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N-Acetylcysteine in Paraquat Toxicity: Toxicological and Histological Evaluation in Rats

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ABSTRACT

The therapeutic effect of N-acetylcysteine (NAC) in paraquat-treated rats was investigated. The animals were divided into four groups: A = control; B = NAC; C = paraquat; D = NAC + paraquat. In the appropriate groups, paraquat 20 mg/kg body weight was administered intraperitoneally and 1 % NAC solution was provided as drinking water. All surviving rats were killed on the seventh day after paraquat exposure. The lungs were graded histologically on the basis of oedema and cellular infiltration.

On histological examination, the lungs of poisoned rats that had received NAC displayed a tendency towards less oedema and cellular infiltration than those of poisoned rats not treated with NAC. It is concluded that NAC might afford some therapeutic effect against paraquat toxicity in rats.

INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'bipyridinium) is a widely used and powerful herbicide. In the last twenty years it has caused a considerable number of deaths following accidental or intentional ingestion (1,8). The predominant organ damaged is the lung, but other organs such as the kidneys and liver are also affected (1,8). The disproportionate pulmonary injury characteristic of paraquat toxicity is attributed, at least partly, to the preferential accumulation of paraquate in the lung (11) and the continuous exposure of the lung to atmospheric oxygen. Under the aerobic conditions prevailing in the lung, paraquat undergoes oxidation-reduction cycling, which leads to two unfavourable events: generation of superoxide anion and other oxygen-free radicals (4), and depletion of NADPH (2). Superoxide anion and other oxygen-free radicals are oxidants, and may serve to initiate lipid peroxidation of cell membranes, causing tissue damage and death (4). Furthermore, the critical depletion of NADPH per se may be sufficient to disrupt vital cell functions and to render the cell more susceptible to lipid peroxidation.

This majority view of the mechanism of paraquat toxicity has important

therapeutic implications. Thus, it is reasonable to speculate that antioxidants may protect against paraquat-induced oxidant injury. N-acetylcysteine (NAC), a known antioxidant, has been found to reduce paraquat toxicity in hepatocytes in vitro (5) and to protect lung epithelial cells in vitro against oxidant injury (13). However, the position of NAC in the protection of lung oxidant injury in vivo is not clearly defined.

In a previous study NAC was demonstrated to have a protecting effect on paraquat-induced lung damage when administered 24 hours before the poison (15). The aim of this study was to study the possible therapeutic effect of NAC when administered after the poison.

MATERIAL AND METHODS

Male Sprague-Dawley rats (Alab, Sollentuna, Sweden), weighing from 200 to 230 g were used. They were housed in separate cages and were given commercial pellets (Ewos, Södertälje, Sweden) and tap water ad libitum throughout the experiment. A paraquat solution was prepared by dissolving paraquat (Methyl Viologen, Sigma Chemical Company, St Louis, USA) in normal saline to obtain a concentration of 10 mg/ml. A 1% NAC solution was freshly prepared on each study day by dissolving NAC (Fluimucil^{R}, Zambon SpA, Milano-Vicenza, Italy) in water.

The rats were divided into four groups: Group A (n=5) received 0.5 ml of normal saline intraperitioneally (i.p.); group B (n=5) received 0.5 ml of normal saline i.p. and 1% NAC in their drinking water; group C (n=15) received paraquat 20 mg/kg body weight i.p.; group D (n=15) received paraquat 20 mg/kg body weight i.p. and 1% NAC instead of drinking water.

The behaviour, water intake and weight of each rat were recorded daily. On the seventh day after paraquat administration, all surviving rats were anaesthesized with pentobarbital (Inactin^{R}, Byk-Gulden, FRG) 125 mg/kg body weight i.p., and exsanguinated via the abdominal aorta. All rats were then processed according to a modification of the technique employed by Hasan et al. (9). The chest wall was opened and the right lung was tied off with a silk thread. The left lung was inflated and perfused with modified Karnovsky solution (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M cacodylate buffer at a pH of 7.2) through a tracheal cannula under a water pressure of 20 cm for 5 to 10 minutes. The right lung was dissected free, blotted gently and weighed. The left lung was then fixed in 4% neutral-buffered formalin solution, embedded in parrafin, sectioned at 5 um and stained with haemotoxylin-eosin; sections were taken from the upper and lower part of the lung in all the rats, regardless of the surface appearance.

The slides were coded and were subsequently read by an observer (TS) who was blind of their identity. For each slide, about 20 high-power fields were examined at 630 times magnification. The severity of the pulmonary damage was graded histopathologically as follows: Grade 0 = normal; grade 1 = mild oedema and cellular infiltration; grade 2 = moderate oedema and cellular infiltration; grade 3 = severe oedema and cellular infiltration.

Data were analysed by using the unpaired t test with Welch's approximation for water intake, body weight and right lung weight, Fisher's exact probability test for mortality, and the Mann-Whitney U test for light microscopic grade. Differences were considered statistically significant when the P value was less than 0.05.

RESULTS

Behaviour and mortality

Following administration of paraquat, the rats became apathetic and inactive. Forty-eight hours later their fur was ruffled in appearance and they were breathing rapidly. Four paraquat-treated rats that did not receive NAC (group C) died spontaneously (two each on the third and the fourth day), and two paraquat-treated rats that received NAC (group D) died on the third day; this difference was not statistical significant. Generally, group C rats were in a poorer general condition than rats of group D. By the fifth day, most of the paraquat-treated rats showed signs of recovery and behaved normally, like the control rats (group A) and the rats that received NAC alone (group B).

Water and NAC intake

The initial water intake on day 1 (Fig. 1) is significantly lowered both by the NAC-addition to the drinking water (groups B and D) and by the paraquat poisoning (groups C and D). Until day 4 the water intake of group D is increased to a significantly higher lewel than that of group C.

The mean daily water intake per animal was 32 ml in group A, 26 ml in group B, 22 ml in group C and 24 ml in group D. This meant that the average daily NAC intake was 1070 mg/kg body weight in group B rats (mean body weight 243 g), and 1182 mg/kg body weight in rats of group D (mean body weight 203 g).

Body weight

Weight loss was observed in all the paraquat-treated rats one day after the poisoning, and reached a nadir on the second day (Fig. 1). Thereafter there was an increasingly quick weight gain and the initial weight was regained on the fifth day. NAC did not abolish this early weight loss. Control and group B rats gained weight steadily throughout the experiment. The only significant

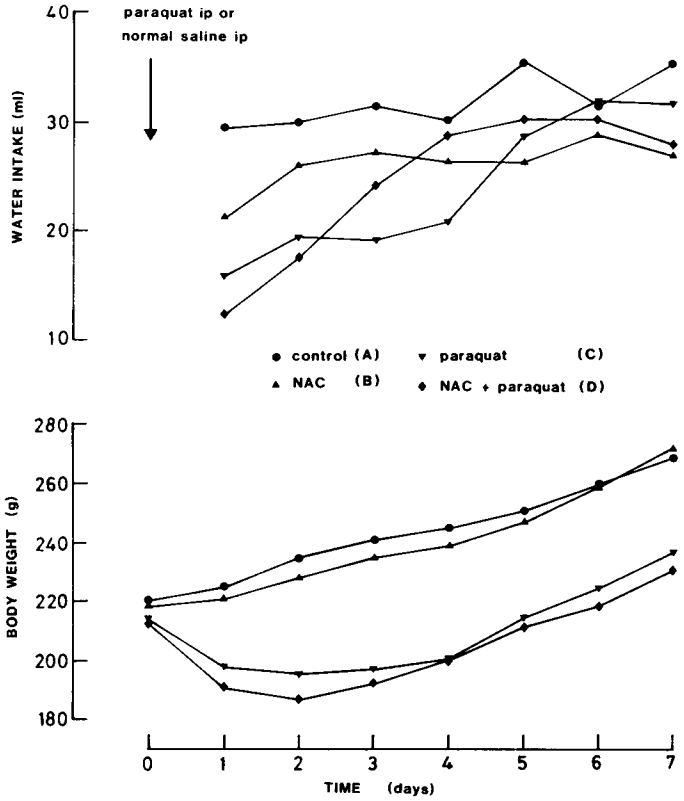


Figure 1. Effect of NAC on the mean daily water intake and the mean daily body weight of rats which recieved paraquat 20 mg/kg body weight i.p. or normal saline 0.5 ml i.p.

difference in body weight was found on day two between groups C and D (Fig. 1).

Right lung weight

The right lung weight of those rats that received paraquat were significantly higher than those that did not receive the poison (Table 1). There was no difference in right lung weight between groups A and B or between groups C and D.

Table 1. Right lung weight (g) in the four experimental groups.

Group	A (control)	B (NAC)	C (paraquat)	D (paraquat + NAC)
Mean	0.74	0.75	0.92	0.92
SD	0.05	0.11	0.21	0.26

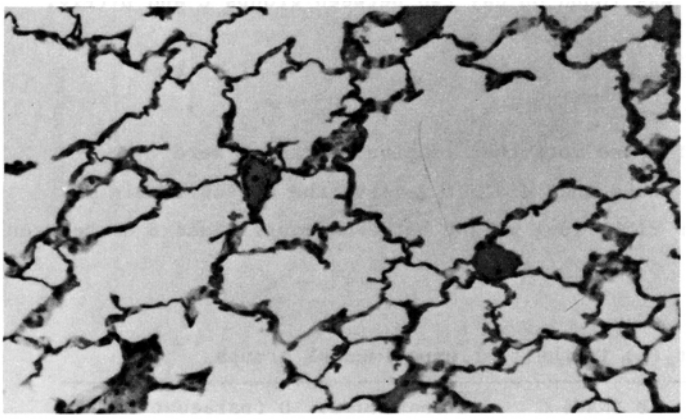
Light microscopy

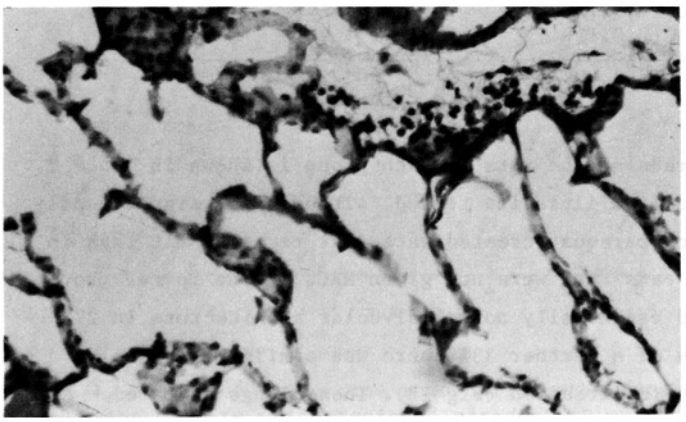
The light microscopic grade of the rats in each group is shown in Table 2. The grade of oedema and cell infiltration tended, although not significantly, to be lower in the lungs of paraquat-treated rats that received NAC than in those of similary treated rats that were not given NAC. In the former group (group D) the lungs showed essentially normal alveolar architecture in 27% (Fig 2A), and in the lungs of a further 33% there was a mild perivascular oedema and little cellular infiltration (Fig 2B). These lungs compared favourably with those of poisoned rats that had not received NAC; in 20% of these the lungs were normal or showed mild changes, but in 47% they displayed atelectasis, marked interstitial and alveolar oedema, and extensive neutrophilic infiltration (Fig 2C). All rats that had only received NAC had normal lungs histologically.

Table 2. Light microscopic grade of the lungs of rats in each group.

Group	Grade: 0	1	2	3
A (Control)	5	-	-	-
B (NAC)	5	-	-	-
C (Paraquat)	1	2	5	7
D (Paraquat + NAC)	4	5	2	4

The light microscopic grade is based on the degree of oedema and cellular infiltration: 0 = normal, 1 = mild, 2 = moderate, 3 = severe.





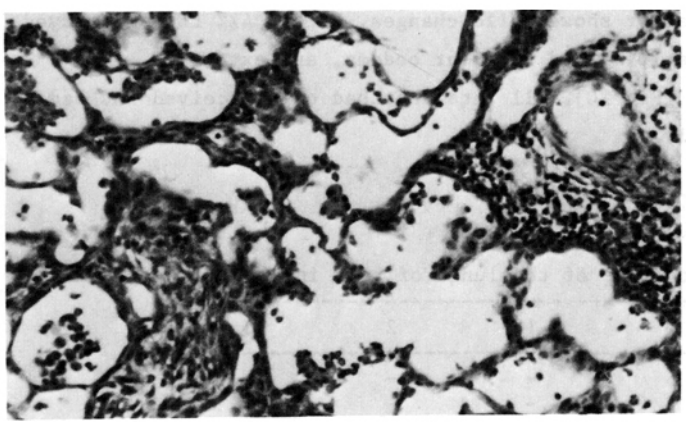


Figure 2. Photomicrographs of rat lungs Hematoxylin staining. Magnification 175 X.

A. Normal lung.

B. Mild perivascular oedema and slight cellular infiltration in paraquat-treated rat that received NAC.

C. Severe interstitial and alveolar oedema, and extensive neutrophilic infiltration in Paraquat-treated rat not given NAC.

DISCUSSION

In this study it was found that NAC, administered after exposure to paraquat, did not prevent the toxicological consequences in rats, although

among poisoned rats receiving NAC there was a tendency towards earlier recovery of normal activity, increased water intake during day 3 and 4 and lower mortality. In a previous study of this laboratory a significant reduction in mortality was noted among rats in which NAC treatment was initiated 24 hours before paraquat administration (15). When the results of both studies are considered together, two conclusions may be drawn: (i) Pre-treatment with NAC has a significant protective effect in paraquat toxicity, and (ii) the effectiveness of NAC is markedly diminished when it is given after the administration of paraquat. The exact protective mechanism of NAC pre-treatment in paraquat toxicity is uncertain. It is conceivable that adequate antioxidant cover by NAC pre-treatment blocks the oxidant chain reaction and buys time for the kidneys to clear the blood of paraquat. Likewise, delayed and inadequate treatment allows paraquat to generate a huge oxidant burden that ultimately overwhelms the antioxidant defence system.

In contrast to the toxicological findings, our histological results indicate that NAC might have some therapeutic effect in paraquat-exposed rat lungs (Table 2 and Fig 2). The histological criteria used here, i.e. oedema and cellular infiltration, are relevant to the evaluation of pulmonary damage. Other authors have reported that pulmonary oedema and haemorrhage accompanied severe paraquat poisoning in man (6,8,10). In previous animal experiments, morphological examination of paraquat-damaged lungs revealed early alveolitis of mainly neutrophils and macrophages (12,14), an observation confirmed in the present study (Fig 2C). Shoenberger et al. (12) have postulated that paraquat induces alveolar macrophages to release neutrophil chemotactic factor (for recruitment of neutrophils), alveolar-macrophage-derived growth factor and fibrinonectin (for recruitment and replication of fibroblasts). Consequently, neutrophils will provide toxic oxidant radicals, and fibroblasts will cause pulmonary fibrosis. Although paraquat has been shown to denude the alveolar walls of their epithelial lining (14), it has been reasoned that pulmonary fibrosis is unlikely to result simply from fibrous repair of damaged alveolar walls (10). For all the above-mentioned reasons, we believe our microscopic findings indicate that NAC rendered some therapeutic effect against paraquat toxicity in rats.

The antioxidant property of NAC has been discussed in previous publications (3,5,10). It is postulated that this compound directly scavenges toxic oxygen-free radicals, or directly impairs the ability of inflammatory cells to generate oxygen-free radicals (3). In addition, it may replenish intracellular glutathione stores by increasing intracellular substrates for the glutathione reductase pathways (3,5,10). Glutathione then accelerates the conversion of toxic lipid hydroperoxides, formed during lipid peroxidation, to less toxic

lipid alcohols. In clinical practice, NAC is used as a mucolytic agent for chronic bronchitis and cystic fibrosis, an antidote for paracetamol poisoning, and a prophylactic for meconium ileus equivalent. Ferrari (7) reported that the common adverse effects associated with such clinical use of NAC were trivial gastric complaints such as nausea, diarrhoea and dyspepsia. The NAC doses used in clinical practice were also high - up to 18 g/day orally or 300 mg/kg body weight over 20 hours intravenously. NAC is thus a relatively non-toxic pharmacological agent.

The lower water intake in the NAC groups (B and D, Fig 2) might be explained by the acrid taste of the NAC. In further experiments a more effective way of administration should be considered.

It is difficult to extrapolate these insignificant results of animal experiments to clinical application in man. We believe that clinical trials of NAC might still be premature.

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REFERENCES

1. Addo, E. & Poon-King, T.: Leucocyte suppression in treatment of 72 patients with paraquat poisoning. Lancet 1:1117-20,1986.
2. Aldrich, T.K., Fisher, A.B. & Forman, H.J.: Paraquat inhibits mixed-function oxidation by rat lung. J Appl Physiol 54:1089-93,1983.
3. Bernard, G.R., Lucht, W.D., Niedermeyer, M.E., Snapper, J.R., Ogletree, M.L. & Brigham, K.L.: Effect of N-acetylcysteine on the pulmonary response to endotoxin in the awake sheep and upon in vitro granulocyte function. J Clin Invest 73:1772-84,1984.
4. Bus, J.S., Cagen, S.Z., Olgaard, M. & Gibson, J.E.: A mechanism of paraquat toxicity in mice and rats. Toxicol Appl Pharmacol 35:501-13, 1976.
5. Dawson, J.R., Norbeck, K., Anundi, I. & Moldeus, P.: The effectiveness of N-acetylcysteine in isolated hepatocytes, against the toxicity of paracetamol, acrolein, and paraquat. Arch Toxicol 55:11-15,1984.
6. Fairshter, R.D., Rosen, S.M., Smith, W.R., Glauser, F.L., McRae, D.M. & Wilson, A.F.: Paraquat poisoning: New aspects of therapy. Quart J Med 180:551-65,1976.
7. Ferrari, V.: Safety and drug interactions of oral acetylcysteine related to utilization date. Eur J Respir Dis 61(Suppl 111):151-57, 1980.
8. Fletcher, K.: Paraquat poisoning. In: Forensic Toxicology (ed. B. Ballantyne), pp 86-98. Wright & Sons, Birmingham, 1974.
9. Hasan, F.M., Teplitz, C., Farrugia, R., Huan, E. & Schwartz, S.: Lung function and structure after Escherichia coli endotoxin in rabbits: Effect of dose and rate of administration. Circ Shock 13:1-19,1984.
10. Onyeama, H.P. & Oehma, F.W.: A literature review of paraquat toxicity. Vet Hum Toxicol 26:494-502,1984.

11. Rose, M.S., Lock, E.A., Smith, L.L. & Wyatt, I.: Paraquat accumulation and species specificity. Biochem Pharmacol 25:419-23,1976.
12. Shoenberger, C.I., Rennard, S.I., Bitterman, P.B., Fukuda, Y., Ferrans, V.J. & Crystal, R.G.: Paraquat-induced pulmonary fibrosis: Role of the alveolitis in modulating the development of fibrosis. Am Rev Respir Dis 129:168-73,1984.
13. Simon, L.M. & Suttorp, N.: Lung cell oxidant injury: Decrease in oxidant mediated cytotoxicity by N-acetylcysteine. Eur J Respir Dis 66(Suppl 139):132-35,1985.
14. Torre, C., Varetto, L., Pavanelli, P.L. & Belliardo, F.: Lung morphology in experimental acute paraquat poisoning: SEM observations. Am J Forensic Med Pathol 5:125-29,1984.
15. Wegener, T., Hillerdahl, G., Özesmi, M., Sandhagen, B. & Saldeen, T.: Die Wirkung der N-Azetylzystein-Behandlung auf Paraquat-vergiftete Ratten. Atemwegs- und Lungenkrankheiten 7:303-06,1986.

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