Low glutathione peroxidase activity in Gpx1 knockout mice protects jejunum crypts from γ-irradiation damage

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Low glutathione peroxidase activity in Gpx1 knockout mice protects jejunum crypts from γ-irradiation damage. Am J Physiol Gastrointest Liver Physiol 279: G426–G436, 2000.—Gpx1 knockout (KO) mice had a higher number of regenerating crypts in the jejunum than did Gpx2-KO or wild-type mice analyzed 4 days after ≥10 Gy γ-irradiation. Without γ-irradiation, glutathione peroxidase (GPX) activity in the jejunal and ileal epithelium of Gpx1-KO mice was <10 and ~35%, respectively, of that of the wild-type mice. Four days after exposure to 11 Gy, GPX activity in wild-type and Gpx1-KO ileum was doubled and tripled, respectively. However, jejunal GPX activity was not changed. Thus the lack of GPX activity in the jejunum is associated with better regeneration of crypt epithelium after radiation. Gpx2 gene expression was solely responsible for the increase in GPX activity in the ileum, since radiation did not alter GPX activity in Gpx2-KO mice. The intestinal Gpx2 mRNA levels of Gpx1-KO and wild-type mice increased up to 14- and 7-fold after radiation, respectively. Although the Gpx1-KO jejunum had higher levels of PGE2 than the wild-type jejunum after exposure to 0 or 15 Gy, these differences were not statistically significant. Thus whether GPX inhibits PG biosynthesis in vivo remains to be established. We can conclude that the Gpx2 gene compensates for the lack of Gpx1 gene expression in the ileal epithelium. This may have abolished the protective effect in Gpx1-KO mice against the radiation damage in the ileum.

Gpx2 gene induction; microcolony survival assay; Gpx2-knockout mice; antioxidant protein 2; gene compensation

THE PREVAILING THEORY on the mechanisms of radiation-induced cell death identifies DNA single- and double-strand breaks as the direct damaging effect. Such damage is produced from direct interaction of the DNA with ionizing radiation or with the hydroxyl free radicals generated from radiolysis of water and other molecules. The cells with unrepaired DNA damage will die of necrosis or apoptosis (14). Although generation of H2O2 is an alternative route for hydroxyl free radical generation (42), there is little evidence that elevated Se-dependent glutathione peroxidase (GPX) activity protects cells from radiation damage (9, 25, 32, 35, 36).

The intestinal crypt epithelial cells are very sensitive to abdominal and pelvic radiation therapy. The protective effect in the mucosal epithelium can result in a variety of symptoms, including diarrhea, electrolyte imbalance, and sepsis. The survival of slowly proliferating crypt stem cells appears to play a central role in mucosal regeneration following injury (8, 31). Several mitogens enhance the survival of intestinal epithelial cells after radiation injury. These include keratinocyte growth factor (KGF), stem cell factor (SCF), and prostaglandins (PGs) (8, 11, 22).

KGF is a newly recognized member of the heparin-binding fibroblast growth factor family. Short-term pretreatment with KGF, and to a lesser extent SCF, from 2 h to 3 days before irradiation provides significant protection of the crypts (11, 22). The exact mechanism for the KGF or SCF enhancement of crypt survival is not clear. KGF is a potent inducer for a human analog of the antioxidant protein 2 (Aop2), which has a weak Se-independent GPX activity (13, 18, 27, 28, 37). Aop2 has no homology with the Se-dependent GPXs. Instead, Aop2 has homology with antioxidant protein 1 (Aop1) family, which used to be known as thiol-specific antioxidant protein (18). The human Aop2 is also known as non-Se GPX, 1-Cys peroxiredoxin, and KGF-regulated gene 1 (12, 13, 20, 21, 27). Aop2 appears to be ubiquitous and is highly expressed in the nasal epithelium, skin, and eye tissues including the ciliary body, retina, and iris (37). Whether Aop2 can contribute significantly to total GPX activity in intestinal epithelium and thus affect crypt hydroperoxide metabolism is not known. Therefore, we have included Aop2 in this study.

PGs are important mediators of epithelial integrity and function in the gastrointestinal tract. Many PGs protect gastric and intestinal mucosa from damaging agents, including γ-irradiation (15). Cyclooxygenases (COXs) catalyze two key steps in PGs biosynthesis: oxygenation and cyclization of arachidonic acid to form PGG2 and the reduction of the hydroperoxide of PGG2 to form PGH2. Indomethacin, an inhibitor of both COX1 and COX2, reduced the number of surviving crypts in the irradiated mice (8). Although peroxy-
trite, the coupling product of nitric oxide and superoxide anion, activates COX in activated macrophages in the presence of GPX (24), hydroperoxides activate COX in vitro, an activity inhibited by GPX (2, 23, 26). Whether GPX inhibits PG biosynthesis in vivo has not been established. In this study, we have investigated the effect of GPX activity on intestinal crypt survival and PGE₂ concentrations after exposure to a high dose of γ-irradiation. An inverse correlation between GPX activity and either crypt survival or PGE₂ concentration would support the inhibitory effect of GPX on PG synthesis in the intestinal epithelium.

There are two major cellular Se-dependent GPX isozymes expressed in the intestinal epithelium: the classic ubiquitous GPX-1 encoded by the Gpx1 gene, and the epithelium-specific GPX-GI encoded by the Gpx2 gene (3, 10). Unlike the rather uniform distribution of GPX-1 in rat small intestine along the cephalo-caudal axis, a higher level of Gpx2 gene expression was detected in the distal section of the mouse small intestine or the ileum (6, 10). We have estimated that GPX-1 contributed >80% of total GPX activity in mouse proximal small intestine, and GPX-1 and GPX-GI contributed ~50 and 35% of total GPX activity in the distal small intestine. Since these two isozymes have very similar substrate specificity, this apparent redundant gene expression suggests that the two isozymes may be regulated differently to serve different physiological functions.

In this manuscript, we describe the making of Gpx2 knockout mouse lines. The homozygous Gpx2 knockout mice are apparently normal. These mice with disrupted Gpx1 or Gpx2 gene expression were analyzed for GPX activity and crypt regeneration after exposure to γ-irradiation. We found that the crypt survival is highly correlated with the lack of GPX activity. Our homozygous Gpx1-KO mice are apparently normal. These mice with disrupted Gpx1 or Gpx2 gene expression were analyzed for GPX activity and crypt regeneration after exposure to γ-irradiation. We found that the crypt survival is highly correlated with the lack of GPX activity. Our result provides the first link between GPX activity and radiation sensitivity in the intestinal epithelium. This study may provide a reason for the development of GPX-specific inhibitors to be used as protective agents on the intestinal epithelium during radiotherapy or accidental exposure to radiation.

**EXPERIMENTAL PROCEDURES**

**Animals.** The generation of mice with disrupted Gpx1 gene expression (Gpx1-KO) has been described previously (17). These homozygous Gpx1-KO mice on a mixed C57BL/6J (B6) and 129/Sv (129) genetic background (B6 × 129) were maintained by inbreeding. The wild-type control mice originated from heterozygous Gpx1-KO parents and were maintained similarly as a mixed B6 × 129 line. Breeding B6 × 129 Gpx1-KO with B6 for six generations generated the second Gpx1-KO subline in a B6 background. Southern blots were used for all genotyping. GPX assays were performed to confirm the phenotypes on the hemolysate from 25 μl of blood after rinsing the red blood cells with PBS. The relative specific activity was estimated as a fixed arbitrary ratio of activity to the A₅₄₀ of Drabkin's reagent-treated hemolysate (1). Wild-type B6 mice were purchased from Jackson Labs (Bar Harbor, MA) and used as the controls for B6 Gpx1-KO mice.

To isolate the mouse Gpx2 gene without interference from its pseudogene (5), intron primers were used for screening by PCR. Three positively identified P1 clones (nos. 8470, 8471, and 8472) were isolated from a genomic library of 129/Sv embryonic stem (ES) cells (Genome Systems, St. Louis, MO). A Hind III fragment (6.5 kb) isolated from the 8471 P1 clone was subcloned into pBluescript (Stratagene, La Jolla, CA) and sequenced (GenBank accession no. AJ249277). It contained 2 kb of 5’ untranscribed region, 0.3 kb of exon 1, 2.2 kb of intron, 0.8 kb of exon 2, and 1.5 kb of 3’ untranscribed region. The Gpx2-KO construct was made by substituting the exon 1 with the neo gene using the pPNT vector (40), which also contains a herpes simplex viral thymidine kinase gene cassette as shown in Fig. 1. The linearized pPNT-Gpx2-KO construct was transfected into W9.5 ES cells of the 129S3/SvImJ (129S3) mouse strain according to procedures previously described (39, 41). ES clones resistant to 0.3 mg/ml G418 and 2 μM ganciclovir were analyzed for the evidence of homologous recombination. Two correctly targeted clones, nos. 8 and 21, were injected into (CBA/CaJ-a/a × B6/F1) × B6 blastocysts and resulting chimeras were mated to B6 females to obtain germ line transmission of the mutation. The heterozygous Gpx2-KO mice were then intercrossed to generate mice homozygous for the mutation, i.e., Gpx2-KO mice. As controls, wild-type Gpx2+/− mice from the same intercross were used. Thus all mice studied were of a mixed 129S3 × B6 genetic background. Occasionally, the B6 × 129 wild-type mice generated for the Gpx1-KO controls were used as the controls for the Gpx2-KO mice.

**Whole body irradiation.** All mice were maintained on a 12:12-h light/dark schedule and given free access to standard lab mouse chow and water. Mice of both genders at 8–16 wk of age were placed into a ventilated Plexiglas pie container during exposure to γ-irradiation. All exposures were done between 7:15 and 8:15 AM to minimize the potential variations caused by circadian rhythm (19). The mice were exposed to a 60Co irradiator (Theratron-80 S/N 140; Atomic Energy of Canada) at 51–56 cGy/min with a source-to-skin distance of ~80 cm. The exposure procedure and all other mouse work were done with the approval of the City of Hope Research Animal Care Committee. At certain time points from 6 h to 7 days after exposure, mice were euthanatized by halothane (Halocarbon Labs, North Augusta, SC).

**Crypt survival assay.** Crypt microcolony survival was measured in animals terminated 4 days after irradiation following the described procedure (30, 43). Two 3-cm sections of the jejunum and the ileum starting from 1 cm distal to the pylorus end of stomach and 1 cm proximal to the cecum were excised. They were rinsed and fixed in Bouin’s fixative for 2–3 h and, after rinsing 3 times with 50% ethanol, were equilibrated in 70% ethanol overnight. They were cut to 1-cm lengths and then embedded longitudinally in paraffin. They were then sectioned onto slides and stained with hematoxylin and eosin. The number of surviving crypts was scored from cross-sections. Two to four complete cross-sections were scored from each region of mice. Two to four mice exposed to each dose of radiation were analyzed in each experiment.

**Assay of GPX activity in intestinal epithelium.** The GPX activity was determined on mouse intestinal epithelium. Jejunal and ileal epithelial cells were isolated from two 12-cm sections (total small intestine is ~36 cm long) of the proximal and the distal small intestine (4, 10). Briefly, after the intestinal lumen was rinsed with buffer A containing PBS and 1 mM dithiothreitol, the epithelial cells were eluted three times with buffer B containing 1.5 mM EGTA in buffer A after incubation at 37°C for 10, 20, and 30 min. These isolated cells were pooled and washed 3 times in PBS. Four
volumes of homogenate buffer containing 0.15 M Tris, pH 7.2, 0.1 M NaCl, 5 mM EGTA, and a cocktail of protease inhibitors were added to the packed cells. The cells were sonicated by five 1-s bursts with the use of a cell sonicator equipped with a microprobe (Branson Cell Disruptor 200; Branson Sonic Power, Danbury, CT). After centrifugation at 22,000 g for 30 min, GSH was added to an aliquot of the supernatant to 5 mM final concentration for the preservation of GPX-GI activity during freezing and thawing. The GPX activity was measured with 60 °C H2O2 and 3 mM GSH at pH 7.3. The protein concentration was determined with a BCA assay (Pierce Chemical, Rockford, IL) with bovine serum albumin as the standard.

Analysis of Southern and Northern blots. Genomic DNA was isolated from ES cells and 1 cm of mouse tails following the described procedure (34). Genotyping of the Gpx1 or Gpx2 genes was done by Southern blot analysis on the BamHI or ApaI digested genomic DNA. After resolving 10 μg of DNA per sample on 0.7% agarose gels, the DNA was denatured with 0.25 M HCl for 10 min and then transferred onto Zeta Probe blotting membranes (Bio-Rad, Richmond, CA) in 0.4 N NaOH overnight. Total RNA was isolated from 60 mg (0.6 cm) of small intestine. After homogenization by polytron in the lysis buffer, the total RNA was isolated with the RNeasy kit (Qiagen, Valencia, CA). Ten micrograms of RNA per sample were resolved in a formaldehyde-denaturing agarose gel containing 1.3% agarose and then transferred to a Zeta Probe blotting membrane with 10× standard saline citrate (34). The hybridization and washing was performed at 62°C in buffers that had high concentrations of SDS, as recomended by the manufacturer. All presented mRNA levels were normalized against β-actin mRNA levels. Similar re-

Fig. 1. Targeted disruption of the mouse Gpx2 gene (Gpx2-KO) and the analysis of Gpx2-KO mice. A: genomic structure and partial restriction map of the wild-type (WT) mouse Gpx2 locus (top), the targeting vector pPNT-Gpx2-KO (middle), and the targeted locus (bottom). Striped boxes contain the mouse gene. Black boxes contain the Gpx2 exons. The pPNT-Gpx2-KO has a neo cassette, which confers neomycin resistance, and a herpes simplex viral thymidine kinase gene cassette (hsv-tk), which contains a herpes simplex viral thymidine kinase gene driven by mouse phosphoglycerate kinase-1 promoter. The recognition sites for restriction enzymes are as follows: A, Apa I; H, Hind III; K, Kpn I; N, Not I; B, BamHI. The exon 1 and exon 2 probes are shown in the open box on the top of the wild-type Gpx2 gene. B: Southern blot of wild-type and Gpx2-KO mouse DNA. The Apa I-digested genomic DNA was probed with the Gpx2 exon 2. Lane 1 shows 7 kb of wild-type Gpx2 DNA and a 14 kb of the Gpx2-ps pseudogene in the homozygous Gpx2+/+ DNA. Lane 2 shows 4.9 kb of the targeted allele in addition to the 7 kb wild-type allele and the 14 kb pseudogene in the heterozygous Gpx2+/− DNA. Lane 3 shows the 4.9 kb of targeted allele and the 14 kb pseudogene in the homozygous Gpx2-KO (−/−) DNA. C: Northern blot of total RNA isolated from the middle small intestine of wild-type, heterozygous, and homozygous Gpx2-KO mice but not homozygous Gpx2-KO mice, as shown at top. The 2nd panel shows a 1-kb wild-type Gpx2 mRNA and a 3-kb Gpx2-KO mRNA with weaker intensity when probed with Gpx2 exon 2. The 3rd and 4th panels show the Gpx1 and β-actin mRNA.
results were obtained with normalization against 28S rRNA, which was also probed initially and later periodically. Quantification of the Gpx1 and Gpx2 mRNA levels was done with phosphor imaging after 6- to 24-h exposure (Molecular Dynamics, Sunnyvale, CA).

The 600 bp of EcoRI fragment of the mouse Gpx1 gene and the 400 bp of the mouse Aop2 cDNA probes have been described previously (4, 27). The 220 bp of the mouse Gpx2 exon 1 probe was generated by PCR with PX212 (GGGGCTCACCTGGCGCTTCA) and PX222 (CTTGGCTTCCCTTGCAACCAA) primers based on the human Gpx2 sequence. The 450 bp of the Gpx2 exon 2 probe was generated by PCR with the mouse mPX201 (GGACGATATTAAGGGAATGCCTT) and the human PX221 (GAGAACTGTCAGAATGAGGAGAT) primers (5). The Gpx2 exon 2 probe was used mostly because it yielded a much stronger signal. Routinely, the blots were probed in the order of Gpx2, β-actin, Gpx1, and Aop2. The old signal was stripped by two incubations of the blots at 80°C in 0.1% SDS and 0.1 × standard saline citrate for 20 min.

Measurement of PGE2 and protein levels. The mice back-crossed to B6 for 7–8 generations were used for this study. About 0.12 g of the jejunum (a 4-cm segment from 1 cm distal to the stomach) and 0.1 g of the ileum (a 4-cm segment from 1 cm proximal to cecum) were processed to measure PGE2 concentrations. These two sections of small intestine were rinsed with PBS and then snap frozen in liquid nitrogen.

Fig. 2. Regenerating jejunal crypt foci after exposure to high-dose γ-irradiation. Cross-sections of the jejunum were obtained from wild-type (Gpx1+/+; A, C, E, and G) and the Gpx1-KO (Gpx1−/−; B, D, F, and H) mice 4 days after exposure to 0, 15, 17, and 20 Gy. The regenerating crypts exhibit darker stained epithelial cells, which locate close to the muscular layer.
Without thawing, they were homogenized in 1 ml of 100% methanol containing 1 mM indomethacin to inhibit COX activity in a prechilled 10 ml cylinder with a polytron. The homogenization buffer was also prechilled on dry ice. After centrifugation at 10,000 g for 15 min, 0.5 ml of supernatant was added to 10 ml of H2O, pH 3. A trace amount of 3H-PGE2 (10,000 dpm) with a high specific activity was added to monitor for the yield through organic extraction. Ten milliliters of diluted supernatant was passed through an activated C-18 Sep-Pak cartridge (Waters, Milford, MA) for solid-phase extraction. The column was washed with 5 ml H2O followed by 5 ml hexane. Prostaglandins were eluted in 5 ml of ethyl acetate containing 1% methanol. Half a milliliter was counted for radioactivity, and another 0.1 ml was lyophilized and resuspended in 0.1–0.5 ml of an enzyme immunometric assay buffer supplied by the manufacturer (Cayman Chemical, Ann Arbor, MI). Duplicate PGE2 assays were performed on an aliquot of each sample with enzyme immunoassay.

Protein concentrations in the clarified methanol extract were determined with BCA assay (Pierce). Bovine serum albumin was used as a standard. The interference of indomethacin was accounted for by assaying the homogenization buffer. The value obtained from the homogenization buffer was subtracted from all sample values. We estimated that 0.5 μg of protein had been extracted from 1 mg of wet tissue.

Statistical analysis. All statistical analysis (ANOVA) was done with a two-tailed Student’s t-test using Microsoft Excel 97. A P value of <0.05 was considered significant.

RESULTS

Generation of Gpx2-KO mice. The target for Gpx2 gene disruption was the 300 bp of exon 1, which contained the UGA codon for the selenocysteine amino acid residue (Fig. 1A). A 2-kb DNA sequence 5' to the translation start site and 2.2 kb of mouse Gpx2 intron were generated by PCR and then inserted 5' and 3' to a 1.8-kb neo gene cassette and flanking sequence in pPNT vector. The 5' end of the neo cassette had an Apa I recognition site. Thus, after homologous recombination, Apa I digestion resulted in a 4.9-kb fragment from the disrupted Gpx2 gene and a 7-kb fragment from the wild-type Gpx2 gene when probed with the exon 2 probe (Fig. 1B). The ~14 kb of Apa I fragment appeared to contain the Gpx2 pseudogene (5).

Lanes 1, 2, and 3 of the Southern blot in Fig. 1B contain mouse DNA isolated from a wild-type, a heterozygous Gpx2-KO, and a homozygous Gpx2-KO mouse, respectively. Expression of the Gpx2 mRNA in each type of mouse is
shown in Fig. 1C. No Gpx2 mRNA was detectable in homozygous Gpx2-KO mice when the Gpx2 exon 1 was used as the probe. A Gpx2 mRNA of higher molecular weight was detected in the Gpx2-KO mice when the Gpx2 exon 2 was used as the probe.

Two ES-transfectant cell lines were used to create two independent mouse knockout lines, designated as nos. 8 and 21. No difference can be detected between these two lines based on Southern blot, Northern blot, and activity assays (data not shown). Therefore, we have combined the results obtained from these two lines.

**Crypt survival.** Whether Gpx1 and Gpx2 gene expression could have any effect on the survival of crypt epithelium after exposure to γ-irradiation was analyzed in the jejunum and the ileum (Fig. 2). The number of cryptlike foci of the surviving epithelial cells was scored on the cross-sections of intestine 4 days after exposure. Each epithelial focus represents survival of one or more clonogenic stem cells able to regenerate a crypt. Figure 2 shows the jejunum of B6 × 129 Gpx1-KO and wild-type mice after exposure to 0, 15, 17, and 20 Gy. Although γ-irradiation destroyed most of the epithelial cells, Gpx1-KO mice had more surviving stem cells proliferating to form regenerative foci than Gpx2-KO mice and wild-type mice (Fig. 3).

The fractional crypt survival was determined by the total number of crypt foci divided by the number of crypts in the nonirradiated intestines. Because the radiation damage follows the first order of kinetics, the percentage of crypt survival is plotted on a log scale. As shown in Fig. 3, after exposure to 15 Gy, there was <10% survival, plotted as log10(10) surviving crypt foci of the control. Since the genetic background can affect crypt survival, we have analyzed this in both B6 and B6 × 129 Gpx1-KO and wild-type mice. The jejunal crypt of Gpx1-KO is more resistant to radiation than wild-type and Gpx2-KO mice regardless of mouse strain background. The jejunal crypts of B6 × 129 Gpx1-KO mice are more resistant to radiation than the B6 × 129 wild-type, but the jejunal crypts of B6 Gpx1-KO mice had similar sensitivity to radiation as those of the B6 wild-type. Thus the protection of Gpx1-KO on the jejunal crypts transcends the strain differences, and the genetic background is as big a factor in the ileal crypts. No difference was found between Gpx2-KO and wild-type mice in both ileum and jejunum.

**Elevation of GPX activity.** To determine whether the crypt survival correlated with GPX activity, we analyzed the GSH-dependent H2O2-reducing activity in the jejunal and ileal crypts. No difference was found between the control and irradiated wild-type mice and between the control and irradiated Gpx1-KO mice. No difference was found between the control and irradiated Gpx2-KO mice.

Fig. 5. Increase of GPX activity in mouse ileal epithelium after exposure to 11 Gy. The ileal epithelium was isolated from wild-type, Gpx1-KO, and Gpx2-KO mice 4 days after exposure to 0 and 11 Gy. The error bars are standard deviations of the means from 38 control and 13 irradiated wild-type mice, and 12 control and 13 irradiated Gpx1-KO mice. Significant difference (P < 0.01) in GPX activity levels is found between the control and irradiated wild-type mice and between the control and irradiated Gpx1-KO mice. No difference was found between the control and irradiated Gpx2-KO mice.
exposed Gpx1-KO mice was higher than that in the unexposed wild-type mice. One each of B6 and B6 × 129 Gpx1-KO mice and two B6 × 129 wild-type mice were assayed at each time point as shown in Fig. 4. Since we did not find any difference in GPX activity between these two lines of Gpx1-KO mice, we have combined the data.

The increase of GPX activity in ileal epithelium appeared to be solely contributed by increased levels of Gpx2 mRNA. As shown in Fig. 5, Gpx2-KO ileal epithelium did not increase GPX activity assayed at the fourth day after exposure to 11 Gy. This is in contrast to the wild-type and Gpx1-KO ileal epithelium, in which the GPX activity increased 50% and twofold, respectively, at the fourth day after exposure. Each column represents the mean of 9–38 mice.

Elevation of the Gpx2 mRNA levels after γ-irradiation. To determine whether the increase in GPX activity in ileal epithelium appeared to be solely contributed by increased levels of Gpx2 mRNA. As shown in Fig. 5, Gpx2-KO ileal epithelium did not increase GPX activity assayed at the fourth day after exposure to 11 Gy. This is in contrast to the wild-type and Gpx1-KO ileal epithelium, in which the GPX activity increased 50% and twofold, respectively, at the fourth day after exposure. Each column represents the mean of 9–38 mice.

The dosage response to γ-irradiation of the increase of Gpx2 mRNA levels was studied in the middle small intestine of B6 × 129 Gpx1-KO and wild-type mice (Fig. 8). When exposed to 0–6 Gy, the Gpx2 mRNA levels in the Gpx1-KO mice were at 50% of wild-type mice assayed at the third day after exposure. When exposed to >10 Gy, the Gpx1-KO and wild-type mice had the same levels of Gpx2 mRNA. Each data point represents the average of 2–4 mice.

Since Gpx1 gene expression appeared to have an impact on the Gpx2 mRNA levels, we had also analyzed whether there was a reciprocal effect between these two genes, i.e., if the Gpx1 mRNA levels were altered in the Gpx2-KO mice. Figure 1C shows the Northern blot analysis of RNA isolated from the middle small intestine of the Gpx2-KO and wild-type mice at the fourth
day after exposure to 0 and 17 Gy. The Gpx2-KO mRNA of higher molecular weight was expressed at 50% of the endogenous Gpx2 mRNA level. The Gpx2-KO mRNA can only be detected with the Gpx2 exon 2 probe but not with the exon 1 probe. The lower level of Gpx2-KO mRNA was evident in the heterozygous mice, as shown in lane 12 of Fig. 1C.

After exposure to γ-irradiation, the Gpx2-KO mRNA level was also elevated in small intestine, as shown in Figs. 1C and 9. The latter figure is the analysis of Fig. 1C. The amount of Gpx2 mRNA was eightfold higher (from 0.58 ± 0.17 to 5.35 ± 2.96 of the normalized Gpx2 mRNA level; P = 0.04) in wild-type mice after exposure to 17 Gy when the amount of Gpx2-KO mRNA was onefold higher in Gpx2-KO mice (from 0.29 ± 0.08 to 0.49 ± 0.25; P = 0.17). There was no change in the Gpx1 mRNA level in the irradiated Gpx2-KO mice (from 0.56 ± 0.03 to 0.65 ± 0.19 of the normalized Gpx1 mRNA level; P = 0.40) and wild-type mice (from 0.51 ± 0.06 to 0.65 ± 0.12; P = 0.09).

Effect of Gpx1 gene expression on PGE2 levels in the small intestine. To determine whether Gpx1 gene expression affects PG biosynthesis in the small intestine, we have measured PGE2 concentrations in jejunum and ileum after exposure to 15 Gy. As shown in Fig. 10, B6 Gpx1-KO jejunum had a higher level of PGE2 than the wild-type jejunum after exposure to 0 and 15 Gy. The basal PGE2 concentration in the Gpx1-KO jejunum was 21.0 ± 8.5 vs. 15.5 ± 10 pg PGE2/µg protein in the wild-type jejunum. The induced PGE2 concentration in the Gpx1-KO jejunum was 215 ± 76 pg PGE2/µg protein after exposure to 15 Gy compared with 146 ± 41 pg...
PGE$_2$/$\mu$g protein in the wild-type jejunum. The Gpx1-KO ileum also had a higher basal level of PGE$_2$ (38 ± 9 pg PGE$_2$/\mu$g protein) than the wild-type ileum (20 ± 10.5 pg PGE$_2$/\mu$g protein). However, the irradiated Gpx1-KO ileum had the same level of PGE$_2$ (101 ± 57 pg PGE$_2$/\mu$g protein) as the exposed wild-type ileum (114 ± 64 pg PGE$_2$/\mu$g protein). Nevertheless, none of the differences are statistically significant. Only the increase of PGE$_2$ level by \gamma-irradiation in both the jejunum and ileum of the Gpx1-KO and the wild-type mice is statistically significant.

**DISCUSSION**

We have previously shown that both GPX-1 and GPX-GI are the major GPX activities in the intestinal epithelium of mouse and rat. The mouse jejunal and ileal epithelia have different levels of GPX-1 and GPX-GI activity (6, 10). To study their function in the crypt epithelium after exposure to \gamma-irradiation, we have generated Gpx1-KO mice. Similar to the Gpx1-KO mice, the Gpx2-KO mice have lower levels of GPX activity in the gastrointestinal tract than the wild-type mice, and there is no unusual phenotype in the Gpx2-KO mice under normal conditions (17). GPX-1 contributes to 80% and 50% of total GPX activity in the jejunum and the ileum, respectively. Four days after exposure to high-dose \gamma-irradiation, the jejunum of both B6 and B6 × 129 Gpx1-KO mice has higher numbers of surviving crypt foci than the wild-type controls. The ileum of the B6 × 129 but not B6 Gpx1-KO mice is also more resistant to radiation. Thus radiation resistance can be associated with lack of GPX activity. The levels of resistance found in the Gpx1-KO mice are comparable to the protection afforded by KGF and PG mimetics (9–11).

It was previously reported that radiation injury decreases the number of surviving crypts in the jejunum. Indomethacin, an inhibitor of COX, further decreases the number of crypt foci. The indomethacin response can be blocked by dimethyl-PGE$_2$ (8). In vitro studies have shown that GPX activity inhibits activation of COX, the rate-limiting enzyme for PGE$_2$ biosynthesis (2, 23). Radiation increases the PGE$_2$ concentration ninefold in the jejunum of the Gpx1-KO and wild-type mice and about threefold in the ileum of the Gpx1-KO and wild-type mice. With the exception of the irradiated ileum, the Gpx1-KO small intestine has higher levels of PGE$_2$ than wild-type small intestine does. Perhaps because of the large variation of PGE$_2$ levels in samples, the differences are not statistically significant. Thus we cannot make any conclusions about the in vivo effect of GPX activity on the PGE$_2$ level. Our PGE$_2$ measurements are comparable to the reported data in FVB/N mice (8). This suggests that the PGE$_2$
level is similarly regulated by γ-irradiation in these two strains of mice.

Although the Gpx2 mRNA was expressed in the jejunal epithelium of the wild-type and Gpx1-KO mice, it did not result in a significant increase of GPX activity, probably due to the lack of GPX-GI production. The juncture between the regions of the intestine showing activity increases was between 33 and 50% of the length of the intestine from the duodenum (data not shown). The lack of GPX-GI activity in the jejunum may be due to the fact that GPX-GI is ultrasensitive to cellular redox status. GPX-1 is already sensitive to oxidative damage (16, 29, 44), and we have found that GPX-GI is more labile than GPX-1 (4). Thus the lack of GPX activity in the jejunum of the Gpx1-KO mice may be due to the rapid inactivation of GPX-GI in this tissue in the absence of GPX-1. In the wild-type mouse jejunum, the high level of GPX-1 activity would have masked the increase of GPX-GI activity.

Contrary to jejunal Gpx2 gene expression, the increase of Gpx2 mRNA level in the ileal epithelium does result in an increase of GPX activity. After exposure to ≥10 Gy, the GPX activity level in Gpx1-KO ileum is higher than that found in the unexposed wild-type mice. This provides the first evidence that the Gpx2 gene can compensate for the lack of Gpx1 gene expression. Unlike the Gpx1 gene, of which mRNA level is constitutively expressed in most tissues, the Gpx2 mRNA level is highly regulated. In addition to this effect of radiation, all-trans retinoic acid can increase Gpx2 mRNA level 10-fold, as shown in MCF-7 breast cancer cells (7). Since γ-irradiation of MCF-7 cells did not increase Gpx2 mRNA levels (data not shown), and maximal levels of Gpx2 mRNA in small intestine occurred at 3–4 days postirradiation, this suggests that the increase in mRNA levels is not a direct response to radiation. Rather, it is a response to some component of secondary injury. The kinetics of Gpx2 mRNA changes is similar to the changes observed with Cox1 in the intestine after exposed to high-dose γ-irradiation (8). This suggests that the Gpx2 and the Cox1 genes are coregulated in this exposure.

Although we have found that most GPX activity in the intestinal epithelium is contributed by GPX-1 and GPX-GI under normal conditions, the recent identification of Aop2 has raised the question of its possible contribution to GPX activity after exposure to γ-irradiation. The Aop2 gene is normally expressed at a low level in the intestinal epithelium but is highly expressed in the eye (13, 27, 37). It has been shown in keratinocytes that the Aop2 gene is highly inducible by KGF, which can also increase crypt survival. We can rule out any significant contribution of Aop2 to total GPX activity in the intestinal epithelium on the basis of the following observations: 1) Aop2 mRNA level was increased only onefold after exposure to high-dose γ-irradiation; 2) Aop2 has low GPX activity, and 3) no increase in GPX activity occurred in Gpx2-KO mice after exposure to radiation.

Our study elucidates a function for Gpx1 gene expression in the intestinal epithelial cells after exposure to γ-irradiation. The fact that the Gpx1-KO and the wild-type ileum have similar sensitivity to γ-irradiation is most likely a result of the compensatory effect of Gpx2 gene expression. To study the function of the Gpx2 gene in the ileal epithelium against high-dose radiation, the crypt survival in Gpx1 and Gpx2 double knockout mice should be analyzed in the future.

Considering the beneficial effect of voiding GPX activity in the crypt epithelium against radiation damage, it may be highly desirable to develop GPX-specific inhibitors as therapeutic reagents. Gold (I)-containing compounds, including aurothioglucone and the antiarthritis drug auranofin, are potent inhibitors for several selenocysteine-containing enzymes (33, 38). Unfortunately, aurothioglucone is a much more potent inhibitor for thioredoxin reductase and less effective on GPX-1 when administered intraperitoneally. To our knowledge, there are no GPX-specific inhibitors reported. We thank Heather Adams for maintaining mouse colonies, Helen Sun for processing mouse tissue samples for histological analysis, Sabine Werner at the Swiss Federal Institute of Technology for providing the mouse Aop2 cDNA clone, and Jason D. Morrow at Vanderbilt University for advice on the POR assay.

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