Cellular Glutathione Peroxidase Is the Mediator of Body Selenium To Protect against Paraquat Lethality in Transgenic Mice[1,2,3]

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ABSTRACT The antioxidative role of Se-dependent cellular glutathione peroxidase (EC 1.11.1.9, GPX1) in vivo has not been established. Our objective was to determine the effects of GPX1 knockout or overexpression on the susceptibility of mice to paraquat toxicity and the contributions of GPX1, compared with other selenoproteins and vitamin E, to body defenses against such acute oxidative stress. Four experiments were conducted using 111 GPX1 knockout or overexpressing mice and the respective controls. Mice were fed diets supplemented with Se (as sodium selenite) at 0–0.4 mg/kg and/or all-*rac*-α-tocopheryl acetate at 0–75 mg/kg before intraperitoneal injections of 12.5, 50 or 125 mg paraquat/kg body weight. All mice that received 50 or 125 mg paraquat/kg died spontaneously, and the survival time of mice was (independent of dietary levels of Se per se or α-tocopheryl acetate) solely a function of tissue GPX1 activity ($P < 0.001$). Severe acute pulmonary interstitial necrosis was found only in the GPX1 overexpressing mice and the controls that had extended survival time. Thiobarbituric acid reacting substances in postmortem liver inversely correlated with the tissue GPX1 activity and dietary levels of Se and/or α-tocopheryl acetate. In contrast, all mice that received 12.5 mg paraquat/kg survived and were killed 2 wk after the injection for tissue collection. Compared with the saline injection, this low dose of paraquat resulted in greater ($P < 0.001$) liver and lung F_2-isoprostanes in both the GPX1 knockout mice and the controls. However, there was no difference in plasma alanine transaminase (EC 2.6.1.2) activity or overt injuries in liver, lung and kidney in either group. Our data indicate that GPX1 is the major, if not the only, metabolic form of body Se that protects mice against the lethal oxidative stress caused by high levels of paraquat; it seems less important, however, in protecting mice against the moderate oxidative stress by the low level of paraquat. J. Nutr. 128: 1070–1076, 1998.

KEY WORDS: • *glutathione peroxidase* • *transgenic mice* • *selenium* • *paraquat* • *antioxidation*

Cellular glutathione peroxidase (EC 1.11.1.9; GPX1)[5] was the first Se-containing enzyme to be identified (Flohé et al. 1973, Rotruck et al. 1973). The in vivo antioxidative role of GPX1 continues to be inconclusive, although it was the only known biochemically functional form of body Se for many years. Because there are no direct links between a specific reduction in GPX1 expression and signs of Se-associated diseases, and expression of GPX1 mRNA and activity in tissues is much more susceptible to dietary Se deficiency than that of the other selenoproteins, such as phospholipid hydroperoxide glutathione peroxidase (EC 1.11.1.12; GPX4) (Bermano et al. 1995, Lei et al. 1995, Weitzel et al. 1990), GPX1 has been suggested as a storage form of body Se to serve a homeostatic function in Se metabolism (Burk 1991, Sunde 1994). In contrast, recent studies in cultured cells have shown that GPX1 is indeed protective against oxidative stress in vitro (Geiger et al. 1993, Mirault et al. 1991). However, no direct evidence has been reported to support such a role of GPX1 in vivo.

Pro-oxidant compounds such as paraquat and diquat have been used to study the metabolic functions of Se, GPX1 and other selenoproteins. Although Se effectively protects several species against oxidative stress induced by these compounds, opposite views have been presented regarding the role of GPX1 as a putative mediator of the protection conferred by Se. Burk et al. (1980 and 1995) suggested that GPX1 was not associated with the protection of Se against diquat toxicity in rats, whereas Mercurio and Combs (1986a and 1986b) considered that GPX1 was responsible for the protection of dietary Se against paraquat or nitrofurantoin toxicity in chicks. Although many factors might have contributed to this controversy, the most apparent difficulty has been to distinguish the effect of GPX1 from that of other multiple selenoproteins in the geneti-

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[5] Abbreviations used: ALT, alanine transaminase; BD, basal diet; GPX1, cellular glutathione peroxidase; GPX1(–), cellular glutathione peroxidase knockout mice; GPX1(+), cellular glutathione peroxidase overexpressing mice; GPX4, phospholipid hydroperoxide glutathione peroxidase; LD_{50}, median lethal dose; TBARS, thiobarbituric acid reacting substances.

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cally intact animals used. Furthermore, differences in species, pro-oxidant compounds and intensities of the oxidative stress among these experiments by different groups have complicated the assessment of the GPX1 role. To overcome these limitations, we used the recently developed GPX1 overexpressing or knockout mice (Cheng et al. 1997a, Ho et al. 1997) in this study. After manipulating their body status of Se and vitamin E by dietary treatments, we challenged these transgenic mice with lethal and low doses of paraquat and compared their biochemical and pathologic responses with the controls. Our objectives were to determine the following: *1*) whether GPX1 protected mice against lethal oxidative stress induced by the high doses of paraquat, and how GPX1 interacted with other selenoenzymes and vitamin E in that protection and *2*) whether GPX1 was also protective against moderate oxidative stress induced by the low dose of paraquat.

MATERIALS AND METHODS

Trangenic mice. The generation and characterization of the GPX1 transgenic mouse models have been described in detail previously (Cheng et al. 1997a, Ho et al. 1997). In brief, the GPX1 knockout mice [GPX1(−)] were derived from the 129/SVJ × C57BL/6 mice (Taconic, Germantown, NY) by microinjecting C57BL/6 blastocysts with recombinant embryonic stem cells carrying a target mutation in the GPX1 gene. The GPX1 overexpressing mice [GPX1(+)] were derived from B6C3 (C57BL × C3H) hybrid mice (Taconic). Weanling mice (3 wk old) from the Se- and vitamin E–adequate breeding colonies were used in all of the following experiments. Our experiments were approved by the Institutional Animal Care and Use Committee at Cornell University and conducted in accordance with the NIH guidelines for the care and use of experimental animals. All chemicals and materials were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated.

Dietary treatments before paraquat injection. A Se-deficient (0.02 mg/kg), vitamin E–unsupplemented torula yeast basal diet (BD; Cheng et al. 1997a) was used to manipulate dietary levels of Se and vitamin E in all four experiments. Experiment 1 (2 × 2 factorial) was to determine whether GPX1 was the major metabolic form of body Se that protected mice against paraquat lethality; 21 GPX1(−) and 22 control mice were fed 0 or 0.3 mg Se/kg (as Na_2SeO_3) in the BD (+ 20 mg all-*rac*-α-tocopheryl acetate/kg of diet) for 5 wk. Experiment 2 (2 × 2 factorial) was to determine whether the protection of GPX1 against paraquat lethality was affected by dietary levels of α-tocopheryl acetate; 14 GPX1(−) and 15 control mice were fed 0 or 20 mg of all-*rac*-α-tocopheryl acetate/kg in the BD (+ 0.3 mg Se/kg diet) for 3 wk. Experiment 3 was to determine whether the GPX1 overexpression, above the physiologic level, was beneficial to protection against paraquat lethality; 9 GPX1(+) and 6 control mice were fed the BD supplemented with Se (0.4 mg/kg) and all-*rac*-α-tocopheryl acetate (20 mg/kg) for 12 wk. Experiment 4 was to determine whether GPX1 was also protective against moderate oxidative stress induced by a low dose of paraquat; 12 GPX1(−) and 12 control mice were fed the BD supplemented with Se (0.4 mg/kg) and all-*rac*-α-tocopheryl acetate (75 mg/kg) for 9 wk. Mice were given free access to distilled water and diets, and housed individually in hanging wire cages in a constant temperature (22°C) animal room with a 12-h light:dark cycle. Body weights of individual mice were recorded weekly.

Paraquat injection and sample collection. At the end of feeding, acute oxidative stress in mice was induced by an intraperitoneal injection of paraquat (methyl viologen or 1,1′-dimethyl-4,4′-bipyridinium dichloride). The doses (mg/kg body weight) were as follows: Experiments 1 and 2, 50; Experiment 3, 125 and Experiment 4, 12.5. The two lethal doses (50 and 125 mg/kg) were chosen from a range of doses used in preliminary trials on the basis of their ability to produce distinguishable histopathology in the targeted organs (e.g., lung) and significant differences in survival time between the control and the GPX1-altered groups, but also to yield a relatively consistent survival time within groups. Similar to the approach by Burk et al. (1995) in a diquat study, the low dose of paraquat in Experiment 4 was one fourth of the lethal amount given to the GPX1(−) mice in Experiments 1 and 2. More importantly, this dose, close to the median lethal dose (LD_{50}) in Se-deficient mice (10 mg/kg) estimated by Cagen and Gibson (1977), was chosen to test whether the GPX1 knockout in Se-adequate mice replicated the effect of Se deficiency on paraquat toxicity. Paraquat was dissolved into isotonic saline and filter-sterilized (0.2 μm, Gelman Sciences, Ann Arbor, MI); injection volumes were controlled at 10 mL/kg body weight.

After the injection, mice in Experiments 1–3 were watched constantly during the first 8 h and continuously thereafter except for a 6-h overnight interval. Immediately after the death of mice, fresh samples of liver, kidney and lung were frozen in liquid nitrogen and stored at −80°C for biochemical assays; the remaining tissues were fixed in situ in 10% buffered formalin. Mice in Experiment 4 were fed the same diet for 2 wk after the injection; after being anesthetized with carbon dioxide, they were killed by exsanguination via heart puncture with the use of a heparinized syringe. Tissue samples were collected and processed as described above.

Biochemical assays and histology. Activities of GPX1 and GPX4 in tissues were measured by the NADPH coupled assay, with the use of hydrogen peroxide and phosphatidylcholine hydroperoxide as a substrate, respectively (Cheng et al. 1997a). Liver α-tocopherol concentration was determined by reverse-phase HPLC (McShane et al. 1991). Plasma alanine transaminase (EC 2.6.1.2; ALT) activity was measured by using a kit (ALT 10) from Sigma Chemical. To assess the protection of GPX1 against paraquat-initiated lipid peroxidation, we measured concentrations of thiobarbituric acid reacting substances (TBARS) in liver of the moribund mice in Experiments 1–3 and of F_2-isoprostanes in liver and lung of the mice killed in Experiment 4. Liver TBARS concentration, expressed as malondialdehyde equivalents, was determined by incubating the total homogenate with 2-thiobarbituric acid, with 1,1,3,3-tetraethoxypropane as a standard (Cheng et al. 1997a). Liver and lung F_2-isoprostanes concentration was determined by gas chromatography/mass spectrometry (model HP 5890 A with a HP 5970 series mass selective ion monitoring, Hewlett-Packard, Palo Alto, CA) (Awad et al. 1994, Morrow and Roberts 1994). Histologic changes due to paraquat toxicity were examined by a board-certified veterinary pathologist after sections of the fixed lung, liver, kidney, heart, pancreas, thymus and diaphragm were embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin. In all experiments, one or two mice from each of the treatment groups were injected with saline and killed 1 or 2 wk after the injection; they served as the histologic and/or biochemical controls of paraquat toxicity.

Statistical analysis. Data from Experiments 1 and 2 were analyzed by 2 × 2 factorial ANOVA, with mouse type [control vs. GPX1(−)] and dietary levels of Se or α-tocopheryl acetate as the main treatments. The Bonferroni *t* test was used for mean comparisons. Data from Experiments 3 and 4 were analyzed by Student's *t* test. Because of the very different means for GPX1 activity and survival time among treatment groups, data were examined for normality and equal variance before statistical analysis. In cases of unequal variance, the transformed (logarithmic) data were analyzed to confirm the conclusions from the original data, and individual SEM instead of pooled SEM were listed. All analyses were conducted using SAS (release 6.11, SAS Institute, Cary, NC).

RESULTS

Before paraquat injection, all mice remained healthy and gained body weight during the feeding periods. There were no differences in body weight gain among treatment groups within the same experiment.

Experiment 1. Dietary Se supplementation resulted in a 14-fold ($P < 0.001$) increase in survival time in the control mice, but had no effect in the GPX1(−) mice (**Fig. 1**). Similarly, a difference (10-fold, $P < 0.001$) in survival time between the GPX1(−) and control mice was shown only in the presence of adequate dietary Se. Thus Se-deficient controls had survival times similar to those of both the Se-deficient and Se-adequate GPX1(−) mice. Dietary Se supplementation

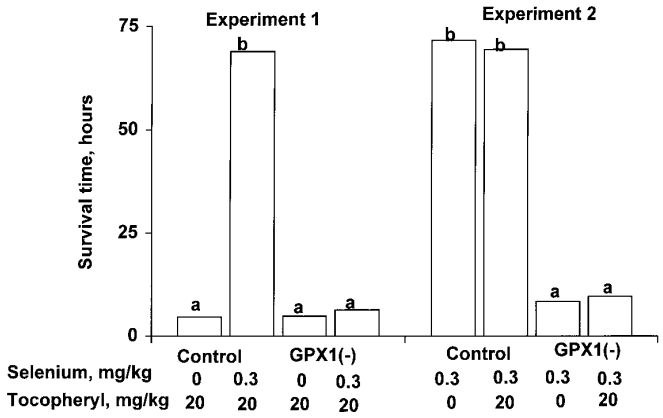

FIGURE 1 Effects of cellular glutathione peroxidase (GPX1) knockout and dietary selenium or α-tocopheryl acetate concentrations on survival time of mice injected with 50 mg paraquat/kg body weight. Values are means, and SEM are as follows: Experiment 1, control (0 mg Se/kg, n = 10) 0.8, control (0.3 mg Se/kg, n = 9) 4.6, GPX1(−) (0 mg Se/kg, n = 9) 0.7 and GPX1(−) (0.3 mg Se/kg, n = 9) 0.4; experiment 2, control (0 mg tocopheryl/kg, n = 6) 6.8, control (20 mg tocopheryl/kg, n = 7) 4.7, GPX1(−) (0 mg tocopheryl/kg, n = 6) 0.6 and GPX1(−) (20 mg tocopheryl/kg, n = 6) 0.9. Bars without the same letter are different ($P < 0.05$) within the same experiment.

resulted in a 95-, 28- and 6-fold increase in GPX1 activity in liver, kidney and lung, respectively, of controls ($P < 0.001$), but did not significantly affect GPX1 activity in these tissues of the GPX1(−) mice (**Table 1**). As expected, controls had 84-, 24- and 9-fold greater ($P < 0.001$) GPX1 activity in liver, kidney and lung, respectively, than the GPX1(−) mice in the presence of adequate dietary Se. There was no significant difference in tissue GPX1 activity between the Se-deficient GPX1(−) mice and controls. Tissue GPX4 activity was affected by dietary Se levels ($P < 0.001$), but not by the GPX1 gene knockout. Selenium-adequate controls had the lowest ($P < 0.05$) liver TBARS concentrations among the four treatment groups. Selenium-deficient controls and GPX1(−) mice had similar liver concentrations of TBARS, which were greater than that of Se-adequate GPX1(−) mice ($P < 0.05$).

Experiment 2. The mouse survival time was again greatly reduced by the GPX1 knockout but was not affected by dietary α-tocopheryl acetate concentrations in either genetic group. Mean survival time of the GPX1(−) mice was approximately one eighth that of the control mice($P < 0.001$) at both levels of dietary α-tocopheryl acetate (Fig. 1). Dietary α-tocopheryl acetate supplementation resulted in a twofold increase in liver α-tocopherol concentrations in both the control and GPX1(−) mice (1.2 vs. 3.8 μg/g tissue, $P < 0.001$), but there was no difference between the two mouse groups (data not shown). Activity of GPX1 in tissues was lower ($P < 0.001$) in the GPX1(−) mice than in controls, whereas activity of GPX4 was similar between the two groups (**Table 2**). Dietary α-tocopheryl acetate level did not affect GPX1 or GPX4 activity. Liver TBARS concentrations were inversely correlated ($P < 0.05$) with GPX1 activity in tissues and/or dietary α-tocopheryl acetate level.

Experiment 3. The mean survival time of the GPX1(+) mice was 10-fold longer ($P < 0.001$) than that of the control mice (**Fig. 2**). Lung and kidney GPX1 activities of the GPX1(+) mice were 1.6-fold and 59% greater ($P < 0.01$) than those of controls, respectively. There were no significant differences in GPX4 activity between these two groups (**Table 3**). As shown previously (Cheng et al. 1997a), liver GPX1 activity was not altered by the GPX1 overexpression in these mice. Liver TBARS concentration in the GPX1(+) mice was only 15% of that in the controls ($P < 0.05$).

Experiment 4. None of the control or the GPX1(−) mice was killed by the low dose of paraquat (12.5 mg/kg). During the 2-wk post-injection period, mice in both groups showed no notable changes in food intake, activity or body weights. When mice were killed 2 wk after the injection, the GPX1(−) mice had lower ($P < 0.001$) GPX1 activity but similar GPX4 activity in liver and lung compared with the controls (**Table 4**). Compared with the injection of saline, this low dose of paraquat caused similar increases (~20-fold, $P < 0.001$) in liver and lung F_2-isoprostanes concentrations in both groups but caused no changes in plasma ALT activity in either group.

Histopathology. In Experiments 1–3, we found severe diffused acute pulmonary interstitial necrosis in the mice that had relatively extended survival time. This typical paraquat

TABLE 1

Activities of selenium-dependent glutathionine peroxidases and thiobarbituric acid reacting substances levels in tissues of control and knockout mice injected with 50 mg paraquat/kg body weight in Experiment 1[1,2]

	Control		Knockout		
	−Se	+Se	−Se	+Se	SEM[3]
	Cellular glutathione peroxidase (GPX1), U/mg protein				
Liver	8.8[a] ± 1.5	844.6[b] ± 38.0	3.6[a] ± 0.8	9.9[a] ± 0.8	
Kidney	19.2[a] ± 6.0	564.4[b] ± 33.2	5.8[a] ± 1.6	22.8[a] ± 5.0	
Lung	14.6[a] ± 2.7	105.6[b] ± 8.2	5.2[a] ± 1.8	10.9[a] ± 1.6	
	Phospholipid hydroperoxide glutathione peroxidase (GPX4), U/mg protein				
Liver	2.5[a]	9.5[b]	3.0[a]	9.6[b]	0.6
Kidney	4.5[a]	14.1[b]	2.6[b]	17.8[b]	1.1
Lung	9.7[a]	15.0[b]	7.7[a]	14.0[b]	1.4
	Thiobarbituric acid reacting substances (TBARS), nmol malondialdehyde/g wet tissue				
Liver	15.2[c]	2.2[a]	16.4[c]	8.8[b]	1.3

[1] Mice were fed diets supplemented with 20 mg all-*rac*-α-tocopheryl acetate/kg and 0 (−Se) or 0.3 (+Se) mg Se/kg. Values are means (n = 3 for GPX1 and GXP4, n = 3–6 for TBARS).

[2] Means without the same superscript letter within the same row are different ($P < 0.05$).

[3] Pooled SEM are listed for measures with equal variance; individual SEM are listed for the measure (GPX1) with unequal variance.

TABLE 2

Activities of selenium-dependent glutathionine peroxidases and thiobarbituric acid reacting substances levels in tissues of control and knockout mice injected with 50 mg paraquat/kg body weight in Experiment 2[1,2]

	Control		Knockout		
	−E	+E	−E	+E	SEM[3]
	Cellular glutathione peroxidase (GPX1), U/mg protein				
Liver	766.6[b] ± 22.6	812.6[b] ± 26.9	8.3[a] ± 1.1	17.5[a] ± 2.6	
Kidney	654.6[b] ± 31.0	585.0[b] ± 33.6	30.1[a] ± 0.9	46.8[a] ± 6.5	
Lung	166.1[b] ± 9.6	165.6[b] ± 8.5	26.3[a] ± 1.1	23.4[a] ± 1.0	
	Phospholipid hydroperoxide glutathione peroxidase (GPX4), U/mg protein				
Liver	12.8	13.3	12.0	10.6	0.5
Kidney	12.3	13.5	14.2	15.3	1.3
Lung	10.1	11.4	10.1	15.0	1.2
	Thiobarbituric acid reacting substances (TBARS), nmol malondialdehyde/g wet tissue				
Liver	6.8[b]	0.8[a]	24.0[c]	6.8[b]	1.5

[1] Mice were fed diets supplemented with 0.3 mg Se/kg and 0 (−E) or 20 (+E) mg all-*rac*-α-tocopheryl acetate/kg. Values are means ($n = 3$ for GPX1 and GXP4, $n = 3$–6 for TBARS).
[2] Means without the same superscript letter within the same row are different ($P < 0.05$).
[3] Pooled SEM are listed for measures with equal variance; individual SEM are listed for the measure (GPX1) with unequal variance.

injury (Bus et al. 1974, Sata et al. 1992, Smith 1987) was characterized by alveolar edema and hemorrhage, perivascular edema and acute inflammation, and alveolar and bronchiolar epithelial necrosis (**Fig. 3**). The rate of such injury was 100% in the Se-adequate controls examined in Experiment 1 (5 of 5), 100% in the controls fed either level of α-tocopheryl acetate in Experiment 2 (13 of 13) and 40% in the GPX1(+) mice examined in Experiment 3 (2 of 5). Severe acute renal tubular necrosis of proximal convoluted tubules was found in the rest of the GPX1(+) mice examined (3 of 5). This was characterized by cytoplasmic eosinophilia, nuclear pyknosis, occasional vacuolar degeneration and sloughing of cells. In contrast, no apparent histopathology in lung, liver, heart, thymus or diaphragm was present in the GPX1(−) mice or the controls that died shortly after paraquat injection. Only minimal renal acute tubular necrosis, likely secondary to hypoxia, was detected in these mice. There was no histopathology in saline-injected mice from any of the treatment groups that were killed 1 wk after the injection.

In Experiment 4, none of the mice in either group showed overt liver necrosis or any injury in lung and kidney. The only sign of paraquat toxicity and difference between the two groups was that the GPX1(−) mice seemed to have more pronounced vacuolar changes in liver than the controls.

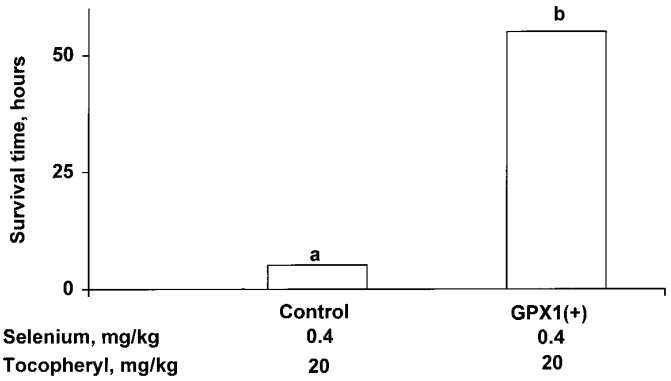

FIGURE 2 Effects of cellular glutathione peroxidase (GPX1) overexpression on survival time of mice injected with 125 mg paraquat/kg body weight ($P < 0.05$). Values are means, and SEM are as follows: GPX1 overexpression ($n = 8$) 9.4, control ($n = 5$) 2.3. Bars without the same letter are different ($P < 0.05$).

DISCUSSION

It is believed (Bus et al. 1974, Smith 1987) that paraquat, through the redox cycling as a futile attempt at its reductive metabolism, produces superoxide radicals ($O_2^{\bullet}$). These superoxide anions are converted to hydrogen peroxide (H_2O_2) by

TABLE 3

Activities of selenium-dependent glutathione peroxidases and thiobarbituric acid reacting substances levels in tissues of control and overexpression mice injected with 125 mg paraquat/kg body weight in Experiment 3[1,2]

	Control	Overexpression	SEM[3]
	Cellular glutathione peroxidase (GPX1), U/mg protein		
Liver	670.5	772.3	69.2
Kidney	640.3[a]	1015.2[b]	34.7
Lung	188.2[a]	486.5[b]	8.6
	Phospholipid hydroperoxide glutathione peroxidase (GPX4), U/mg protein		
Liver	9.7	9.2	1.5
Kidney	16.2	11.7	1.4
Lung	13.1	15.9	0.8
	Thiobarbituric acid reacting substances (TBARS), nmol malondialdehyde/g wet tissue		
Liver	17.2[b]	2.6[a]	0.9

[1] Mice were fed the diet supplemented with selenium (0.4 mg/kg) and all-*rac*-α-tocopheryl acetate (20 mg/kg). Values are means ($n = 4$ for GPX1 and GPX4, $n = 3$ for TBARS).
[2] Means without the same superscript letter in the same row are different ($P < 0.05$).
[3] Pooled SEM.

TABLE 4

Activities of selenium-dependent glutathione peroxidases, activity of alanine transaminase, and concentrations of F_2-isoprostanes in tissues of control and knockout mice injected with 12.5 mg paraquat/kg body weight in Experiment 4[1,2]

	Control	Knockout	SEM[3]
	Cellular glutathione peroxidase (GPX1), U/mg protein		
Liver	845.0[b] ± 23.8	3.6[a] ± 0.3	
Lung	179.7[b] ± 9.0	15.5[a] ± 0.6	
	Phospholipid hydroperoxide glutathione peroxidase (GPX4), U/mg protein		
Liver	8.3	7.8	0.3
Lung	12.4	12.7	1.4
	Alanine transaminase (ALT), U/L		
Plasma	25.2 (26.8)[4]	22.9 (30.2)	3.2 (3.4)
	F_2-isoprostanes, ng/g wet tissue		
Liver	189.7 (8.6)	178.3 (9.8)	14.1 (2.2)
Lung	190.1 (6.2)	188.7 (8.6)	7.4 (4.5)

[1] Mice were fed with diet supplemented with Se (0.4 mg/kg) and all-*rac*-α-tocopheryl acetate (75 mg/kg) before and after the injection of paraquat. Values are means ($n = 5$ for GPX1, GPX4 and F_2-isoprostanes; $n = 10$ for ALT).

[2] Means without the same superscript letter in the same row are different ($P < 0.05$).

[3] Pooled SEM are listed for measures with equal variance; individual SEM are listed for the measure (GPX1) with unequal variance.

[4] Basal values ($n = 2$) of plasma ALT activity and F_2-isoprostanes in liver and lung in saline-injected control or GPX1(−) mice are listed in the parentheses.

superoxide dismutases. If hydrogen peroxide fails to be metabolized to water by catalase and peroxidases in a timely way, it undergoes a series of iron-catalyzed reactions to produce hydroxyl free radicals ($OH^{\bullet}$). These highly toxic radicals are extremely reactive with macromolecules and result in pulmonary and/or multiple organ injuries, leading to death in several species (Shu et al. 1979, Steffen and Netter 1979). In this study, we have shown that GPX1(−) mice survived significantly less time, whereas GPX1(+) mice survived much longer than the respective controls after the injection of lethal doses of paraquat. Thus normal expression of GPX1 is important and overexpression of GPX1 is helpful for mice to protect against lethal oxidative stress, indicating an in vivo role of GPX1 in reducing hydrogen peroxide and/or other hydroperoxides. Apparently, this antioxidative role of GPX1 is not redundant with those of catalase, superoxide dismutases and other antioxidant enzymes (Ho et al. 1997). Earlier, several groups showed that overexpression of GPX1 in various cell lines enhances resistance to clastogenic oxidant stress by prooxidants (Chu et al. 1990, Hockenbery et al. 1993, Kelner et al. 1995). In contrast, suppression of GPX1 expression by transfecting the antisense of GPX1 gene in DG44 cells increases the cell susceptibility to paraquat or adriamycin toxicity (Taylor et al. 1993). Unprecedentedly, the transgenic mouse models have enabled us to clarify the presumed antioxidative role of GPX1 in vivo in a very straightforward way. In turn, our data provide justification for the current use of tissue GPX1 activity to determine the Se requirement (Levander and Whanger 1996).

Deprivation of Se has been shown to potentiate paraquat toxicity in several species (Burk et al. 1980, Cagen and Gibson 1977, Mercurio and Combs 1986a). Indeed, we have confirmed the same protection of dietary Se against paraquat toxicity in the control mice in Experiment 1. But, there was no difference in survival time between the Se-adequate and Se-deficient GPX1(−) mice. Because the Se-supplemented diets contained >0.30 mg Se/kg, a full expression of all known selenoproteins (Bermano et al. 1995, Christensen et al. 1995, Vadhanavikit and Ganther 1993, Weiss et al. 1996, Yang et al. 1989), except selenoprotein W (Yeh et al. 1995), was expected. Evidently, increases in the expression of GPX4 and other Se-dependent proteins by dietary Se supplementation, in the absence of GPX1, did not afford a major protection against paraquat lethality. Therefore GPX1 is the major, if not the only, selenoperoxidase responsible for the differences in survival time among these treatment groups. Because the method that we used to measure GPX1 activity in the supernatant, with hydrogen peroxide as a substrate, did not exclude contributions from other selenoperoxidases, the activity might be stated more accurately as hydrogen peroxidase GPX rather than GPX1.

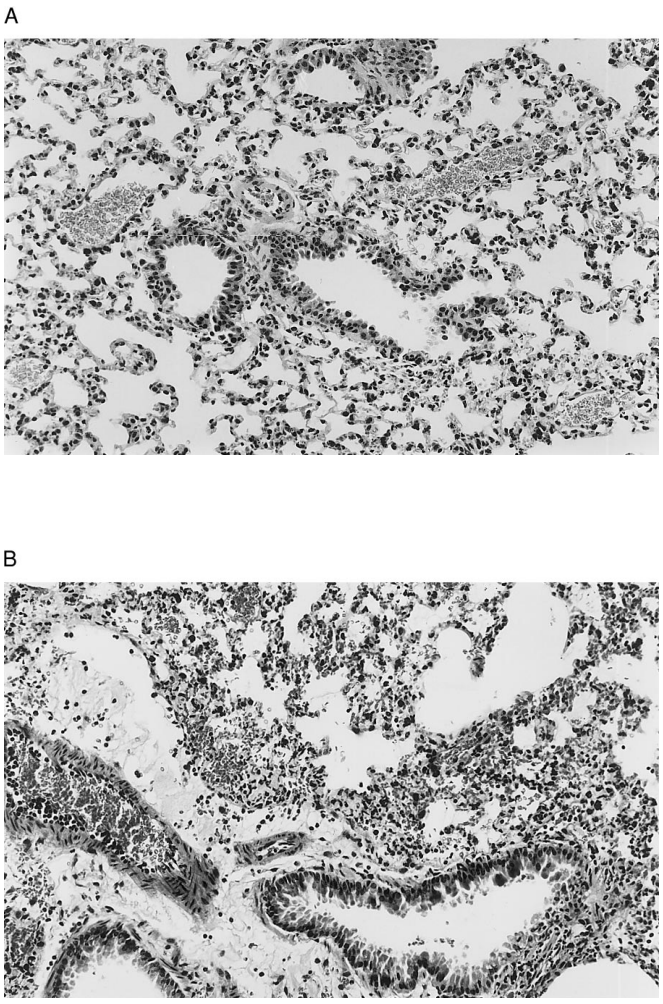

FIGURE 3 Comparison of histologic changes in lung of the GPX1 knockout mouse (*A*) and control (*B*) injected with 50 mg paraquat/kg body weight (magnification, X175). Both mice were fed the diet supplemented with Se (0.30 mg/kg) and all-*rac*-α-tocopheryl acetate (20 mg/kg) for 3 wk before intraperitoneal injection of paraquat. Severe diffused acute lung injury was present in the control mouse. The injury was characterized by alveolar edema and hemorrhage, perivascular edema and acute inflammation, and alveolar and bronchiolar epithelial necrosis. In contrast, lung of the GPX1 knockout mouse dying acutely after paraquat injection remained as normal as that of the saline-injected mouse.

However, this should not weaken our conclusion that the protection of dietary Se against paraquat lethality in the control mice is mediated through GPX1. In fact, the contribution of the other selenoperoxidases to the apparent total GPX activity was negligible because there was only minute residual GPX activity detected in the tissues of the GPX1(−) mice. More convincingly, dietary Se supplementation produced minimal increases in tissue GPX activity and essentially no changes in the survival time in the GPX1(−) mice.

To record the accurate survival time of mice in Experiments 1–3, we had to use the liver of moribund animals for TBARS analysis; this might not be the most reliable marker of lipid peroxidation in vivo (Awad et al. 1994). Nevertheless, we observed an additive reduction of hepatic TBARS by dietary supplementation of tocopheryl acetate and Se and GPX1 expression. It is unclear why the GPX1(+) mice had a lower hepatic TBARS concentration than the controls, given the similar liver GPX1 activities. However, it is obvious that dietary tocopheryl acetate did not affect survival time or histopathology in either the GPX1(−) mice or controls, in spite of its effects on liver TBARS concentrations of these mice. Because there was no correlation between the hepatic TBARS concentration and the survival time of mice or the extent of tissue lesions, lipid peroxidation (Bus et al. 1975) was not likely to have been responsible for paraquat lethality (Shu et al. 1979, Steffen and Netter 1979). Although TBARS are not considered the most reliable indicator of lipid peroxidation, there was no distinct histopathology in the GPX1(−) or the Se-deficient controls that died shortly after paraquat injection. Likely, these mice were killed by paraquat through rapidly depleting NADPH and other reducing equivalents. A sharp decrease in the ratios of NADPH to NADP in the lung was seen in rats 60 min after an intravenous injection of paraquat (40 mg/kg), and the decrease persisted for ≥24 h (Witschi et al. 1977). In the deficiency of GPX1 expression, the depletion of NADPH (and other reducing agents) by the lethal dose of paraquat could be greatly aggravated because of the rapid accumulation of hydroperoxides. Thus the redox status and many NADPH-dependent cellular or subcellular functions could be severely altered within a short period of time (Witschi et al. 1977), resulting in a total collapse of the metabolic system and sudden death of mice. However, caution should be taken in considering all of these potential changes because our experiments were designed to determine the antioxidative role of GPX1, not to study the mechanism of paraquat toxicity.

Contrary to its remarkable effects on paraquat lethality, the GPX1 knockout did not make mice prone to the moderate oxidative stress presented by the low dose of paraquat (12.5 mg/kg). The GPX1(−) mice, like the controls, had no change in plasma ALT activity or sign of overt injury in lung, liver or kidney after the injection compared with the saline-injected animals. Although liver and lung F_2-isoprostanes concentrations in both the GPX1(−) and control mice were elevated over the basal lines (saline-injected) by this low dose of paraquat, these changes were similar between the two groups and resulted in no tissue lesions. Thus GPX1 knockout did not aggravate F_2-isoprostanes formation. Earlier, Cagen and Gibson (1977) reported that Se deficiency in mice resulted in a reduction of the LD_{50} of paraquat from 30 to 10 mg/kg and a shift in the organ specific toxicity from lung toward liver. They observed substantial increases in the plasma transaminase activity and liver damages in Se-deficient mice injected with 8–12 mg paraquat /kg, but not in Se-adequate mice injected with 30 mg paraquat/kg. Our results are in line with their notion that paraquat alone is not hepatotoxic in Se-adequate mice. However, the GPX1 knockout in Se-adequate mice does not replicate the effects of Se deficiency on potentiating toxicities of the low dose of paraquat. Other Se-dependent factors may be more important than GPX1 in that process. Burk et al. (1995) reported that selenoprotein P might be the mediator of the injected Se that protected against mortality and hepatic F_2-isoprostane formation induced by low levels of diquat in Se-deficient rats.

Recently, several groups have shown a limited metabolic role of GPX1 in GPX1 transgenic mice under normal physiologic conditions (Ho et al. 1997, Mirochnitchenko et al. 1995, Spector et al. 1996). The GPX1 overexpression or knockout did not affect the mouse susceptibility to dietary Se and vitamin E deficiency (Cheng et al. 1997a and 1997b). Compared with the lethal doses of paraquat, dietary Se and vitamin E deficiency or low doses of pro-oxidants might produce insufficient oxidative stress to show a full function of GPX1. This may partially explain the discrepancy on the assessment of the role of GPX1 between previous experiments by Burk et al. (1980) and Mercurio and Combs (1986a). Clinically, all of the genetic defects of the pentose phosphate shunt (favism) and GSH regeneration that lead to a functional deficiency of GPX1 in erythrocytes are asymptomatic unless the patients are stressed with pro-oxidant xenobiotics (Flohé 1989). Most likely, there is a critical threshold of oxidative stress (Toussaint et al. 1993) that regulates the actual antioxidative function of GPX1 in vivo. Although the mechanism of such presumed regulation (Garberg and Thullberg 1996) remains to be defined, our data, along with all of the others, have demonstrated that GPX1 serves as a major metabolic form of Se that protects mice against acutely lethal oxidative stress by high levels of paraquat, but might be less important in protecting against relatively low levels of oxidative stress.

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