -5.72*	3 ± 7 (n = 14) t = 5.36‡
Lung weight (g)	11.76 ± 1.71 (n = 22)	19.34 ± 6.38 (n = 29) t = -5.41*	14.40 ± 7.89 (n = 25) t = -2.54†
Lung volume (ml)	83.5 ± 11.9 (n = 22)	78.7 ± 13.2 (n = 29)	98.9 ± 11.8 (n = 25) t = -4.46, † t = -5.88‡

*Comparison between control and acute groups.

†Comparison between control and recovery groups.

‡Comparison between acute and recovery groups. In all cases $p < 0.05$.

PMN = Polymorphonuclear leukocytes.

Results

The control group seemed well throughout the experiment with no evidence of breathing abnormalities. Most experimental animals developed snuffles and laboured respiration two days after exposure to paraquat; these animals showed less interest in eating, drinking, or sexual activity in the exposure chamber during the second exposure. The animals allowed to recover without further exposure regained behaviour and feeding patterns similar to that of the controls about one week after the second exposure to paraquat.

The table compares control, acute, and recovery values for eight dependent variables. The data show that severe gas exchange and ventilatory abnormalities develop and must resolve when exposure is discontinued and a recovery period allowed. Animals killed after the second exposure to paraquat showed significant changes in all categories except lung volume, which was reduced compared with control values. The recovery animals differed significantly from acute animals except for PaO₂, which was higher in the recovery animals. The recovery group was indistinguishable from the controls except for a significantly increased lung volume and an increased body weight.

All the control rabbits had grossly normal lungs (fig 1). Nevertheless, rare foci of alveolar infiltrates

similar to those in the animals recovering from exposure to paraquat were seen in most control rabbits microscopically. These foci tended to measure only a few alveoli in diameter and were infrequent.

Rabbits killed three days after the second dose of paraquat showed a variable gross appearance. A few lungs showed no obvious infiltrates, whereas other lungs showed infiltrates affecting as much as two thirds or more of the total sectioned surface of both lungs. The infiltrates were of two types: greyish tan, sharply demarcated nodular infiltrates scattered either diffusely or focally in the lung and indistinct firm reddish brown infiltrates that tended to be more diffuse. Microscopically, the nodular infiltrates were related to terminal airways and consisted of areas of bronchopneumonia characterised by an interstitial infiltrate of histiocytes and smaller numbers of neutrophils, proliferation of bronchiolar and alveolar epithelium, and an alveolar infiltrate of macrophages and smaller numbers of neutrophils (fig 2). The alveolar septa were thickened by cellular infiltrate and oedema. Small blood vessels were often surrounded by a mixture of large and small lymphoid cells; in some cases both large and small airways contained a neutrophilic infiltrate. Microscopically, the reddish brown infiltrate consisted of areas of acute haemorrhage in the alveoli mixed with an intra-alveolar network of fibrin which was occasionally associated with hyaline membranes.

Fig 1 Histology of a typical terminal airway and alveolar tissue from a control rabbit. (Haematoxylin and eosin, $\times 150$).

The lung usually showed both histological patterns, although occasionally only one or other would be seen.

Some of the rabbits killed six weeks after the last dose of paraquat showed areas of focal pleural fibrosis and retraction of the pleura. The sectioned alveolar tissue was grossly unremarkable, however, without obvious fibrosis. Some rabbits did not show these pleural alterations and the lungs were completely normal. Microscopically, in some of these animals tiny foci of fibrosis were seen in relation to terminal airways (fig 3), producing the pleural "puckering." The degree of fibrosis, however, was minimal. In a few of these rabbit's lungs microscopic foci of macrophagic alveolar infiltrate together with the same cellular infiltrate in adjacent alveolar septa could be seen.

Discussion

Most animals exposed to paraquat aerosol develop a pneumonitis and characteristic pathophysiological changes (table). The more acute changes seen after two doses of paraquat in these rabbits (haemorrhage, fibrin, hyaline membranes) are similar to those previously described in acute inhalation para-

quat injury in rabbits,^{10,12} and are also similar to those described by various authors after intraperitoneal or oral administration.¹³

The lack of any pertinent pathological alteration in the rabbits exposed to paraquat after six weeks of recovery is impressive and, even allowing for individual differences in sensitivity to paraquat among rabbits, the lack of any significant long term injury is surprising. Apparently the rabbit possesses tissue defences adequate to detoxify the paraquat before it produces irreversible alveolar injury, and the resolution of the inflammatory exudate from even two occasionally lethal doses (LD_{5-10}) of paraquat is nearly complete. Butler and Kleinerman and Gage noted that rabbits may be resistant to exposure to parenteral paraquat.^{14,15} Martin also noted this resistance in explanted lung cells.¹⁶

In vivo evidence of recovery supports previous reports of biochemical adaptation to oxidant lung injury.¹⁷ These findings show that animals exposed to paraquat rarely sustain permanent synthetic injury unless a lethal dose is given.

Previous work with oxidant injury has shown severe pathological changes after acute exposure and death.^{13,18} The pneumonitis that these authors describe is similar to that seen when animals are

Fig 2 Histology of the terminal lung unit in a rabbit exposed acutely to paraquat. Note interstitial and alveolar infiltrate that obliterates alveoli and proliferation of bronchiolar and alveolar epithelium. (Haematoxylin and eosin, $\times 150$).

killed three days after the second exposure in our system. Montgomery *et al* described persistence of focal lesions and vascular lesions in rat lungs 28 days after sublethal exposure to paraquat.¹⁷ Values for drug metabolism were back to control levels at 28 days after initial reduced metabolic activity. Recovery has not been described. We allowed a longer recovery interval than Montgomery *et al* or Popenoe and used an LD_{10} as opposed to the higher doses given by Popenoe (LD_{30}).^{12,17} Montgomery *et al* did not specify the lethality of the dose of paraquat given. The recovery period was chosen while we followed results of physiological tests, weights, and breathing frequencies. At six weeks, we saw no reason to continue observation as controls and experimentals were indistinguishable. We found this result on both experimental runs that allowed a recovery period.

The findings of decreased frequency of breathing after injury and return to control levels has been puzzling. This observation has been made repeatedly and confirmed statistically with a two way analysis of variance test (parameters are time and experimental group). This pattern of breathing may be used by the animals exposed to paraquat

because of mechanical advantages to minimise dead space breathing, but this finding has not been reported by others. The increased lung volume indicates that despite absence of significant pathology on light microscopic examination, injury has occurred. We noted this previously as did Vizek *et al* who attributed the abnormality to increased vagal tone.¹⁹ The volume decreased after bilateral cervical vagotomy in rats. The increased lung weight would also indicate that inflammation persists but to a lesser degree than in animals killed three days after the last exposure. These abnormalities occurred despite minimal pathological abnormalities; the relative amount of gross disease was noted in multiple sections before standardised and directed sections were taken.

Some animals do not develop pneumonitis or abnormal gas exchange when exposed to a sublethal dose of paraquat. These animals are indistinguishable from controls throughout the study. We postulate that these animals may be subjected to less oxidant injury. Lipid peroxidation may be less, due to increases in antioxidant defences in these animals.^{3,6} Conversely, most affected animals respond to injury with stimulated antioxidant defences and

Fig 3 Histology of a terminal lung unit in a rabbit six weeks after paraquat inhalation. A small fibrous scar (arrow) is present adjacent to terminal bronchiole. (Haematoxylin and eosin, $\times 150$).

eventual architectural restoration, if the injury is sublethal. Recovery as gauged by pathology, morphometrics, bronchoalveolar lavage, arterial blood gases, and compliance is seen in the animals surviving paraquat injury. Recovery is more common with this exposure than progressive interstitial pneumonitis and fibrosis.

Supported in part by the General Medical Research Service of the Veterans Administration.

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