VETERINARSKI ARHIV 74 (2), 141-155, 2004

Effect of paraquat intoxication and ambroxol treatment on hydrogen peroxide production and lipid peroxidation in selected organs of rat

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ANGUELOV, A., M. CHICHOVSKA: Effect of paraquat intoxication and ambroxol treatment on hydrogen peroxide production and lipid peroxidation in selected organs of rat. Vet. arhiv 74, 141-155, 2004.

ABSTRACT

Experiments with the herbicide paraquat were carried out on Wistar rats. The concentration of hydrogen peroxide (H_2O_2) and various lipid peroxidation products (LPP), such as conjugated dienes (CD), lipid peroxides (LH), malonyl-dialdehyde (MDA) and Schiff bases in selected organs of the rat given a single intra-peritoneal dose of 35 mg/kg^{-1} paraquat was examined. The influence of a mucolytic and probably antioxidant drug, ambroxol, on paraquat-induced changes in the concentration of H_2O_2 and LPP was also examined. Paraquat increased the pulmonic, cardiac and hepatic concentration of H_2O_2, CD, LH and MDA approximately fourfold. Although the dose of paraquat was nearly twice the LD_{50} dose, it did not noticeably increase the concentration of these substances in the kidney. Ambroxol alleviated the increase of H_2O_2 in the liver but did not reduce the concentration of CD, LH and CD. The results indicate that ambroxol did not alleviate the increase the pulmonic concentration of H_2O_2, but protected the increase in the concentration of CD and MDA. Moreover, the drug administration alone induced lipid peroxidation in the liver. Ambroxol alone acts as a pro-oxidant.

Key words: paraquat, ambroxol, hydrogen peroxide, lipid peroxidation

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ISSN 0372-5480
Printed in Croatia

Introduction

Paraquat (Pq) is a contact herbicide used worldwide. It may be a cause of incidental and accidental poisonings, most of which are fatal. The critical organs in Pq toxicity are the lung, and changes, both in animals and humans, occur in two phases: an acute or destructive phase with extensive destruction of alveolar epithelial cell and clinical signs of pulmonary oedema lasting for 1-4 days, followed by a chronic or proliferating phase leading to massive lung fibrosis (SMITH et al., 1973; DEARDEN et al., 1978). Pathological changes were also observed in kidney, liver and heart at high doses (DAVIES et al., 1977; BEEBEEJAUN et al., 1971), but death is usually associated with respiratory insufficiency. The proposed mechanism of Pq toxicity is based on its ability to undergo one-electron reduction catalyzed by microsomal cytochrome P-450 reductase and NADPH, with subsequent reoxidation of Pq radical and formation of superoxide anion (O_2^-). In the reaction catalysed by superoxide dismutase (SOD), O_2^- can be dismutated to H_2O_2, part of which is decomposed by catalase. In the presence of transition metal ions H_2O_2 may be a source of highly toxic hydroxyl radical (Î Í$^-$) which can directly destroy proteins, nucleic acids, polysaccharides and cause peroxidation of membrane lipids, leading to cell death (BUS and GIBSON, 1984). Although several in vitro studies show that Pq can induce lipid peroxidation in various biological materials (BUS et al., 1975; KADIISKA et al., 1993; TOMITA and OKUYAMA, 1993), *in vivo* experiments give conflicting results. Quantification of lipid peroxidation in rats by analysis of ethane exhalation showed a four-fold increase within 6 h. after a median lethal dose (LD_{50}) of Pq (BURK et al., 1980). In another study conjugated dienes (CD) were not elevated in the lungs of mice treated with twice the LD_{50} dose (SHU et al., 1979). Malonyldialdehyde (MDA) levels were elevated in the lungs after Pq treatment of rats fed a selenium-deficient diet, but not in those fed selenium-adequate diets (OMAYE et al., 1978).

Although Pq toxicity was enhanced in mice fed selenium- or vitamin E-deficient diets, and also in those depleted of reduced glutathione (GSH) (BUS et al., 1975), various antioxidant drugs applied in the treatment of Pq intoxication proved unsuccessful (FAIRSHTER, 1981). Some expectations, however, may be linked with a bromhexine derivative ambroxol (trans-4[2-amino-3.5-dibrombenzyl-amino]-cyclohexan hydrochloride), a drug

from a class of expectorants which has been used in adjuvant therapy of chronic obstructive pulmonary disease and other chronic respiratory disorders (OLIVIERI et al., 1987; FRASCHINI et al., 1988). It increases the production of surfactant by type II pneumocytes (CERUTTI and KAPANCI, 1979), accelerates the maturation of foetal lung (PETTEN et al., 1978), and therefore has also been used in the treatment of infant respiratory distress syndrome (SALZER et al., 1986). Recently, a wide spectrum of its antioxidant activity has been reported: ambroxol protected α_1 -antiprotease against oxidative inactivation by chloramine-T (ROZNIECKI and NOWAK, 1987), inhibited the generation of reactive oxygen species from activated phagocytes in vitro (WINSEL and BECHER, 1992) and protected lung and heart lipids from oxidative stress provoked by intravenous injection of lipopolisaccharide in mice (NOWAK et al., 1993). Ambroxol also revealed a protective effect on acute Pq poisoning in rats, significantly improving the survival rate (DONNINI et al., 1990). It has had a protective effect on Pq-induced reduction of total poisoning in rats, significantly improving survival rate (SALMONA et al., 1992).

Considering the above, we decided to evaluate whether a single i.p. dose of Pq increases the concentration of H_2O_2 and lipid peroxidation products, such as conjugated dienes, lipid hydroperoxides, malonyldodialdehyde and Schiff bases in liver, heart, lung, and kidney, and also whether ambroxol treatment influences the Pq-induced changes in the concentration of H_2O_2 and lipid peroxidation products in internal organs of the rat.

Materials and methods

Paraquat (methyl viologen), Hoechst 33258, horseradish peroxidase, homovanilic acid, sodium dodecyl sulphate, butylated hydroxytoluene and tetra-methoxy-propane were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), ambroxol-HCl (trans-4[2-amino -3.5-dibrombenzyl-amino]-cyclohexanhydrochloride) was obtained from Boehringer Ingelheim (Vienna, Austria), Brietal (methohexital sodium), Thiobarbituric acid (TBA), pyridiene, trichloracetic acid (TCA), EDTA, phosphate-buffered saline (PBS), cyclohexane, N-butanol, acetic acid, methanol and other reagents were procured from Fluka, (Switzerland).

Forty male locally-bred Wistar rats weighing 185-200 g were used. Animals were fed with standard diet and were allowed free access to both food and water. All administered solutions were sterile and were injected after disinfection with disposable syringes and needles. Twenty animals were injected intraperitoneally (i.p.) once daily for three consecutive days three doses of 70 mg/kg^{-1} ambroxol-HCl, diluted in 1ml of 0.9% NaCl, while the other 20 animals were injected with 1 ml of diluent alone. On day 3, 30 min. after ambroxol (or saline) injection, ten animals from each treatment group received a single i.p. dose of 35 mg/kg^{-1} Pq according to DONNINI et al. (1990) dissolved in 1ml of 0.9% NaCl, whereas the remaining animals were injected with 1 ml of diluent alone. This dose of Pq being 1.75-fold, the LD_{50} dose, was chosen because of interest in the mechanism whereby Pq causes severe, often fatal, intoxication. On day 4 rats received a final i.p. dose of ambroxol (or saline) in the same manner as on days 1-3. Thus, animals were finally divided into four groups of ten rats each: paraquat (Pq), ambroxol/paraquat (A+Pq), ambroxol (A), and saline control (C). On day 5 (48 h. after Pq injection) animals were sacrificed with Brietal (50 mg i.p.) and dissected. Organs were immediately transferred at -80 ^{o}C and stored no longer than 7 days. Storing at this temperature prevents redox cycling of Pq and artifacticious peroxidation of lipid membranes. On the day of measurement organs were homogenized in ice-cold polytron homogenizer PBS (125 mg tissue /1 ml^{-1}).

Conjugated dienes were measured according to method of OHAKAWA et al. (1976). A 500 ěl of homogenate was mixed with 7 ml chloroform/methanol mixture (1:2, v/v), shaken for 2 min. and centrifuged at 1500 g for 5 min. A 5-ml aliquot of the lower layer was mixed with 2 ml distilled water acidified with HCl to pH 2.5.The solution was shaken again for 2 min. and centrifuged as above. The chloroform layer was aspirated and dried under a flow of nitrogen. Residues were diluted with 2 ml heptan and absorbance was read against a heptan blank at 233 nm using Ultrospec III (Pharmacia LKB). Results were presented in absorbance units.

Lipid hydroperoxides (LH) were measured using the method of BUEGE and AUST (1978). A 0.1 ml of homogenate was mixed with 0.1 ml of 8.1% sodium dodecyl sulphate, 0.75 ml of 20% acetic acid buffered to pH 3.5, 0.75 ml of 0.8% TBA, and 0.3 ml of distilled water. The solution was

boiled for 60 min., allowed to cool to room temperature and the chromogen formed was extracted into an n-butanol-pyridine mixture (25:1, v/v) by vigorous shaking for 30 s. Following centrifugation (1500 g for 10 min. at 25 °C), LH conjugated with TBA were measured spectrophotometrically at 532 nm in butanol-pyridine layer against a butanol-pyridine mixture. Results were presented in arbitrary absorbance units.

Malonyldialdehyde (MDA) concentration was estimated with the method of YAGY (1987). A 100 µl aliquot of homogenate was added to 1 ml of 0.05 M sulphuric acid and 0.5 ml of 1.2 M TCA. Following mixing for 20 s. the solution was allowed to stand for 5 min.

After centrifugation (at 1500 g for 10 min. at 4 °C) supernatant was discarded and the pellet resuspended in 2 ml of TBA solution (0.67 g in 100 ml with distilled water and diluted 1:1 with glacial acetic acid). The solution was boiled for 30 min., allowed to cool at room temperature and the chromogen formed was extracted into 2.5 ml of butanol by vigorous shaking for 30 s. Following centrifugation (at 1500 g for 10 min. at 25 °C), TBA-reactive substances in the butanol layer were measured spectrofluorometrically using Perkin Elmer Luminescence Spectrometer LS-50/Norwalk, CT (operating in the read mode). Excitation and emission were measured at 515 nm and 546 nm, respectively. Readings were converted into nanomoles per litre by use of regression equation $Y = 0.021 X - 0.076$ (Y = nmol l^{-1} MDA; X = intensity of emission at 546 nm expressed in arbitrary units). The regression equation was prepared from three series of calibration experiments with six increasing concentrations of tetra-methoxy-propane, used as a standard of MDA (0.1-10 nmol l^{-1}). The lower limit of detection was 0.1 nmol l^{-1}.

Schiff bases (SB) measurements were performed according to BUEGE and AUST (1978). A 70-ěl aliquot of homogenate was mixed with 980 ěl of chloroform-methanol mixture (2:1, v/v), adjusted to pH 2.3 with 280 ěl of distilled water and then vigorously shaken and centrifuged (at 1500 g for 10 min.) The SB were determined spectrofluorometrically. Excitation was measured at 430 nm and emission at 360 nm. Results were expressed in arbitrary units of fluorescence.

Measurements of DNA were performed according to the method of LABARCA and PAIGEN (1980). A 1 ěl of homogenate was mixed with 3 ml of

buffer (5 mM Na_2HPO_4, 2 M NaCl, 2 mM EDTA, pH 7,4) and with 15 ěl of Hoechst 33258 (200 ěg ml^{-1} H_2O). DNA concentration was determined spectrofluorometrically. Extraction and emission were measured at 365 nm and 458 nm, respectively. Readings were converted into ng ml^{-1} standard simple using regression equation Y = 1.23 X - 3.15 (Y = ng DNA homogenate; X = intensity of emission at 458 nm expressed in arbitrary units) obtained from three series of calibration experiments with eight increasing (12.5 - 500 ng ml^{-1}) DNA concentrations. The lower limit of DNA detection was 12.5 ng ml^{-1}.

Hydrogen peroxide concentration was determined according to the method of RUCH et al. (1983). A 10 ěl aliquot of homogenate was mixed with 90 ěl of PBS and 100 ěl of horseradish peroxidase (1 U ml^{-1}) containing 400 ěmol/l homovanilic acid (HVA) and incubated for 60 min. at 37 °C. Afterwards, the sample was mixed with 300 ěl of PBS and 125 ěl of 0.1 M glycine-NaOH buffer (pH 12.0) with the addition of 25 mM EDTA. The HVA-derived oxidation product, as a measure of the amount of H_2O_2, was determined spectrofluorometrically. Excitation was measured at 312 nm and emission at 420 nm. Readings were converted into nanomoles per litre using regression equation Y=0.038 X - 0.32 (Y = nmol H_2O_2 l^{-1} homogenate; X = intensity of emission at 420 nm expressed in arbitrary units) obtained from a series of calibration experiments with 19 increasing (0.01-10 nmol l^{-1}) H_2O_2 concentrations. The lower limit of H_2O_2 detection was 0.01 nmol l^{-1}.

All measurements were performed in duplicate. Reproducibility in all assays was better that 90%. The mean value for separate samples was calculated and standardized for ng DNA. Results are expressed as the mean ± standard deviation. The analysis of variance (ANOVA) was applied to estimate statistical significance. All calculations were performed by use of Microsoft-EXCEL 5.0.

Results

All animals survived the trial for 48 h. after Pq administration (groups Pq and A+Pq) but were severely dyspnoeic, with a rapid and shallow respiratory rate and with red-coloured droplets forming around the month. At autopsy, lungs were hyperaemic. Mean weight of lungs was 1.4 ± 0.4 g

in the Pq group ($P \leq 0.007$), 1.6 ± 0.5 g in the A+Pq group ($P \leq 0.004$), and 0.9 ± 0.1 g in the control group. Animals treated with ambroxol alone did not manifest any visible distress; the mean weight of lung in this group was 1.0 ± 0.1 g (NS).

Paraquat increased H_2O_2 concentration in liver homogenates approximately four-fold (4.02 ± 1.98 nmol ng^{-1} DNA, vs 0.85 ± 0.65 nmol ng^{-1} DNA ; $P \leq 0.003$), in lung homogenates 3.9-fold (1.45 ± 0.98 nmol ng^{-1} DNA, vs 0.37 ± 0.31 nmol ng^{-1} DNA, $P < 0.02$ and in heart homogenates 1.6-fold (5.36 ± 1.52 nmol ng^{-1} DNA , vs 3.31 ± 1.15 $x10^{-1}$ nmol ng^{-1} DNA (NS) (Table 1). Ambroxol treatment decreased Pq-induced H_2O_2 concentration to approximately two-fold of the paraquat group level (1.96 ± 1.04 nmol ng^{-1} DNA vs 4.02 ± 1.98 nmol ng^{-1} DNA for paraquat group) in liver, and more weakly in heart and kidney (3.95 ± 1.35 nmol ng^{-1} DNA vs 5.36 ± 1.52 nmol ng^{-1} DNA in paraquat group and 3.61 ± 1.39 nmol ng^{-1} DNA vs 4.20 ± 1.80 nmol ng^{-1} DNA in paraquat group, respectively) (Table 1).

Table 1. Concentration of hydrogen peroxide (nmol ng^{-1} DNA) in homogenates of lung, heart, liver and kidney after a single i.p. dose of paraquat of 35 mg/kg^{-1}, three doses of 70 mg/kg^{-1} ambroxol before and one dose after paraquat treatment. Control group received 1 ml/day i.p 0.89%NaCl for 4 days (mean ± SD).

		Groups			
	Organs	Control n = 10	Paraquat n =10	Paraquat+ambroxol n =10	Ambroxol n =10
1	Lung	0.37 ± 0.31	1.45 ± 0.98**	2.31 ± 1.81	0.81 ± 0.69
2	Heart	3.31 ± 1.15	5.36 ± 1.52*	3.95 ± 1.35	4.86 ± 1.14
3.	Liver	0.85 ± 0.65	4.02 ± 1.98***	1.96 ± 1.04*	1.81 ± 1.22
4.	Kidney	3.85 ± 1.11	4.20 ± 1.80	3.61 ± 1.39	5.89 ± 3.01

* $P < 0.04$ vs control; **$P < 0.02$ vs control; ***$P < 0.003$ vs control

Ambroxol treatment did not reduce concentration of H_2O_2 in lung compared to Pq group (2.31 ± 1.81 nmol ng^{-1} DNA vs 1.45 ± 0.98 nmol ng^{-1} DNA, respectively), but reduced concentrations of CD and MDA in this organ. (Table 1).

Ambroxol alone induced H_2O_2 concentration slightly in all organs compared to control group (Table 1).

We also found slight changes in H_2O_2 concentration in Pq and A groups compared to control level in the kidney (Table 1).

Table 2. Concentration of conjugated dienes (absorbance units ng^{-1} DNA) in homogenates of lung, heart, liver and kidney after a single i.p. dose of paraquat of 35 mg/kg^{-1}, three doses of 70 mg/kg^{-1} ambroxol before and one dose after paraquat treatment. Control group received 1 ml/day i.p. 0.89% NaCl for 4 days (mean ± SD).

		Groups			
	Organs	Control n = 10	Paraquat n = 10	Paraquat + ambroxol n = 10	Ambroxol n = 10
1.	Lung	2.25 ± 2.09	3.35 ± 1.80	2.82 ± 1.51	1.08 ± 0.39
2.	Heart	2.48 ± 1.95	4.05 ± 1.97	3.15 ± 2.60	3.79 ± 2.35
3.	Liver	1.19 ± 0.80	4.05 ± 1.97**	4.21 ± 2.61**	3.06 ± 1.43*
4.	Kidney	2.45 ± 1.55	2.54 ± 1.46	2.52 ± 1.29	2.78 ± 1.26

*P <0.03; **P< 0.007 vs control

Table 3. Concentration of lipid hydroperoxide (absorbance units ng^{-1}DNA) in homogenates of lung, heart, liver and kidney after a single i.p. dose of paraquat of 35 mg/kg^{-1}, three doses of 70 mg/kg^{-1} ambroxol before and one dose after paraquat treatment. Control group received 1 ml/day i.p. 0.89% NaCl for 4 days (mean ± SD).

		Groups			
	Organs	Control n = 10	Paraquat n = 10	Paraquat + ambroxol n = 10	Ambroxol n =10
1.	Lung	0.49 ± 0.41	0.92 ± 0.39	1.39 ± 0.69	0.50 ± 0.15
2.	Heart	1.39 ± 0.42	2.25 ± 1.21	2.35 ± 1.07	2.61 ± 1.26
3.	Liver	0.50 ± 0.29	1.61 ± 0.80**	1.81 ± 0.98**	1.19 ± 0.71*
4.	Kidney	0.60 ± 0.38	0.82 ± 0.50	1.29 ± 0.71	0.95 ± 0.59

*P<0.04 vs control; **P<0.03 vs control

Paraquat induced lipid peroxidation in the liver. Concentrations of CD, LH and MDA were significantly increased in the Pq-treated group and were as follows: 4.05 ± 1.97 $x10^{-3}$ U ng^{-1} DNA vs 1.19 ± 0.8 $x10^{-3}$ nmol ng^{-1} DNA for control group ($P \leq 0.007$), 1.61 ± 0.90 $x10^{-3}$ U ng^{-1} DNA vs 0.5 ± 0.2 x 10^{-3} nmol ng^{-1} DNA ($P \leq 0.04$) and 2.75 ± 0.1.0 $x10^{-1}$ nmol ng^{-1} DNA vs 0.96 ± 0.5 x 10^{-1} nmol ng^{-1} DNA ($P \leq 0.05$), respectively (Tables 2, 3, 4). Ambroxol did not reveal any protective effect. Moreover, ambroxol administered alone induced lipid peroxidation in the liver (Table 3). Concentrations of CD and LH after ambroxol treatment were significantly

higher, and mean values were $3.06 \pm 1.5 \times 10^{-3}$ Ung^{-1} DNA vs $1.19 \pm 0.8 \times 10^{-3}$ ($P \leq 0.03$) and $0.98 \pm 0.51 \times 10^{-3}$ Ung^{-1} DNA vs $0.5 \pm 0.29 \times 10^{-3}$ nmol ng^{-1} DNA / $P \leq 0.03$/, respectively. Paraquat did not cause any increase in concentration of lipid peroxidation products in the lung, a critical organ in

Table 4. Concentration of malonyldialdehyde (nmol ng^{-1} DNA) in homogenates of lung, heart, liver and kidney after a single i.p. dose of paraquat of 35 mg/kg^{-1}, three doses of 70 mg/kg^{-1} ambroxol before and one dose after paraquat treatment. Control group received 1 ml/day i.p. 0.89%NaCl for 4 days (mean ± SD).

		Groups			
	Organs	Control n = 10	Paraquat n = 10	Paraquat+ambroxol n = 10	Ambroxol n = 10
1.	Lung	0.89 ± 0.48	2.90 ± 1.68*	1.90 ± 1.54	1.26 ± 1.02
2.	Heart	2.84 ± 2.21	2.71 ± 2.45	2.09 ± 1.26	2.79 ± 2.08
3.	Liver	0.96 ± 0.50	2.75 ± 1.00*	1.71 ± 0.99	1.49 ± 1.28
4.	Kidney	1.81 ± 1.01	1.50 ± 1.12	1.70 ± 1.10	2.00 ± 1.45

*P<0.05 vs control

Table 5. Concentration of Schiff bases (emission units ng^{-1} DNA) in homogenates of lung, heart, liver and kidney after a single i.p. dose of paraquat of 35 mg/kg^{-1}, three doses of 70 mg/kg^{-1} ambroxol before and one dose after paraquat treatment. Control group received 1 ml/day i.p. 0.89%NaCl for 4 days (mean ± SD).

		Groups			
	Organs	Control n = 10	Paraquat n = 10	Paraquat + ambroxol n = 10	Ambroxol n = 10
1.	Lung	0.41 ± 0.20	0.82 ± 0.41	1.30 ± 0.60*	0.65 ± 0.48
2.	Heart	1.09 ± 0.72	0.39 ± 0.30	2.01 ± 0.59	1.11 ± 0.60
3.	Liver	1.30 ± 0.60	1.85 ± 0.72	3.19 ± 2.21	1.52 ± 0.72
4.	Kidney	2.19 ± 1.56	1.94 ± 1.34	1.16 ± 0.89	2.71 ± 1.61

*P< 0.03 vs control

its toxicity. Only combination treatment (A + Pq) resulted in an increase of concentration of SB, the final product of lipid peroxidation ($1.3 \pm 0.6 \times 10^{-1}$ vs $0.41 \pm 0.2 \times 10^{-1}$ nmol ng^{-1} DNA) (Table 5) ($P \leq 0.03$). We found no significant changes in lipid peroxidation products in heart and kidney. The results of CD, LH, MDA and SB in all examined organs are presented in Tables 2, 3, 4 and 5.

Discussion

In our experiment, Pq increased concentration of H_2O_2 and products of lipid peroxidation in the lung, a key organ in Pq toxicity, and is consistent with other investigations (BUS and GIBSON, 1984; BUS et al., 1976).This observation is consistent also with those of TAKENAKA and GOTO (1994), who measured TBA reactive substance (TBARS) in various organs of mice following i.p. administration of 60 mg/kg^{-1} Pq (twice the LD_{50} dose for mice).They showed the maximum level of TBARS at 9 h. in the liver, 12 h. in the kidney and 15 h. in the serum, although they observed no increase in TBARS in the lung. Moreover, they did notice a decrease in TBARS concentration in this organ at 15 h., accompanied by an increase in the concentration of catalase and SOD. In the study carried our by BUS et al. (1976), Pq administered at a dose of LD_{50} to mice significantly decreased GSH in the liver, but not in the lung. In the same study, exposure of rats to Pq in drinking water for 3 weeks significantly increased the activity of pulmonary GSH reductase and glucose-6-phosphate dehydrogenase. In another study, GSH peroxidase, GSH reductase and SOD activities were increased 48 h. after a single dose of Pq (REDDY et al., 1977). The ability of Pq to stimulate the antioxidant protection mechanism was also observed in red blood cells (BAINY et al.1994). Induction of the synthesis of antioxidant enzymes, particularly in lung, may also explain decreased lipid peroxidation in this organ in the present study. However, our results do not accord with the hypothesis that peroxidation of lung lipid is the key mechanism in Pq toxicity.

Our results further indicate that after 48 h. Pq induces the production of H_2O_2, CD, LH and MDA in the liver. After both the oral and i.p. routes of administration, this is the main organ of the metabolism and it probably explains the incidence of marked peroxidation. There is also a possibility that Pq is stored in this organ, where it may be a substrate for lipid peroxidation. There are reports of biphasic elimination of Pq from the plasma of rats after i.p. injection (SHARP et al. 1972), and oral administration (MURRAY and GIBSON, 1972), with a rapid phase of 20-30 min. half-life and a slow phase of 56 h.

In our experiment, administration of ambroxol for four consecutive days (3 days of pre-treatment plus 1 day after Pq injection) prevented the

Pq-induced increase in H_2O_2 production in the liver, but it did not protect against lipid peroxidation. Moreover, ambroxol administered alone stimulated lipid peroxidation in liver. NOWAK et al. (1994) reported that ambroxol administered i.p. to mice (0.169 mmol kg^{-1}) once daily for three consecutive days protected lung lipids from peroxidation induced ex vivo either by heat or H_2O_2. It was equipotent to N-acetylcysteine and stronger than methionine. Ambroxol, however, did not protect heart and liver lipids. In another study they examined in detail its antioxidant properties in vitro (NOWAK et al., 1993). Ambroxol was found to be an effective scavenger of HClO and OH^- radicals and also revealed a capacity to decompose H_2O_2 (an observation consistent with our results), although its protective effect on O_2^--mediated auto-oxidation of pyrogalol was weak. The latter observation may explain the lack of protection afforded by ambroxol against lipid peroxidation induced by Pq, a compound generating O_2^-.

The ability of ambroxol to induce lipid peroxidation in the liver could point to the possibility of its pro-oxidant activity. Such activity has been observed in many systems containing antioxidants. Ascorbic acid in the presence of transition metal ions can generate $OH^\cdot$ in chemical (Fe^{2+}- EDTA - H_2O_2), enzymatic (xanthine-xanthine oxidase- Fe^{2+}- EDTA) or cellular systems (human polymorphonuclear leucocytes or murine peritoneal macrophages) (NOWAK et al., 1991; NOWAK et al., 1991a). á-tocopherol in high concentrations also acts as a pro-oxidant (PORTER et al., 1995). It seems probable that ambroxol in certain conditions can easily become a pro-oxidant and promotes lipid peroxidation. Conditions for revealing such activity by this particular substance need further investigation.

Our observations, however, do not refute the potential usefulness of this drug in the treatment of Pq intoxication. In another study, ambroxol alleviated the Pq-induced reduction of total phosphorolipid content and normalized the decreased ratio of palmatic acid to stearic acid in bronchoalveolar lavage fluid of rats (SALMONA et al., 1992), and may explain its protective effect in acute Pq intoxication in rats (DONNINI et al., 1990). The mechanism of Pq toxicity seems to be complex and, despite the peroxidation of lipid membranes and depletion of cellular NADPH, may involve other mechanisms such as surfactant depletion or the generation of nitric oxide (BERISHA et al., 1994) and pro-inflammatory cytokines (BIANCHI et al., 1993).

In conclusion our results indicate that:

a. Paraquat induce H_2O_2 production and lipid peroxidation in the critical organ, the lung. It also has no effect on the connection of H_2O_2 and lipid peroxidation products in kidney.

b. Paraquat increases the hepatic concentration of H_2O_2 and lipid peroxidation products.

c. Ambroxol inhibits the Pq-induced increase in concentration of H_2O_2 in the liver, without protecting it against lipid peroxidation. Moreover, the drug administered alone may act as a pro-oxidant.

References

BAINY, A. C., M. A. S. SILVA, M. KOGAKE, L. A. VIDELA (1994): Influence of lindane and paraquat on oxidative stress-related parameters of erythrocytes in vitro. Hum. Exp. Toxicol. 13, 461-465.

BEEBEEJAUN, A. R., G. BEEVERS, W. N. ROGERS (1971): Paraquat poisoning. Prolonged excretion. Clin. Toxicol. 4, 397-407.

BERISHA, H. I., H. PAKBAR, A. ABSOOD, S. SAID (1994): Nitric oxide as a mediator of lung injury due to paraquat. Proc. Natl. Acad. Sci. USA 91, 7445-7449.

BIANCHI, M., G. FANTUZZI, R. BERTINI, L. PERINI (1993): mRNA in human mononuclear cells and pulmonary epithelial cells. Cytokine 5, 525-530.

BUEGE, J. A., S. D. AUST (1978): Microsomal lipid peroxidation. Methods Enzymol. 52, 302-310.

BURK, R. F., R. A. LAWRENCE, J. M. LANE (1980): Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. J. Clin. Invest. 65, 1024-1031.

BUS, J. S., J. E. GIBSON (1984): Paraquat: model for oxidant-initiated toxicity. Environ. Health Perspect. 55, 37-46.

BUS, J. S., S. D. AUST, J. E. GIBSON (1975): Lipid peroxidation: a possible mechanism for paraquat toxicity. Res. Commun. Chem. Pathol. Pharmacol. 11, 31-38.

BUS, J. S., S. Z. CAGEN,M. OLGAAD, J. E. GIBSON (1976): A mechanism for paraquat toxicity in mice and rats. Toxicol. Appl. Pharmacol. 35, 501-513.

CERUTTII , P., Y. KAPANCI (1979): Effect of metabolite VIII of bromhexine (Na 872) on type II epithel of the lung. An experimental and morphological study with reference to surfactant secretion. Respiration 37, 241-251.

DAVIES, D. S., G. M. HAWKSWOTH, P. N. BENNET (1977): Paraquat poisoning. Proc. Eur. Soc. Toxicol. 18, 21-26.

DEARDEN, L. C., R. D. FAISHTER, D. M. McREA, W. R. SMITH, F. L. GLASE (1978): Pulmonary ultrastucture of the late aspects of human paraquat poisoning. Am. J. Pathol. 93, 667-676.

DONNINI, M., M. LUIDETTI, L. DIOMEDE (1990): Ambroxol reduces paraquat toxicity in the rat. Prog. Respir. Res. 25, 329-332.

FAIRSHTER, R. D. (1981): Paraquat toxicity and lipid peroxidation. Arch. Intern. Med. 141, 1121-1123.

FRASCHINI, F., F. SCAGLIONE, G. SCARPAZZA, F. MARCHI (1988): Effect of mucolytic agent on the bioavailability of antibiotics in patients with chronic respiratory diseases. Curr. Ther. Res. 43, 734-742.

KADIISKA, M. B., P. M. HANNA, R. P. MASON (1993): In vivo ESR. spin trapping evidence for hydroxyl radical-mediated toxicity of paraquat and copper in rats. Toxicol. Appl. Pharmacol. 123, 187-192.

LABARCA, C., K. PAIGEN (1980): A simple, rapid and sensitive DNA assay procedure. Anal. Biochem. 102, 344-352.

MURRAY, R. E., J. E. GIBSON (1972): A comparative study of paraquat intoxication in rats, guinea pigs and monkeys. Exp. Mol. Pathol. 17, 317-325.

NOWAK, D., T. PIETRAS, A. ANTCZAK, M. KROL, G. PIASECKA (1993): Ambroxol in-hibits endotoxin -induced lipid peroxidation in mice. Pol. J. Pharmacol. 45, 317-322.

NOWAK, D., A. ANTCHAK, T. PIERAS, P. BIALASIEWICZ, M. KROL (1994): Protective effect of ambroxol against heat- and hydrogen peroxide-induced damage to lung lipids in mice. Eur. Respir. J. 7, 1629-1634.

NOWAK, D., G. PIASECKA, A. ANTCZAK, T. PIETRAS (1991): Effect of ascorbic acid on hydroxyl radical generation by chemical, enzymatic and cellular systems. Importance for antioxidant prevention of pulmonary emphysema. Biomed. Biochem. Acta 50, 265-272.

NOWAK, D., G. PIASECKA, T. PIETRAS, A. ANTCZAK (1991a): Effect of ascorbic acid on killing of lymphocytes and macrophages by hydrogen peroxide. Biomed. Bioche. Acta 50, 1079-1086.

OHAKAWA, H., O. OHISHI, K. YAGI (1976): Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351-358.

OLIVIERI, D., G. ZAVATTINI, G. TOMASINI, S. DAMIOTTI (1987): Ambroxol for the prevention of chronic bronchitis exacerbations: long-term multicenter trial. Respiration 51 (suppl. 1) 42-51.

OMAYE,S. T., K. A. REDDY, C. E. CROSS (1978): Enhanced lung toxicity of paraquat in selenium-deficient rats. Toxicol. Appl. Pharmacol. 43, 237-247.

PETTEN, G. R., G. J. MEARS, P. J. TAYLOR (1978): The effects of Na 872 on pulmonary maturation in the fetal lamb and rabbit. Am. J. Obs. Gynecol. 130, 35-40.

PORTER, N., S. E. CALDWELLl, K. A. MILLS (1995): Mechanisms of free radical oxidation of unsaturated lipids. Lipids 30, 277-290.

REDDY, K. A., R. E. LITOV, T. OMAVE (1977): Effect of pretreatment with antiinflammatory agents on paraquat toxicity in the rat. Res. Commun. Chem. Pathol. Pharmacol. 17, 87-100.

ROZNIECKI, J., D. NOWAK (1987): Effect of ambroxol on the chloramines T-induced decrease of serum elastase inhibitory capacity in vitro. Lung Respir. 4, 14-15.

RUCH, W., P. H. COOPER, M. BAGGOLINI (1983): Assay for H_2O_2 production by macrophages and neutrophils with homovanilinic acid and horse-radish peroxidase. J. Immunol. Methods 63, 347-357.

SALZER, H., H. WEIDINGER, G. SIMBRUNER (1986): Ambroxol versus Betamethason zur Forderung der antepartalen Lungenreife-eine multizentrische Studie. Z. Geburtshilfe. Perinatol. 190, 49-59.

SALMONA, M., M. DONNINI, L. PERIN, L. DOMEDE, M. ROMANO (1992): A novel pharmacological approach for paraquat poisoning in rat and A 549 cell line using ambroxol, a lung surfactant synthesis inducer. Food Toxicol . 30, 789-794.

SHARP, C. W., A. OTTOLENGHI, A. S. POSNER (1972): Correlation of paraquat toxicity with tissue concentration and weight loss of the rat. Toxicol. Appl. Pharmacol. 22, 241-251.

SMITH, D. J., D. HEATH, M. RAY (1973): The pathogenesis and structure of paraquat induced pulmonary fibrosis in rat. J. Pathol. 114, 57-67.

SHU, H., R. E. TALCOTT, S. A. RICE, E. T. WEI (1979): Lipid peroxidation and paraquat toxicity. Biochem. Pharmacol. 28, 327-331.

TAKENAKA, T., F. GOTO (1994): Alteration of lipid peroxidation and the activity of peroxide metabolism enzymes in the liver, kidney and lung following the administration of paraquat in mice. Masui J. 43, 34-40.

TOMITA, M., T. OKUYAMA (1993): Effect of paraquat on the malonyldialdehyde level in rat liver microsomes (*in vitro*). Arch. Toxicol. 68, 187-192.

WINSEL, K., G. BECHER (1992): Effect of ambroxolon chemiluminescence of phagocytic cells and Na-arachidonate induced bronchoconstriction in guinea pigs. Eur. Respir. 5 (suppl. 15), 298.

YAGY, K. (1987): Lipid peroxides and human diseases. Chem. Phys. Lipids 45, 337-351.

Received: 26 June 2003
Accepted: 15 March 2004

ANGUELOV, A., M. CHICHOVSKA: Učinak otrovanja parakvatom i liječenja ambroksolom na stvaranje vodikova peroksida i peroksidaciju lipida u određenim organima štakora. Vet. arhiv 74, 141-155, 2004.

SAŽETAK

Pokusi herbicidom parakvat provedeni su na štakorima Wistar. Istražena je koncentracija vodikova peroksida (H_2O_2) i različitih proizvoda peroksidacije masti kao što su konjugirani dieni, lipidni peroksidi, malonil-dialdehid i Schiffove baze u određenim organima štakora kojima je intraperitonealno dana doza od 35 mg/kg^{-1} parakvata. Također je vrednovan utjecaj mukolitičkog i vjerojatno antioksidacijskog lijeka ambroksola na promjene u koncentraciji H_2O_2 i proizvoda peroksidacije lipida potaknute parakvatom. Parakvat je doveo do približno četverostrukog povećanja koncentracije H_2O_2, konjugiranih diena, lipidnih peroksidaza i malonil-dialdehida u plućnom, srčanom i jetrenom tkivu. Iako je doza parakvata bila blizu dvostruke letalne doze, ipak nije zabilježeno povećanje koncentracije te tvari u bubrežnom tkivu. Ambroksol je ublažio povećanje koncentracije H_2O_2 u jetri, ali nije smanjio koncentraciju konjugiranih diena i lipidnih peroksida u njoj. Rezultati naznačuju da ambroksol nije ublažio povećanje koncentracije H_2O_2 u plućima, ali je spriječio povećanje koncentracije konjugiranih diena i malonil-dialdehida. Povrh toga i samo davanje lijeka je potaklo peroksidaciju lipida u jetri. Sam ambroksol je djelovao kao pro-oksidant.

Ključne riječi: parakvat, ambroksol, vodikov peroksid, peroksidacija lipida