Cellular Glutathione Peroxidase Protects Mice Against Lethal Oxidative Stress Induced by Various Doses of Diquat (44440)

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Abstract. **This study was to determine if cellular glutathione peroxidase (GPX1) protects against acute oxidative stress induced by diquat. Lethality and hepatic biochemical indicators in GPX1 knockout mice [GPX1(–/–)] were compared with those of wild-type mice (WT) after an intraperitoneal injection of diquat at 6, 12, 24, or 48 mg/kg of body weight. Although the WT survived all the doses, the GPX1(–/–) survived only 6 mg diquat/kg and were killed by 12, 24, and 48 mg diquat/kg at 52, 4.4 and 3.9 hr, respectively. Compared with those of surviving mice that were sacrificed on Day 7, the dead GPX1(–/–) had diquat dose-dependent increases ($P < 0.05$) in plasma alanine aminotransferase (ALT) activities. The GPX1(–/–) also had higher ($P < 0.05$) liver carbonyl contents than those of the WT, but the differences were irrespective of diquat doses. Whereas hepatic total GPX and phospholipid hydroperoxide glutathione peroxidase activities or hepatic GPX1 protein was not significantly affected by the diquat treatment, liver thioredoxin reductase and catalase activities were lower ($P < 0.05$) in the GPX1(–/–) injected with 12 mg diquat/kg than those of other groups. In conclusion, normal GPX1 expression is necessary to protect mice against the lethality, hepatic protein oxidation, and elevation of plasma ALT activity induced by 12–48 mg diquat/kg.**
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Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals, are produced during aerobic metabolism. If ROS are not removed in a timely manner by an antioxidant system, mammalian cells may encounter oxidative stress that causes destruction of macromolecules and abnormal function (1). Glutathione peroxidases (GPX), along with superoxide dismutases (SOD) and catalase, are considered the main antioxidant enzymes in mammals. Cellular GPX (glutathione: H_2O_2 oxidoreductase, EC.1.11.1.9, GPX1) was the first identified selenoprotein (2, 3) and is the most abundant biochemical form of body selenium (Se) (4, 5). Despite general presumption (6), its *in vivo* antioxidant function has not been clarified. Recently, Ho *et al.* (7) have developed the GPX1 knockout mice [GPX1(–/–)]. Using this mouse model, we demonstrated that GPX1 is the mediator of body Se that protects against the lethal, acute oxidative stress by a pro-oxidant, paraquat (8). Similar observation has been reported by another group (9) using independently developed GPX1(–/–). In contrast, others failed to show any impact of the GPX1 knockout on pulmonary defense against hyperoxia (7) or susceptibility of eye lenses to high levels of hydrogen peroxide (10). Earlier, Burk *et al.* (11, 12) reported that GPX1 was not associated with the protection against a relatively low dose of diquat, another pro-oxidant, afforded by the injected Se in Se-deficient rats. These conflicting observations raise the issue of whether the antioxidant function of GPX1 is pro-oxidant, species- and/or tissue-specific.

Diquat is a bipyridyl herbicide that uses molecular oxygen to produce superoxide anion radical and subsequently hydrogen peroxide (13). Because the major target organ of diquat is liver (11, 12, 14) that has the most abundant expression of GPX1 (4), administering diquat to the GPX1(–/–) provides us an ideal model to study the *in vivo* antioxidant function of GPX1 and the related biochemical

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and physiological events. Our objectives were to examine the following: 1) whether or not GPX1 was protective against lethal, acute oxidative stress induced by diquat; and 2) whether the protection was diquat dose-dependent and associated with other seleno- and antioxidant enzymes.

Materials and Methods

The GPX1 Knockout Mice. The GPX1(–/–) were kindly provided by Dr. Y-S Ho (Wayne State University, Detroit, MI), and were generated from the 129/SVJ × C57BL/6 lines (7). Knockout of GPX1 gene expression was characterized by completely undetectable GPX1 mRNA and 80%–99% reduction in total GPX activities in various tissues compared with the wild-type mice (WT) (4, 7). Our experiments were approved by the Institutional Animal Care and Use Committee at Cornell University and conducted in accordance with the NIH guidelines for animal care.

Body Selenium Status and Oxidative Stress. All chemicals and kits were purchased from Sigma Chemical Co. (St. Louis, MO) unless indicated otherwise. The 24 GPX1(–/–) and 24 WT weanling mice (3 weeks old) were fed an Se-adequate (0.4 mg/kg as sodium selenite) torula yeast diet (4) supplemented with 75 mg of all-*rac*-α-tocopheryl acetate/kg for 5 weeks prior to the oxidative challenge of diquat. Diquat (dibromide monohydrate, Chem Service, West Chester, PA) was dissolved in isotonic saline and filter-sterilized. The injection (ip) volume was controlled at 10 ml/kg of body weight. Six GPX1(–/–) and six WT were injected with 6, 12, 24, or 48 mg diquat/kg of body weight. Mice were watched constantly after the injection except for a 6-hr overnight interval until Day 7 when all of the surviving mice were euthanized to collect tissue samples.

Sample Collection. Immediately after the mice died spontaneously or were euthanized by exsanguination, fractions of liver, kidney, lung, heart, and brains were collected and processed for histopathology (8). The rest of the liver was rinsed with ice-cold saline, frozen in liquid nitrogen, and stored at –80°C before analyses. Plasma samples were collected and assayed for alanine aminotransferase (EC 2.6.1.2, ALT) activity immediately.

Biochemical Assays. Tissue total carbonyl content was used as the indicator of protein oxidation (15) and was measured spectrophotometrically (at 360 nm), using absorbance coefficient of 22,000 M^{-1} cm^{-1} (16). Plasma ALT activity was measured using a Sigma kit (ALT 10). Liver samples were homogenized in 0.25 *M* sucrose, 0.1 *M* Tris-HCl, pH 7.4, and centrifuged at 105,000*g* for 1 hr at 4°C for assaying total GPX, phospholipid hydroperoxide GPX (EC 1.11.1.12, GPX4), and thioredoxin reductase (EC 1.6.4.5, TR) activities. Activities of total GPX (17) and GPX4 (18) were measured by the coupled assay of NADPH oxidation using hydrogen peroxide and phosphatidylcholine hydroperoxide as substrate, respectively. The enzyme unit was defined as 1 nmol of GSH oxidized per minute. Activities of TR were determined using the NADPH-dependent reduction of 5,5′-dithiobis-(2-nitrobenzoic acid) method (19). Activity was defined as 2 nmol TNB (5′-thionitrobenzoic acid) formed per minute. Hepatic catalase (EC 1.11.1.6) activity was measured as described by Aebi (20), and the activity was defined as 1 μmol H_2O_2 consumed per minute. Protein concentration was determined as described by Lowry *et al.* (21)

Western Blot Analysis of Liver GPX1 Protein. The liver homogenates (50 μg protein), as prepared for the above assay of total GPX activity, were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 12%). The separated proteins were transferred to a nitrocellulose membrane (Protran, Schleicher & Schuell, Keene, NH) and detected by an anti-human GPX1 antibody (kindly provided by Drs. Q. Shen and P.E. Newburger, University of Massachusetts Medical School, Worcester, MA). The goat anti-rabbit IgG horseradish peroxidase system (Bio-Rad Laboratories, Hercules, CA) was used to detect the relative amount of GPX1 protein. The protein band intensities were determined by an IS-1000 Digital Imaging System (Alpha Innotech Co., San Leandro, CA).

Statistical Analysis. Two-way factorial (2 × 4) analysis of variance was used to examine the main effects of mouse type [GPX1 (–/–) vs WT] and diquat dose (6, 12, 24, and 48 mg/kg). The Bonferroni *t* test was used for mean comparisons. All the analyses were conducted using SAS (release 6.11, SAS Institute, Cary, NC).

Results

Survival Time and Histopathology. The GPX1(–/–) survived 6 mg diquat/kg, but died of 12, 24, and 48 mg diquat/kg at 52, 4.4, and 3.9 hr after the injection, respectively (Table I). In contrast, the WT mice survived all four doses and were sacrificed on Day 7 along with those surviving GPX1(–/–). There was no noticeable change in activity, food intake, or water consumption in all the surviving groups after the injection. Those GPX1(–/–) that received 12 mg diquat/kg became fairly weak before death.

Table I. Effects of the GPX1 Knockout on Survival Times of the Se-Adequate Mice Injected with Various Doses of Diquat

Mouse	Diquat (mg/kg body weight)	*n*	Survival time (hr after injection)
GPX1 (–/–)	6	6	Survived[c]
	12	6	51.8[b] ± 8.8
	24	6	4.4[a] ± 0.3
	48	6	3.9[a] ± 0.4
WT	6	6	Survived[c]
	12	6	Survived[c]
	24	6	Survived[c]
	48	6	Survived[c]

[a,b] Values are means ± SE and differ ($P < 0.05$) without sharing a common superscript letter.
[c] Mice survived and were euthanized on Day 7 to collect tissue samples for analysis.

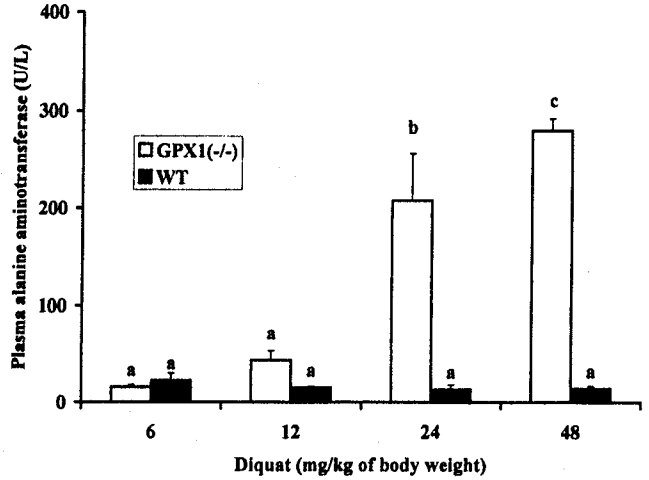

Figure 1. Plasma alanine aminotransferase activities of the GPX1 knockout mice [GPX1(–/–)] and the wild-type mice (WT) injected with various doses of diquat. Values (n = 4) differ ($P < 0.05$) without sharing a common letter.

None of the mice showed any heart injury or overt liver necrosis except for the moderate diffuse vacuolar changes. Moderate lung injury was seen in only one GPX1(–/–) mouse injected with 48 mg/kg of diquat. Moderate mineralization in kidney was seen in those GPX1(–/–) that died of the injection acutely.

Plasma Alanine Aminotransferase Activity. When all the surviving mice (the WT and the GPX1(–/–) that received the 6 mg/kg diquat) were sacrificed on Day 7, their plasma ALT activities were similarly low, regardless of diquat doses. However, those GPX1(–/–) that died spontaneously had significant elevation ($P < 0.05$) of plasma ALT activities, and the increase was proportional to the diquat dose (Fig. 1).

Liver Carbonyl Content. All the GPX1(–/–) had higher ($P < 0.05$) liver carbonyl contents than the WT (Fig. 2), and the contents were similar among the four diquat doses within each of the mouse groups. Despite surviving,

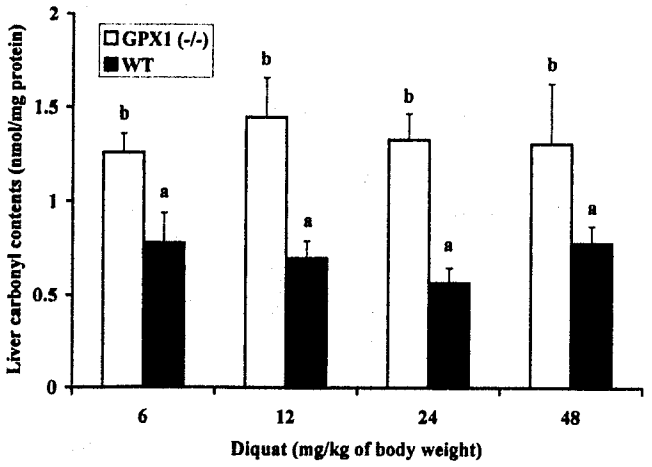

Figure 2. Liver carbonyl contents in the GPX1 knockout mice [GPX1(–/–)] and the wild-type mice (WT) injected with various doses of diquat. Values (n = 3) differ ($P < 0.05$) without sharing a common letter.

the GPX1(–/–) mice that were injected with 6 mg diquat/kg still had a high carbonyl content similar to those of the other GPX1(–/–).

Liver GPX Activity and GPX1 Protein. As expected, the GPX1(–/–) had only residual liver GPX activity that was not changed by diquat treatment (Table II). Numerically, liver GPX activities in the WT exhibited a diquat-dose-related decrease. However, the relationship was not significant, and there were no significant ($P < 0.05$) differences between any two doses. Using the anti-human GPX1 antibody, we detected a specific band of about 23 kDa in liver samples from the WT, but not from the GPX1 (–/–) (data not shown). The band intensities were highly correlated (r = 0.75, $P < 0.005$) with hepatic GPX activities, but the diquat dose effect was not significant (Table II).

Other Antioxidant Enzymes Activities. The GPX1(–/–) injected with 12 mg diquat/kg had the lowest TR activity in liver (Table III) that was significantly different ($P < 0.05$) from those of the other GPX1(–/–) and the WT injected with 12 or 48 mg diquat/kg. Likewise, liver catalase activity in that group of GPX1 (–/–) mice (12 mg diquat/kg) was also lower ($P < 0.05$) than those of the GPX1(–/–) injected with 6 or 24 mg diquat/kg and the WT injected with 6 or 48 mg diquat/kg. There was no significant effect of diquat treatment on liver GPX4 activity in either GPX1(–/–) or WT.

Discussion

Our data clearly indicate that knockout of the GPX1 gene expression rendered mice more susceptible to acute oxidative stress induced by diquat at 12 mg/kg or higher doses. These GPX1(–/–) died spontaneously after the injection, and their mean survival times (4–52 hr) were inversely related to the diquat doses, whereas the WT survived all the

Table II. Effects of the GPX1 Knockout on Hepatic Activities of Glutathione Peroxidase (GPX) and Relative Amounts of GPX1 Protein in the Se-Adequate Mice Injected with Various Doses of Diquat

Mouse	Diquat (mg/kg body weight)	GPX (U/mg protein)	GPX1 protein (relative concentration (%)*)
GPX1 (–/–)	6	$6.2^a \pm 0.2$	ND[c]
	12	$5.1^a \pm 0.8$	ND[c]
	24	$6.6^a \pm 0.6$	ND[c]
	48	$5.7^a \pm 1.6$	ND[c]
WT	6	$929^b \pm 103$	$100^a \pm 5.4$
	12	$909^b \pm 43$	$90.3^a \pm 4.4$
	24	$857^b \pm 67$	$85.8^a \pm 5.2$
	48	$754^b \pm 187$	$88.1^a \pm 9.7$

[a,b] Values are means ± SE (n = 3) and differ ($P < 0.05$) without sharing a common superscript letter within a column.
[c] Not detectable.
* The GPX1 protein concentrations were expressed as relative percentages to that in the 6 mg/kg group (100%).

Table III. Effects of the GPX1 Knockout on Activities of Hepatic Phospholipid Hydroperoxide Glutathione Peroxidase (GPX4), Thioredoxin Reductase (TR), and Catalase in the Se-Adequate Mice Injected with Various Doses of Diquat

Mouse	Diquat (mg/kg body weight)	GPX4 (U/mg protein)	TR (U/mg protein)	Catalase (U/mg protein)
GPX1 (–/–)	6	$9.4^{a} \pm 1.0$	$21.0^{bc} \pm 1.5$	$419^{b} \pm 24$
	12	$9.3^{a} \pm 1.2$	$16.1^{a} \pm 0.6$	$182^{a} \pm 49$
	24	$10.1^{a} \pm 0.7$	$21.9^{ca} \pm 2.0$	$359^{ba} \pm 60$
	48	$8.4^{a} \pm 1.0$	$20.7^{bc} \pm 1.0$	$292^{ab} \pm 23$
WT	6	$9.7^{a} \pm 1.3$	$18.1^{ab} \pm 0.7$	$369^{b} \pm 64$
	12	$11.4^{a} \pm 0.6$	$21.2^{bc} \pm 0.7$	$320^{ab} \pm 43$
	24	$9.9^{a} \pm 1.2$	$19.2^{abc} \pm 1.3$	$336^{ab} \pm 80$
	48	$9.7^{a} \pm 0.4$	$20.3^{bc} \pm 0.6$	$359^{b} \pm 72$

^{a,b,c} Values are means ± SE ($n = 3$) and differ ($P < 0.05$) without sharing a common superscript letter within a column.

doses until the end of the experiment (for 7 days). These GPX1(–/–) also showed high plasma ALT activities and increased liver protein oxidation. In contrast, the WT had none of these responses and were apparently healthy during the 7 days of surveillance. In a parallel experiment, we found that Se-deficient WT and Se-adequate or deficient GPX1(–/–) were all killed (100% mortality) by an injection (ip) of 24 mg diquat/kg, whereas Se-adequate WT survived the same insult (22). The Se-deficient WT and the Se-adequate GPX1(–/–) had similar survival times and oxidative injuries. Thus, the GPX1 deficiency, due to either dietary Se deficiency or the GPX1 gene knockout, is the primary cause for the increased susceptibility of these mice to the diquat-induced oxidative stress. Likewise, we showed that the survival time of mice that received 50 or 125 mg paraquat/kg was solely a function of tissue GPX1 activity, independent of dietary Se levels *per se* (8). Clearly, GPX1 exhibits similar protections against the lethalities of both diquat and paraquat although these two compounds have different primary target organs and toxicokinetics (23, 24).

It is interesting to note that the GPX1(–/–) survived 6 mg diquat/kg and did not show any tissue lesions or increase in plasma ALT activity. Apparently, other enzymatic or nonenzymatic antioxidant systems, in the absence of GPX1, were sufficient to cope with this relatively mild oxidative stress. In our previous study, the GPX1(–/–) were as tolerant as the WT to a low dose of paraquat (12.5 mg/kg) (8). A dose-related susceptibility of the GPX1(–/–) to paraquat was also shown by de Haan *et al.* (9). Along with its limited role in normal development (7), the protection of GPX1 may not be indispensable under moderate oxidative stress (8). Earlier, Burk *et al.* (11, 12) suggested that GPX1 was not associated with the protection against diquat toxicity afforded by the injected Se to the Se-deficient rats. In fact, they used a relatively low dose of diquat (19.5 μmol/kg or 6.7 mg/kg). If there is no distinct difference in susceptibility to diquat toxicity between their rats and our mice, their interpretation is rather consistent with our observation from the lowest dose of diquat (6 mg/kg). Because the protection of GPX1 is so closely associated with the level of the oxidative stress, the physiological significance of its antioxidant role could not be determined or generalized using a single level of pro-oxidant.

In our previous experiments, we found that untreated GPX1(–/–) and WT had similar antioxidative (except for GPX1) or oxidative status, and the injections of diquat (22) and paraquat (25) caused little or no oxidative injuries in the Se-adequate WT. The increased plasma ALT activities in the GPX1(–/–) of the present study indicate that their hepatic cells were damaged by the diquat-induced oxidative stress (11, 12). Although the increase was diquat dose–dependent, it seemed to be marginal compared with those in cases of concomitant overt liver necrosis (12). Such an observation could explain why there was no overt liver necrosis in any of the GPX1(–/–) that were killed by diquat, suggesting that these animals died of other physiological failures caused by diquat.

Protein carbonyl groups are among the targets modified under oxidative stress, and the carbonyl contents are commonly used to measure oxidative injury (15, 26). Physiologically important proteins under *in vivo* and *in vitro* conditions (27) may be oxidized by ROS directly (28), or modified by aldehydic products of lipid peroxidation (i.e., 4 hydroxynonenal) or glycoxidation (29, 30). In this study, we found that a diquat-induced increase of liver carbonyl content was higher in the GPX1 (–/–) than in the WT, irrespective of the diquat doses. Even though the GPX1(–/–) survived 6 mg diquat/kg, their hepatic carbonyl content was similar to those of the mice that were killed by higher doses of diquat. Therefore, tissue carbonyl content may not be directly related to the lethality of diquat, but may, more likely, reflect secondary damage.

The diquat-induced oxidative stress seems to exert a marginal effect on the status of mouse liver antioxidant enzymes. First, there was a tendency for liver GPX activity and GPX1 protein to decrease with the increasing doses of diquat although the changes were insufficient to be statistically significant. Others have also seen decreases (31) or increases (9) in tissue GPX activities caused by pro-oxidants in rodents. Because of the strong correlation between hepatic GPX activity and GPX1 protein and the lack of differences in liver GPX4 activities among treatment

groups, GPX1 is likely the major contributor to the total GPX activity decrease observed in the present study. Second, liver TR and catalase activities in the GPX1(–/–) injected with 12 mg diquat/kg were lower than those of the other groups. As a newly identified selenoenzyme, TR may possess different antioxidant functions from those of GPX1 and other selenoproteins (19, 32, 33). In addition, it has been reported to reduce a couple of peroxides (34). Catalase shares the same substrate, H_2O_2, with GPX1, and may function at high concentrations of H_2O_2 (35). Because 12 mg diquat/kg did not produce oxidative stress as drastic as 24 and 48 mg diquat/kg to kill the GPX1(–/–) acutely, it allowed these mice an extended survival time (52 hr) for the development or accumulation of oxidative damages. This might give a unique window opportunity for both TR and catalase to be involved in the protection. Because Se-dependent GPX4 was not altered in this group, the reduction of TR activity does not seem to reflect a possible change of Se status in these animals. In contrast, there was no significant decrease of TR and catalase activities in the two highest diquat dose groups of the GPX1(–/–). Probably, these mice died too soon to develop such changes. A high dose of paraquat caused a sharp reduction of NADPH/NADP and NADH/NAD ratios in lung and liver of the GPX1(–/–) and the Se-deficient WT (25). The acute disruption of redox status and NADPH-dependent pathways may cause the sudden death of animals without severe tissue lesions. On the other hand, the GPX1(–/–) that received relatively low doses of diquat (12 mg/kg) had a delayed death and might die of mechanisms yet to be determined.

In summary, GPX1 is necessary for mice to protect against the lethality, hepatic protein oxidation, and plasma ALT activity rise induced by 12–48 mg diquat/kg of body weight. The acute oxidative stress might affect liver GPX activity and GPX1 protein in the WT or liver TR and catalase activities in the GPX1(–/–), but these responses and the physiological implication remain to be clarified.

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1. Evans MD, Griffiths HR, Lunec J. Reactive oxygen species and their cytotoxic mechanisms. Adv Mol Cell Biol **20:**25–73, 1997.
2. Flohé L, Günzler WA, Schock HH. Glutathione peroxidase: A selenoenzyme. FEBS Lett **32:**132–134, 1973.
3. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science **179:**588–590, 1973.
4. Cheng W-H, Ho YS, Ross DA, Valentine BA, Combs GF Jr., Lei XG. Cellular glutathione peroxidase knockout mice express normal levels of selenium-dependent plasma and phospholipid hydroperoxide glutathione peroxidase in various tissues. J Nutr **127:**1445–1450, 1997.
5. Behne D, Wolters W. Distribution of selenium and glutathione peroxidase in the rat. J Nutr **113:**456–461, 1983.
6. Hoekstra WG. Biochemical function of selenium and its relation to vitamin E. Fed Proc **34:**2083–2089, 1975.
7. Ho YS, Magnenat JL, Bronson RT, Cao J, Gargano M, Sugawara M, Funk CD. Mice deficient in cellular glutathione peroxidase develop normally and show no increased sensitivity to hyperoxia. J Biol Chem **272:**16644–16651, 1997.
8. Cheng W-H, Ho YS, Valentine BA, Ross DA, Combs GF Jr., Lei XG. Cellular glutathione peroxidase is the mediator of the body selenium to protect against paraquat lethality in transgenic mice. J Nutr **128:**1070–1076, 1998.
9. de Haan JB, Bladier C, Griffiths P, Kelner M, O'Shea RD, Cheung NS, Bronson RT, Silvestro MJ, Wild S, Zheng SS, Beart PM, Hertzog PJ, Kola I. Mice with a homozygous null mutation for the most abundant glutathione peroxidase, GPX1, show increased susceptibility to the oxidative stress-inducing agents paraquat and hydrogen peroxide. J Biol Chem **273:**22528–22536, 1998.
10. Spector A, Yang Y, Ho YS, Magnenat JL, Wang RR, Ma W, Li WC. Variation in cellular glutathione peroxidase activity in lens epithelial cells, transgenic, and knockouts does not significantly change the response to H_2O_2 stress. Exp Eye Res **62:**521–540, 1996.
11. Burk RF, Lawrence RA, Lane JM. Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration. J Clin Invest **65:**1024–1031, 1980.
12. Burk RF, Hill KE, Awad JA, Morrow JD, Kato T, Cockell KA, Lyons PR. Pathogenesis of diquat-induced liver necrosis in selenium-deficient rat: Assessment of the role of lipid peroxidation and selenoprotein P. Hepatology **21:**561–569, 1995.
13. Farrington JA, Ebert M, Land EJ, Fletcher K. Bipyridylium quaternary salts and related compounds. V. Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implication for the mode of action of bipyridyl herbicides. Biochim Biophys Acta **314:**372–381, 1973.
14. Awad JA, Burk RF, Roberts LJ. Effect of selenium deficiency and glutathione-modulating agents on diquat toxicity and lipid peroxidation in rats. J Pharmacol Exp Ther **270:**858–864, 1994.
15. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl contents in oxidatively modified protein. Meth Enzymol **186:**464–478, 1990.
16. Reznick AZ, Packer L. Oxidative damages to protein: Spectrophotometric method for carbonyl assay. Meth Enzymol **233:**357–363, 1994.
17. Lawrence RA, Sunde RA, Schwartz GL, Hoekstra WG. Glutathione peroxidase activity in rat lens and other tissues in relation to dietary selenium intake. Exp Eye Res **18:**563–569, 1974.
18. Maiorino M, Gregolin C, Ursini F. Phospholipid hydroperoxide glutathione peroxidase. Meth Enzymol **186:**448–457, 1990.
19. Holmgren A, Björnstedt M. Thioredoxin and thioredoxin reductase. Meth Enzymol **252:**199–208, 1995.
20. Aebi H. Catalase *in vitro*. Meth Enzymol **105:**121–126, 1984.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193:**265–267, 1951.
22. Fu YX, Cheng W-H, Porres JM, Ross DA, Lei XG. Knockout of cellular glutathione peroxidase gene render mice susceptible to diquat-induced oxidative stress. Free Radic Biol Med **7:**605–611, 1999.
23. Rose MS, Smith LL. Tissue uptake of paraquat and diquat. Gen Pharmacol **8:**173–176, 1977.
24. Rose MS, Lock EA, Smith LL, Wyatt I. Paraquat accumulation: Tissue and species specificity. Biochem Pharmacol **25:**419–423, 1976.
25. Cheng W-H, Fu YX, Porres JM, Ross DA, Lei XG. Selenium-dependent cellular glutathione peroxidase protects mice against a pro-oxidant-induced oxidation of NADPH, NADH, lipids, and protein. FASEB J **13:**1467–1475, 1999.
26. Shacter E, Williams JA, Lim M, Levine RL. Differential susceptibility of plasma proteins to oxidative modification: Examination by western blot immunoassay. Free Radic Biol Med **17:**429–437, 1994.
27. Barbara SB, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem **272:**20313–20316, 1997.
28. Stadtman ER. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu Rev Biochem **62:**797–821, 1993.
29. Uchida K, Stadtman ER. Modification of histidine residues in proteins

by reaction with 4-hydroxynonenal. Proc Natl Acad Sci U S A **89:**4544–4548, 1992.
30. Lee Y, Shacter E. Role of carbohydrates in oxidative modification of fibrinogen and other plasma proteins. Arch Biochem Biophys **321:**175–181, 1995.
31. Garberg P, Thullberg M. Decreased glutathione peroxidase activity in mice in response to nafenopin is caused by changes in selenium metabolism. Chem Biol Interact **99:**165–177, 1996.
32. Gladyshev VN, Jeang KT, Stadtman TC. Selenocysteine, identified as the penultimate C-terminal residue in human T-cell thioredoxin reductase, corresponds to TGA in the human placental gene. Proc Natl Acad Sci U S A **93:**6146–6151, 1996.
33. Gladyshev VN, Factor VM, Housseau F, Hatfield DL. Contrasting patterns of regulation of the antioxidant selenoproteins, thioredoxin reductase, and glutathione peroxidase, in cancer cells. Biochem Biophys Res Commun **251:**488–493, 1998.
34. Björnstedt M, Hamberg M, Kumar S, Xue J, Holmgren A. Human thioredoxin reductase directly reduces lipid hydroperoxides by NADPH, and selenocystine strongly stimulates the reaction *via* catalytically generated selenols. J Biol Chem **270:**11761–11764, 1995.
35. Makino N, Mochizuki Y, Bannai S, Sugita Y. Kinetic studies on the removal of extracellular hydrogen peroxide by cultured fibroblasts. J Biol Chem **269:**1020–1025, 1994.