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Short communication

Effect of preinduction of metallothionein on paraquat toxicity in mice

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Abstract. The effect of pretreatment with metallothionein (MT)-inducing metals (Zn, Cu, Bi, Co, Cd or Hg) on paraquat (PQ) toxicity was investigated in mice. PQ lethality was remarkably reduced by pretreatment with the above MT-inducing metals. The protective effect of pretreatment with these metals on PQ lethality was significantly correlated with MT levels in the lung, a target tissue of PQ toxicity, in mice administered MT-inducing metals, but not with MT in the liver or kidney. The increase in pulmonary lipid peroxidation in mice treated with PQ was significantly inhibited by Zn pretreatment. Zn was the most effective of the MT-inducing metals used in this experiment in protecting mice against PQ lethality. Of those monitored, the only pulmonary free radical scavenging factor increased by Zn pretreatment was MT. Other free radical scavenging factors (activities of superoxide dismutase, glutathione peroxidase and catalase, and concentration of non-protein thiols level) were not influenced by Zn treatment. These results indicate that the induction of pulmonary MT protects against the lethality and lung toxicity of PQ. Pulmonary MT may scavenge free radicals produced by PQ, thereby protecting against lethal pulmonary toxicity.

Key words: Metallothionein – Zinc – Paraquat – Free radicals – Toxicity

Introduction

Metallothionein (MT) is a low molecular weight metal binding protein whose synthesis is induced by heavy metals, glucocorticoids and many other factors (Webb 1979). MT has high affinity for heavy metals since it contains many cysteine residues which account for approximately 30% of the total amino acids in this protein molecule (Kojima et al. 1976). Although the physiological role of MT is still unclear, it is known that MT participates in detoxication of heavy metals or maintenance of Zn and Cu homeostasis (Webb 1979). Recently, some investigators have reported that MT shows free radical scavenging ability in vitro (Shiraishi et al. 1982; Thornalley and Vasak 1985). Moreover, cell lines expressing high MT levels are resistant to free radical-inducing agents such as *t*-butyl hydroperoxide (Ochi 1988), adriamycin (ADR) (Webber et al. 1988; Imura et al. 1989), bleomycin (BLM) (Imura et al. 1989) and ionizing radiation (Bakka et al. 1982; Renan and Dowman 1989).

Paraquat (PQ) is a widely used bipyridilium herbicide. PQ toxicity is characterized by delayed development of pulmonary lesions (Bullivant 1966; Clark et al. 1966; Kimbrough and Gaines 1970; Copland et al. 1974), expressed as initial pulmonary edema which progresses to interstitial fibrosis (Murray and Gibson 1972). The mechanism of these adverse effects is presumed to be due to lipid peroxidation mediated through free radical formation (Bus et al. 1976).

The object of this study was to investigate the effect of preinduction of MT on toxicity of PQ in vivo. We have examined the influence of preadministration of several MT-inducing metals on PQ lethality in mice, and the correlation of the protective effect of metals with induced MT levels in mouse tissue.

Materials and methods

Animals and chemicals. Male ICR mice (20–24 g) were purchased from Charles River Japan Inc., Atsugi, Japan. PQ was purchased from Sigma Chemical Company, St Louis, Missouri. Metal compounds and other chemicals were purchased from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. $^{203}HgCl_2$ (2.4 mCi/mg) was purchased from New England Nuclear, Boston, Mass. Metal compounds and PQ were dissolved in saline.

Treatment of animals. Effect of MT preinduction on PQ lethal toxicity: mice were subcutaneously (s.c.) administered $ZnCl_2$ (400 μmol/kg/day), $CuSO_4$ (40 μmol/kg/day), $Bi(NO_3)_3$ (50 μmol/kg/day), $CoCl_2$

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Table 1. Effect of pretreatment with metal compounds on lethality of PQ in mice

Pretreatment		Injection of PQ	No. of survivals							Survival rate of mice
			Days after PQ injection							
Metal	Dose[a]		0	1	2	3	5	6	20	(%)[b]
Control	–	–	7	7	7	7	7	7	7	100
–	0	+	7	6	4	3	2	0	0	0
$ZnCl_2$	400×2	+	7	7	7	6	6	6	6	86
$CuSO_4$	40×2	+	7	6	5	5	4	3	3	43
$Bi(NO_3)_3$	50×2	+	7	7	7	4	3	2	2	29
$CoCl_2$	250×2	+	7	5	5	3	2	1	1	14
$CdCl_2$	5×2	+	7	7	5	4	3	3	3	43
$HgCl_2$	4×2	+	7	7	4	4	3	2	2	28

[a] μmol/kg/day (s.c.)
[b] Determined 20 days after the injection of PQ. Mice were preinjected with the metal compound once a day for 2 days. PQ (200 μmol/kg, i.p.) was injected 24 h after the last injection of the metal compounds

(300 μmol/kg/day), $CdCl_2$ (5 μmol/kg/day) or $HgCl_2$ (4 μmol/kg/day) once a day for 2 days. PQ (200 μmol/kg/day) was administered intraperitoneally (i.p.) to the mice 24 h after the last administration of each metal. MT concentration in mouse tissue was determined 24 h after the last administration of metal compounds.

Effect of MT preinduction on lipid peroxidation induced by PQ. Mice were pretreated with $ZnCl_2$ (400 μmol/kg/day) once a day for 2 days and PQ was injected 24 h after the last administration of $ZnCl_2$. Pulmonary lipid peroxidation was determined 24 h after PQ administration. Superoxide dismutase, catalase and glutathione peroxidase activities, and levels of pulmonary non-protein thiols were determined 24 h after the last administration of $ZnCl_2$ (400 μmol/kg/day x 2, s.c.) without injection of PQ.

Analysis. Lipid peroxidation was determined by measuring the amounts of thiobarbiturate reactive substances (TBA-RS) according to the methods of Ohkawa et al. (1979) and expressed as nmol malondialdehyde (MDA)/g of lung. MT levels were determined by the ^{203}Hg-binding assay (Kotosonis and Klaassen 1977) as modified by us (Naganuma et al. 1987) and expressed as nmol ^{203}Hg bound to MT.

The 5% lung homogenate was prepared with 0.25 M sucrose and centrifuged at 105000 x *g* for 60 min. The supernatant was analyzed for superoxide dismutase activity by the method of Imanari et al. (1977) and for glutathione peroxidase activity by the method of Lawrence and Burk (1976) using H_2O_2 or cumene hydroperoxide as a substrate. Catalase activity in the lung supernatant (1000 x *g*, 10 min) was measured by the method of Cohen et al. (1970). Non-protein thiol level in the lung was

Table 2. MT levels in lung, liver and kidney of mice treated with metal compounds

Metal	Dose[a]	MT (Hg bound nmol/g tissue)		
		Lung	Liver	Kidney
Control		20.4± 7.1	24.8± 9.7	28.3± 3.7
$ZnCl_2$	400×2	82.5± 6.4*	284.8±66.7*	116.8±29.8*
$CuSO_4$	40×2	45.9±14.4	292.2±35.2*	31.3± 3.2
$Bi(NO_3)_3$	50×2	33.8± 9.4	36.2± 7.4	196.6±26.3*
$CoCl_2$	250×2	32.9± 9.8	242.4±21.5*	32.3± 7.1
$CdCl_2$	5×2	49.1±15.4	263.9±26.3*	44.3± 5.7
$HgCl_2$	4×2	28.9±10.1	34.6±10.1	232.6±27.9*

[a] μmol/kg/day (s.c.)
Mice were administered above metal compounds once a day for 2 days. MT was determined 24 h after the last injection of the metal compound. The values are means ± SD for four mice
* Significantly different from control (p <0.001)

determined by the method of Naganuma et al. (1988 a) using 5,5'-dithiobis(2-nitrobenzoic acid). HPLC determination showed that above 99% of lung non-protein thiol was glutathione.

Statistical calculation. Student's *t*-test was used for statistical analysis of data.

Results

The effect of pretreatment with MT-inducing metals on PQ lethality was examined (Table 1). Eighty-six per cent of the Zn-pretreated mice survived, however all mice administered PQ alone died within 6 days after the administration. Pretreatment with other metals were also effective to a greater or lesser degree in depressing the lethal toxicity of PQ. Table 2 shows MT levels in the tissues of mice 24 h after the last administration of MT-inducing metals. MT levels in the lung were significantly increased only by administration of Zn. Moreover, significant increases of hepatic MT levels in mice receiving Zn, Cu, Co or Cd, and in the kidney of mice receiving Zn, Bi or Hg were observed. The survival rate of mice administered PQ after pretreatment with MT-inducing metals significantly correlated only with pulmonary MT concentration ($r = 0.97$, p <0.001) and not with either hepatic ($r = 0.61$) or renal ($r = 0.16$) MT concentration (Fig. 1).

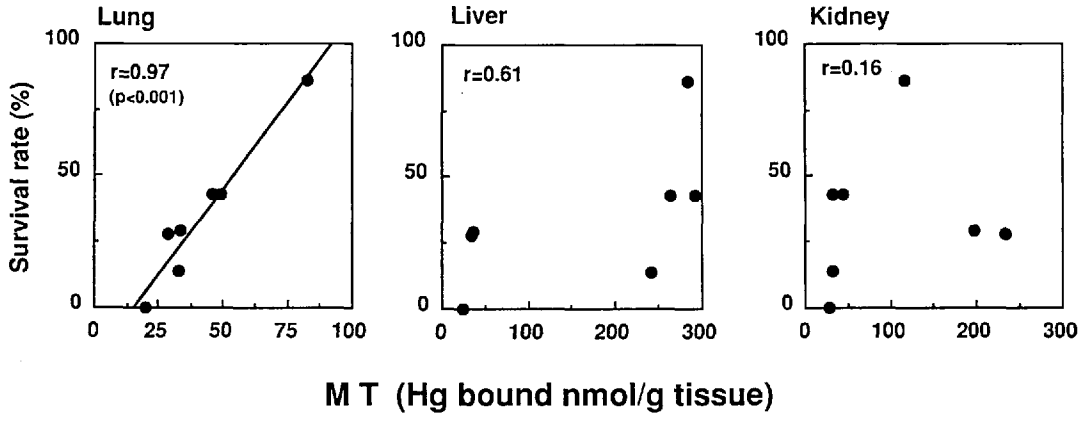

Fig. 1. Correlation between survival rate of mice administered PQ and MT levels in the lung, liver and kidney of mice pretreated with metal compounds

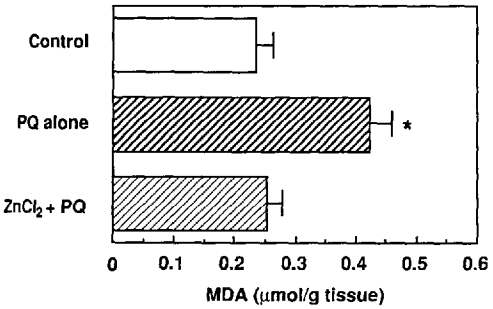

Fig. 2. Effect of pretreatment with zinc on PQ-induced lipid peroxidation in mouse lung. Mice were preadministered $ZnCl_2$ (400 μmol/kg, s.c.) once a day for 2 days. PQ (200 μmol/kg, i.p.) was injected 24 h after the last administration of injection of $ZnCl_2$. MDA in the lung homogenate was determined 24 h after the injection of PQ. The values are mean ± SD for four mice. * Significantly different from control ($p<0.001$)

Table 3. Activities of superoxide dismutase, catalase and glutathione peroxidase, and non-protein thiol content in the lungs of mice treated with $ZnCl_2$[a]

	Control	$ZnCl_2$
Superoxide dismutase (units/mg protein)	7.9 ±1.8	7.2 ±0.8
Catalase (H_2O_2 μmol/min/mg protein)	2.9 ±0.6	2.7 ±0.2
Glutathione peroxidase (NADPH μmol/min/mg protein)		
H_2O_2[b]	0.20±0.01	0.22±0.04
cumene-OOH[b]	0.28±0.02	0.30±0.03
Non-protein thiol (μmol/g tissue)	0.96±0.17	1.01±0.08

[a] Determined 24 h after the last injection of $ZnCl_2$
[b] Substrate used
Mice were administered $ZnCl_2$ s.c. once a day for 2 days. The values are means ±SD for four mice

Since the lung is a target tissue for PQ toxicity and since Zn was the most effective metal in protecting against the lethality of PQ, the effect of pretreatment with $ZnCl_2$ on pulmonary lipid peroxidation was examined (Fig. 2). MDA values determined as an indicator of lipid peroxidation in lungs of mice given PQ alone significantly increased compared with controls. This increase was reduced to control levels by Zn pretreatment. Pulmonary hemorrhage was observed in the lungs of mice given PQ alone, but not in those pretreated with Zn (data not shown). Table 3 compares the levels of free radical scavenging factors in the lung of mice administered Zn. No significant changes in superoxide dismutase, catalase and glutathione peroxidase activities, and non-protein thiol level including glutathione were observed between the Zn-treated group and the controls.

Discussion

Results of the present investigation indicate that preinduction of MT synthesis by administration of appropriate metal compounds protects mice against PQ lethality. PQ is considered to be selectively retained in lung tissue and to induce pulmonary lesions (Kimbrough and Gaines 1970; Sharp et al. 1972). A significant correlation of the protective effect of MT-inducing metals against PQ lethality with MT levels in the lung, but not with those in the liver or kidneys, of mice treated with these metals was observed in this study. This suggests that pulmonary MT induced by these metals may protect against PQ toxicity. These results support findings from an earlier study indicating that increased MT synthesis in the target tissue (heart) protects animals against the toxicity resulting from administration of adriamycin, an active oxygen-inducing substance (Satoh et al. 1988).

MT has recently been recognized as an antidote versus oxidative stress. Bakka et al. (1982) reported that cell lines having high MT content were resistant to X-ray irradiation. Ochi (1988) observed that preinduction of MT synthesis by Zn in cultured cells protected these cells against *t*-butyl hydroperoxide-induced growth inhibition. Furthermore, preinduction of MT synthesis in vivo has reduced lethality of oxidative stress-inducing substances, such as carbon tetrachloride (Cagen and Klaassen 1979), adriamycin (Naganuma et al. 1988b; Satoh et al. 1988) and γ-ray irradiation (Matsubara et al. 1986; Satoh et al. 1989). However, Lohre and Robson (1989) reported that MT overexpression in CHO cells did not prevent cytotoxicity of ionizing radiation and bleomycin. Thus, although contradictory results have been obtained in in vitro experiments cited above, the results of the present study and those of earlier animal experiments indicate that induction of MT synthesis is highly efficacious in protecting animals against the toxicity of substances which produce oxidative stress.

Toxicity of oxidative-stress-inducing agents may be due to the generation of oxygen-free radicals in animals. Some investigators have proposed that MT may have free-radical scavenging ability (Shiraishi et al. 1982; Thornalley and Vasak 1985). Bus et al. (1976) suggested that the lipid peroxidation, initiated by free radicals (superoxide radicals) generated in the cyclic reduction-oxidation of PQ, is responsible for PQ pulmonary toxicity. In the present study, the pulmonary lipid peroxidation induced by PQ was significantly attenuated by preadministration of an MT inducer, Zn. The administration of Zn significantly increased pulmonary MT concentration without affecting the levels of other free-radical scavenging factors, i.e., superoxide dismutase, catalase, glutathione peroxidase and glutathione. These results suggest that MT induced in the lung prevents PQ-induced lipid peroxidation by scavenging free radicals.

Hydroxy radicals may be generated in tissue from superoxide radicals formed by PQ. Furthermore, Sinha et al. (1989) detected the formation of hydroxy radicals in PQ-treated cells. Thornalley and Vasak (1985) indicated that MT quenched both superoxide radicals and hydroxy radicals, and that hydroxy radicals were approximately 10^7-fold more reactive with MT than were superoxide radicals. Abel and Ruiter (1989) reported that in vitro hydroxy radical-induced DNA damage was inhibited by MT. Therefore, a potential mechanism responsible for the protective

effect of MT against PQ toxicity may be the scavenging of hydroxy radicals generated by PQ.

In summary, the present findings indicate that pulmonary MT has a significant protective effect against PQ toxicity in mice. These findings support the view that MT can function as a potent free radical scavenger in vivo.

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