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Enhancement of Radiation-Inducible Hepatic Glutathione-*S*-Transferases *Ya*, *Yb1*, *Yb2*, *Yc1*, and *Yc2* Gene Expression by Oltipraz: Possible Role in Radioprotection

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SUMMARY

Previous studies have shown that radiation in combination with oltipraz enhances hepatic microsomal epoxide hydrolase expression. The effects of γ-ray radiation exposure in combination with oltipraz on the expression of hepatic glutathione-*S*-transferase (GST) subunits *Ya*, *Yb1*, *Yb2*, *Yc1*, and *Yc2* were examined in the rat. Northern RNA blot analyses revealed that GST mRNA levels were altered in response to daily 3- or 0.5-Gy doses of radiation. The hepatic GST mRNA levels were transiently decreased at 3 and 8 hr after a single 3-Gy dose of radiation. The GST Ya, Yb1, Yb2, Yc1, and Yc2 mRNA levels were increased by 2–4-fold at 15 and 24 hr after irradiation with 3 Gy, followed by return to the levels of untreated rats at 48 hr after treatment. The treatment of animals with oltipraz alone resulted in dose-related increases in the GST Ya, Yb1, Yc1, and Yc2 mRNA levels, whereas Yb2 mRNA levels were minimally increased. Although a single dose of oltipraz (30 mg/kg orally) caused a minimal 2-fold elevation in the hepatic GST Ya mRNA level, exposure of animals to both oltipraz and 3-Gy radiation resulted in a 4-fold relative increase in GST Ya mRNA level, indicating that the Ya mRNA expression was additively enhanced by the combination treatment. The Yb1/2 and Yc1/2 mRNA expressions were also enhanced by oltipraz in combination with radiation. Multiple exposure of rats to daily 0.5-Gy radiation caused time-related increases in GST gene expression. The greatest enhancement in GST expression was observed at 24 hr after a single 0.5-Gy dose of radiation in conjunction with oltipraz (e.g., a 9-fold relative increase in GST *Ya*), whereas the relative additive increases in GST mRNA were less pronounced at day 3 or 5 after treatment. These increases in the GST mRNA levels were consistent with those in the immunochemically detectable GST protein levels. Histopathological examinations revealed that exposure of rats to radiation (0.5 Gy/day for 3–5 days) caused mild-to-moderate hepatocyte degeneration with sinusoidal congestion, whereas oltipraz (30 mg/kg/day for 3 days) was effective in blocking the radiation-induced liver injury. The enhanced expression of these GST isoforms by oltipraz may be associated in part with its hepatoprotective effect against the injury caused by ionizing radiation.

Ionizing radiation can excite water molecules and leads to the production of the activated oxygen species, including hydroxyl free radicals, hydrated electrons, and hydrogen atom, from water (1). Ionizing radiation induces multiple biological effects through direct interaction with DNA or through formation of free radical species. It has been shown that small doses of hydrogen peroxide can induce protection against subsequent doses of ionizing radiation, which supports the hypothesis that oxidative species or single-strand breaks function as inducers. Ionizing radiation increases oxidative stress followed by GSH depletion as a result of enhanced detoxification of hydrogen peroxide and hydroxyl free radical by GSH peroxidase and oxidation of GSH to oxidized GSH (2). Oxidative stress is removed through a compensating increase in *de novo* GSH synthesis. Endogenous levels in hepatic GSH contribute to the maintenance of cell homeostasis through scavenging free radicals generated from irradiation and/or xenobiotics. Thus, it is possible that the enzymes associated with GSH levels are altered in response to ionizing radiation.

A number of studies have shown that activated oxygen species are involved in the induction of xenobiotic detoxification enzymes, including mEH and GSTs (3–6). The induction of protective enzymes during oxidative stress will result in a decrease in the levels of reactive oxygen species. Studies have shown that ARE-mediated induction of GST *Ya* gene expression in hepatoma cells by adriamycin correlates with their

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ABBREVIATIONS: GSH, glutathione; ARE, antioxidant responsive element; GST, glutathione-*S*-transferase; mEH, microsomal epoxide hydrolase; SDS, sodium dodecyl sulfate; SSC, standard saline citrate.

oxygen radical production and that intracellular GSH levels affect expression of certain GST genes (7). Intracellular redox potential is maintained by regulating the levels of reactive oxygen species via the GSH peroxidase/GSH reductase.

Inductive expression of certain gene and protein up-regulations seems to be associated with radioprotection, which may address a modulation of repair processes (8–11). Nevertheless, little information is available regarding the regulation of transcriptional control by ionizing radiation. The current study was designed to establish radiation-inducible changes in the GST gene expression, which may address in part the induction of radioresistance. The role of oltipraz in conjunction with radiation in the expression of major GST detoxification enzymes was also assessed under the hypotheses that chemical modulation of GST expression assists in detoxifying free radicals generated from radiation exposure and that oltipraz protects the liver against radiation-induced injury through modulation of phase II detoxification enzyme expression. In addition, expression in GST *Ya* and *Yc* genes in response to radiation and/or certain xenobiotics, including radioprotective or chemoprotective agents, may play a role in the detoxification of toxicants (e.g., aflatoxin B1) (12). In this study, we address the induction of hepatic GSTs as part of the complete study of the transcriptional regulation of hepatic detoxification enzymes in response to radiation- and oltipraz-induced potential modulation of radioresistance.

Experimental Procedures

Materials. [α-^{32}P]dCTP (>110 TBq, 3000 Ci/mmol) and [γ-^{32}P]ATP (>110 TBq, 3000 Ci/mmol) were purchased from New England Nuclear Research Products (Boston, MA). Biotinylated goat anti-rabbit IgG, streptavidin-conjugated horseradish peroxidase, and random prime labeling and 5′-end labeling kits were purchased from GIBCO BRL (Gaithersburg, MD). Form-specific polyclonal rabbit anti-rat liver GST Ya (GSH transferase 1), Yb1 (GSH transferase 3), Yb2 (GSH transferase 4), and Yc (GSH transferase 2) antibodies were purchased from Biotrin International (Dublin, Ireland). Most of the reagents in the molecular studies were obtained from Sigma Chemical (St. Louis, MO). Oltipraz was a gift from Rhone-Poulenc Rorer (Vitry-sur-Seine, France).

Animal treatment. Male Sprague-Dawley rats were obtained from Daehan Laboratory Animals (Seoul, Korea) and maintained at a temperature of 20–23° with a relative humidity of 50%. Animals were caged, breathed filtered pathogen-free air, and were given food (rodent chow; Cheiljedang, Ichon, Korea) and water *ad libitum*. Rats (180–220 g) were treated with either 3 or 0.5 Gy of radiation/day. Rats were subjected to total-body irradiation at a dosage of 12.5 cGy/min from a ^{60}Co radiation source. Rats were placed in an acrylic chamber that allowed little movement. The chamber was designed to fill a radiation field of 47.5 × 47.5 cm^2. In a time-dependent study, animals were killed at 3, 8, 15, 24, or 48 hr after a single dose of γ-ray radiation. Rats were fasted for 16 hr before they were killed. Each value is derived from pooled samples from treatment groups consisting of three animals. Results were confirmed using different groups of animals.

Oltipraz was suspended in 0.1% high viscosity carboxymethylcellulose and administered to rats at doses of 5–200 mg/kg. Animals were gavaged with oltipraz 3 hr before irradiation. Animals exposed to multiple daily radiation doses were killed at 24 hr after the last dose.

Isolation of cytosolic proteins. Liver cytosolic fraction was prepared by differential centrifugation and stored at −70° until use. Protein content was determined according to the method of Lowry *et al*. (13).

Immunoblot analysis. SDS-polyacrylamide gel electrophoresis analysis was performed according to Laemmli (14) using a Hoefer gel apparatus. Immunoblot analysis was performed according to previously published procedures (15–18). Briefly, GST proteins were separated by 12% SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose paper. The nitrocellulose paper was incubated with rabbit anti-rat GST Ya, Yb1, Yb2, or Yc antibodies. Biotinylated goat anti-rabbit IgG was used as the secondary antibody. Immunoreactive protein was visualized through incubation with streptavidin-horseradish peroxidase, followed by the addition of both 4-chloro-1-naphthol and hydrogen peroxide. Band intensities in immunoblots were assessed by loading different amounts of cytosolic proteins to avoid saturation, followed by the use of scanning densitometry. The relative fold increases were determined from multiple immunoblottings using four groups of animals.

Isolation of total RNA. Total RNA was isolated using the improved single-step method of acid guanidinium thiocyanate-phenol-chloroform RNA extraction according to the methods of Chomczynski and Sacchi (19), as modified by Puissant and Houdebine (20).

Preparation of cDNA probes for major GST subunits. cDNAs for major GSTs were prepared as described previously. Specific cDNA probes for GST genes *Ya* (287–684), *Yb1* (643–963), *Yb2* (415–942), *Yc1* (122–488), and *Yc2* (122–530) were amplified by reverse-transcriptase polymerase chain reaction using the selective primers for each gene, as described previously (21–25). Polymerase chain reaction-amplified DNA products using a cDNA derived from hepatic poly(A)$^+$ RNA obtained from rats treated with pyrazine as a template were cloned in a pGEM$^+$T vector (Promega, Madison, WI).

Northern blot hybridization. Northern blotting was carried out according to procedures described previously (18, 25). Briefly, total RNA isolated from rat livers was resolved through electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde and then transferred to supported nitrocellulose paper through capillary transfer. The nitrocellulose paper was baked in a vacuum oven at 80° for 2 hr. The blot was incubated with hybridization buffer containing 50% deionized formamide, 5× Denhardt's solution [5× = 0.1% Ficoll, 0.1% polyvinylpyrrolidine, and 0.1% bovine serum albumin (Pentex Fraction V)], 0.1% SDS, 200 μg/ml sonicated salmon sperm DNA, and 5× SSPE (1× SSPE is composed of 0.15 M NaCl, 10 mM NaH_2PO_4, and 1 mM Na_2EDTA, pH 7.4) at 42° for 1 hr without probe. Hybridization was performed at 42° for 18 hr with a heat-denatured cDNA probe, which was random prime-labeled with [α-^{32}P]dCTP. Filters were washed twice in 2× SSC (1× SSC = 150 mM NaCl, 15 mM sodium citrate)/0.1% SDS for 10 min at room temperature and twice in 0.1× SSC/0.1% SDS for 10 min at room temperature. Filters underwent a final wash in the 0.1× SSC/0.1% SDS solution for 60 min at 60°. After quantification of GST mRNA levels, the membranes were stripped and rehybridized with ^{32}P-end-labeled poly(dT)$_{16}$ to quantify the amount of RNA loaded onto the membranes. Duplicate Northern blot analyses were performed on different mRNA samples.

Scanning densitometry. Scanning densitometry was performed with a Microcomputer Imaging Device (model M1; Imaging Research, St. Catharines, Ontario, Canada). The area of each lane was integrated using Microcomputer Imaging Device (MCID) software version 4.20, Revision 1.0, followed by background subtraction.

Results

Effects of a sublethal dose (3 Gy) of ionizing radiation. Changes in GST gene expression after a subtoxic dose of γ-ray radiation were comparatively assessed. Northern blot analyses were performed to examine changes in GST Ya, Yb1, Yb2, Yc1, or Yc2 mRNA levels in response to 3 Gy of radiation. A single radiation exposure resulted in a biphasic response in the expression of GSTs (Fig. 1). Hepatic GST Ya mRNA levels were transiently decreased at 3 and 8 hr after irradiation to 60% and 50% of the levels in untreated animals

(Fig. 1, A and B). Then, the Ya mRNA levels were increased by 2- and 3-fold at 15 and 24 hr after treatment, respectively, relative to untreated animals. Radiation-increased GST Ya mRNA level returned to that of untreated rats at 48 hr after treatment. The levels of GST Yb1 and Yb2 mRNA were also increased by ~2-fold at 15 and 24 hr after irradiation, followed by a return to the levels of untreated rats at 48 hr after treatment. Thus, radiation exposure affected GST Yb1 and Yb2 mRNA levels to lesser extents. Ionizing radiation also elevated GST *Yc1* and *Yc2* gene expression by 2- and 3-fold, respectively, at 24 hr after treatment relative to control (Fig. 1, A and B).

The mRNA levels in major GST subunits were determined at 24 hr after a single dose of oltipraz. Oltipraz treatment resulted in 1-, 2-, 12-, 16-, and 12-fold increases in GST Ya mRNA at 24 hr at doses of 5, 20, 50, 100, and 200 mg/kg, respectively (Fig. 2, A and B). The levels of GST Yb1 mRNA were increased 1-, 2-, 6-, 9-, and 9-fold at the doses used, respectively, whereas Yb2 mRNA levels were increased by ~2-fold at the same doses. Increases in GST Yc1 mRNA level were less substantial than those in GST Ya mRNA (~9-fold increase). Expression in GST Yc2 mRNA was substantially elevated at doses of >50 mg/kg (i.e., >10-fold increase) (Fig. 2, A and B). The dose of 30 mg/kg oltipraz was used in subsequent experiments because GST mRNA levels were moderately affected.

Studies were designed to establish whether animals treated with 30 mg/kg oltipraz before receiving γ-ray radiation exhibit modulation in the major hepatic GST mRNA levels and whether a common molecular mechanism exists between the radiation- and chemical-inducible alterations in GST gene expression. Although animals irradiated with 3 Gy of γ-ray radiation showed a 3-fold increase in the GST Ya mRNA level at 24 hr after treatment and a single dose of oltipraz (30 mg/kg orally) caused a ~2-fold elevation in the mRNA level, animals exposed to both oltipraz and γ-ray radiation exhibited a 4.5-fold increase in GST Ya mRNA, which demonstrates that the radiation-inducible increase in the GST *Ya* gene expression was additively enhanced by concomitant oltipraz treatment (Fig. 3, A and B).

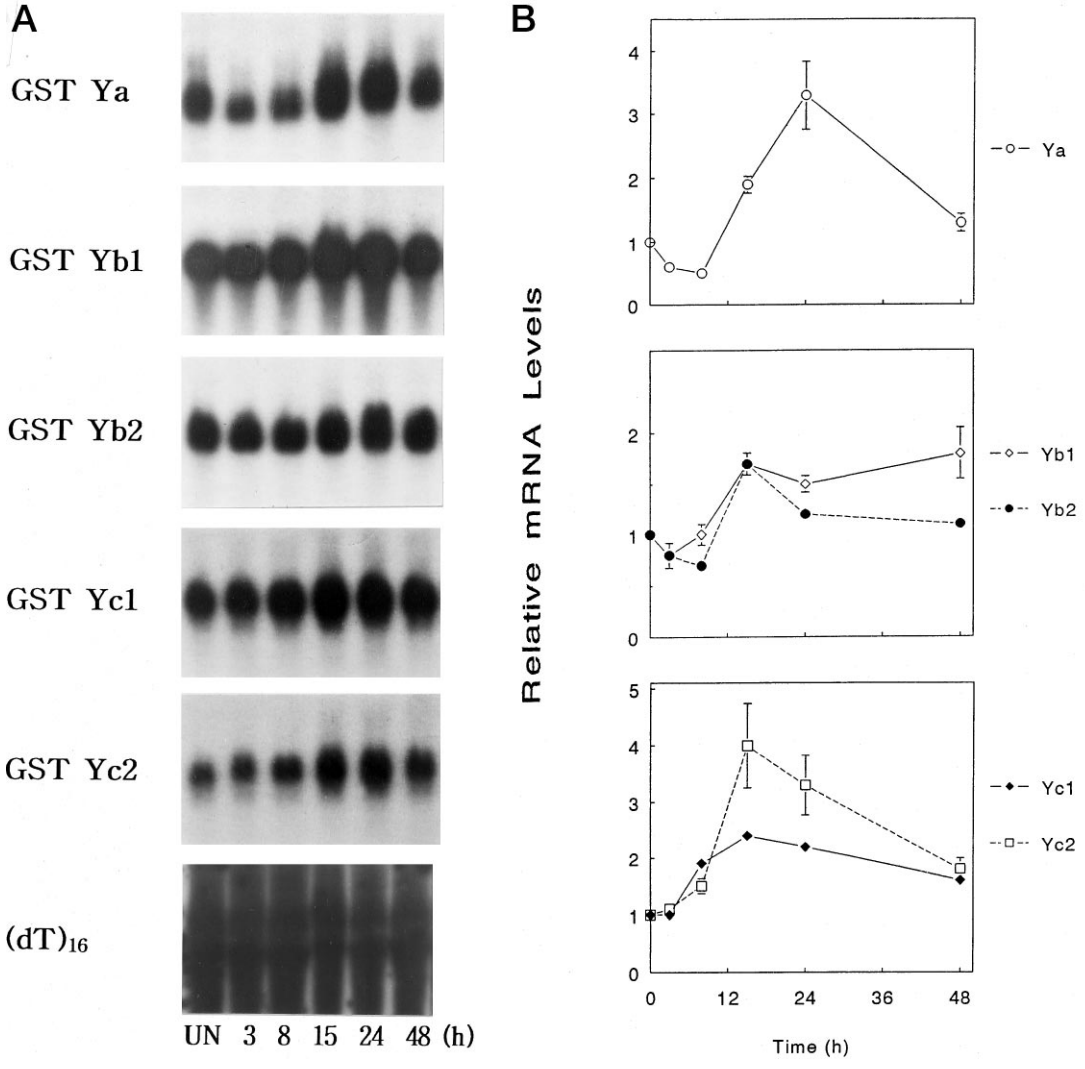

Fig. 1. RNA blot analyses of hepatic GST mRNA levels in rats after 3 Gy of γ-ray radiation. A, Northern blot analysis was performed to examine mRNA levels for GST *Ya*, *Yb1*, *Yb2*, *Yc1*, and *Yc2* in total RNA fractions (20 μg of each) isolated from untreated animals (*UN*) or from rats at 3, 8, 15, 24, and 48 hr after a single 3-Gy dose of radiation. The amount of RNA loaded onto each lane was assessed by rehybridization of the stripped membrane with ^{32}P-labeled poly(dT)$_{16}$. B, Relative changes in GST mRNA levels after irradiation with 3 Gy. Each value is the mean ± standard deviation from four determinations.

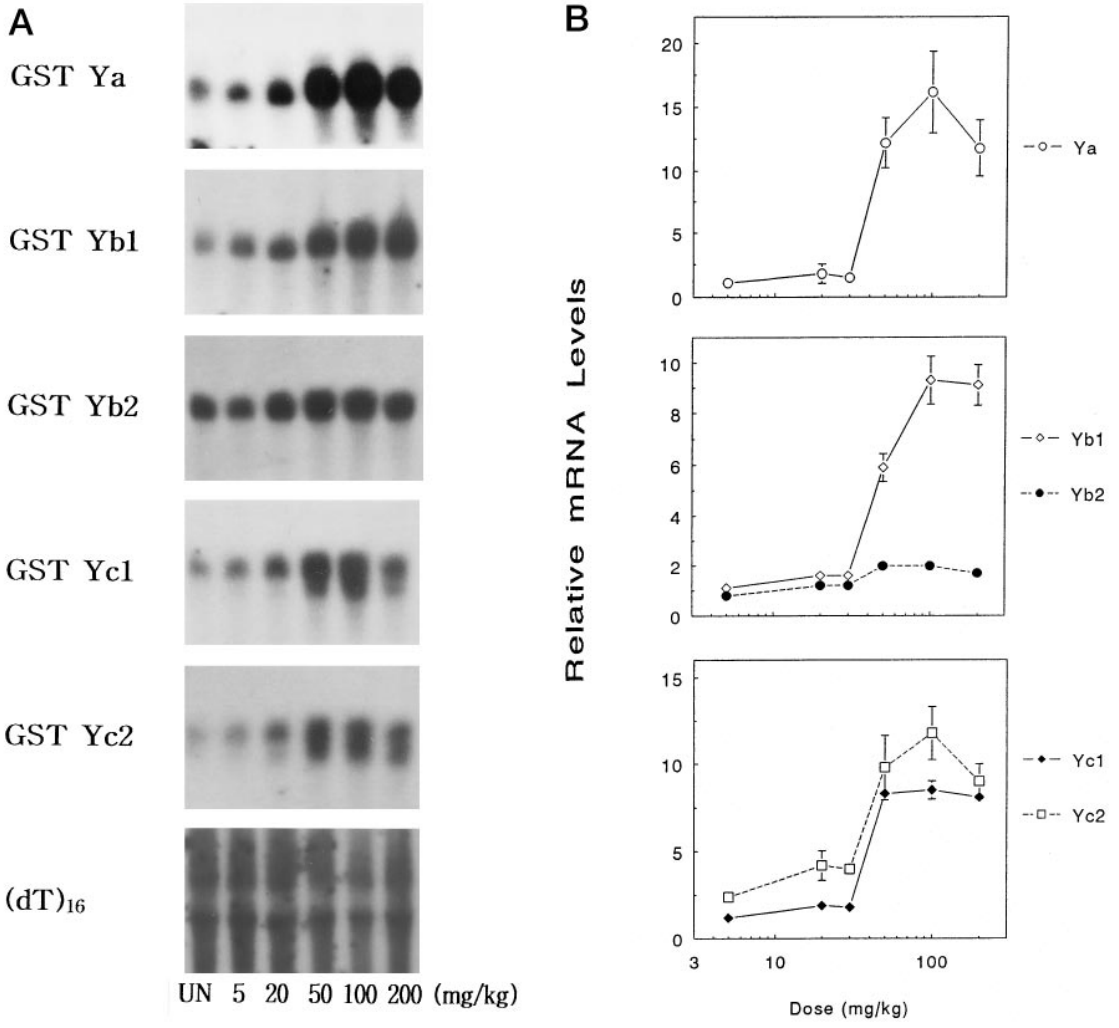

Fig. 2. Hepatic GST mRNA levels in rats after treatment with oltipraz. A, Dose-dependent increases in GST mRNA levels at 24 hr after a single dose of oltipraz (5, 20, 50, 100, or 200 mg/kg body weight orally). *UN*, untreated animals. Equal mRNA loading in each lane was assessed by rehybridization of the stripped membrane with ^{32}P-labeled poly(dT)$_{16}$. B, Relative GST mRNA levels. Each value is the mean ± standard deviation from four determinations.

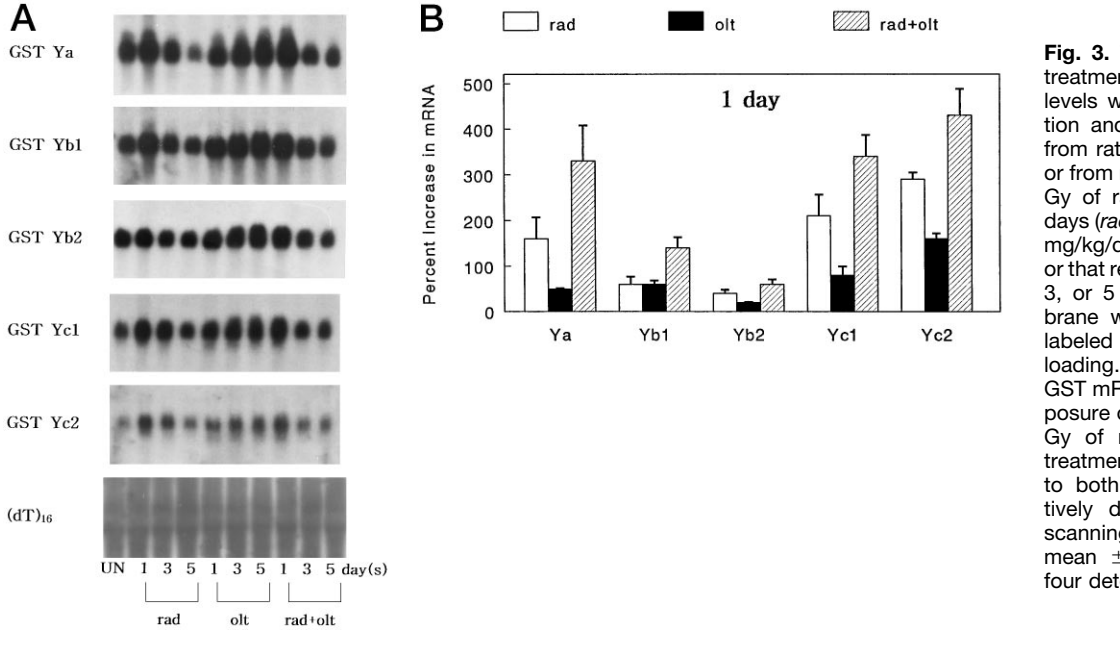

Fig. 3. The effects of combination treatment of rats on GST mRNA levels with both 3-Gy γ-ray radiation and oltipraz. A, Samples are from rats that were untreated (*UN*) or from rats that were exposed to 3 Gy of radiation/day for 1, 3 or 5 days (*rad*); that received oltipraz (30 mg/kg/day) for 1, 3, or 5 days (*olt*); or that received both (*rad*+*olt*) for 1, 3, or 5 days. The stripped membrane was rehybridized with ^{32}P-labeled poly(dT)$_{16}$ to assess mRNA loading. B, Percent increases in GST mRNA levels at 24 hr after exposure of rats to a single dose of 3 Gy of radiation (*rad*), to oltipraz treatment (30 mg/kg orally) (*olt*), or to both (*rad*+*olt*) were comparatively depicted after the use of scanning densitometry. *Points*, the mean ± standard deviation from four determinations.

The extent of changes in GST Ya mRNA level was less after three consecutive daily radiation exposures (i.e., a total of 9 Gy of radiation) than that after a single 3-Gy exposure, resulting in a minimal additive change (Fig. 3A). Five consecutive daily irradiation (i.e., a total of 15 Gy) failed to show increases in GST mRNA levels due to radiation-induced liver injury.

The GST Yb1 and Yb2 mRNA levels were elevated ~2-fold at 24 hr in response to 3-Gy radiation, probably because these GST forms are constitutively expressed. Rats treated with both oltipraz and radiation exhibited ~2-fold increases in Yb1 and Yb2 mRNA levels compared with control animals, indicating that oltipraz failed to further enhance radiation-inducible increases in GST μ gene expression. Multiple daily radiation exposures again resulted in no enhancement due to liver cell death (Fig. 3A).

GST *Yc* expression was minimally affected by either 3 Gy of radiation or oltipraz (i.e., 2-fold). In contrast, oltipraz pretreatment before irradiation resulted in 4-fold increases in either Yc1 or Yc2 mRNA levels at 24 hr after treatment (Fig. 3, A and B). Three consecutive daily radiation exposures resulted in a slight reduction from the maximal increase. Five-day exposure failed to increase the Yc mRNA level due to cytotoxicity.

Effects of a low dose (0.5 Gy) ionizing radiation. Additional studies were designed to determine the effects of low dose ionizing radiation (0.5 Gy) on the expression of hepatic GST forms. Rats irradiated with a single dose of 0.5 Gy of γ-ray radiation exhibited a slight suppression in GST gene expression at 3 hr after treatment, followed by transient increases in gene expression at 8–15 hr (Fig. 4, A and B). Although a single dose of 0.5 Gy of radiation failed to alter GST mRNA levels at 24 hr after treatment, consecutive daily 0.5-Gy treatments of animals for 3 or 5 days (i.e., for a total of 1.5 or 2.5 Gy) caused a 6-fold elevation in GST Ya mRNA levels relative to control animals (Fig. 5, A and B). Concomitant exposure of animals to both oltipraz at the daily dose of 30 mg/kg and 0.5 Gy of ionizing radiation resulted in enhanced elevation in GST *Ya* gene expression, resulting in 9-, 11-, and 7-fold increases in Ya mRNA level after 1-, 3-, and

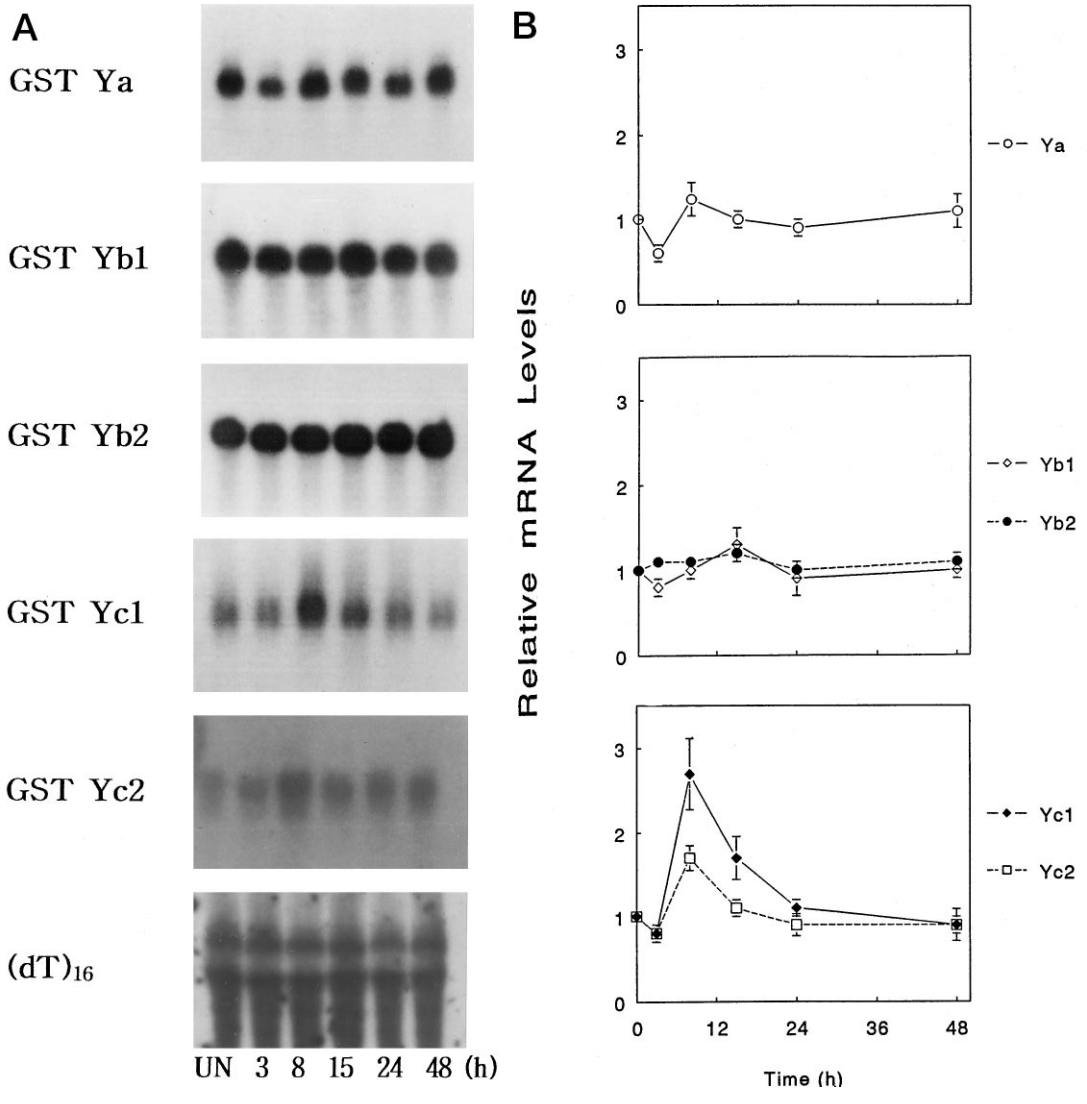

Fig. 4. Time course of hepatic GST mRNA levels in rats irradiated at a single dose of 0.5-Gy γ-ray radiation. A, Northern blot analyses show GST mRNA levels in the samples (20 μg of each) isolated from untreated animals (*UN*) or from rats at 3, 8, 15, 24, or 48 hr after 0.5 Gy of radiation. The amount of RNA loaded onto each lane was assessed by rehybridization of the stripped membrane with ^{32}P-labeled poly(dT)$_{16}$. B, Relative GST mRNA levels. Each value is the mean ± standard deviation from four determinations.

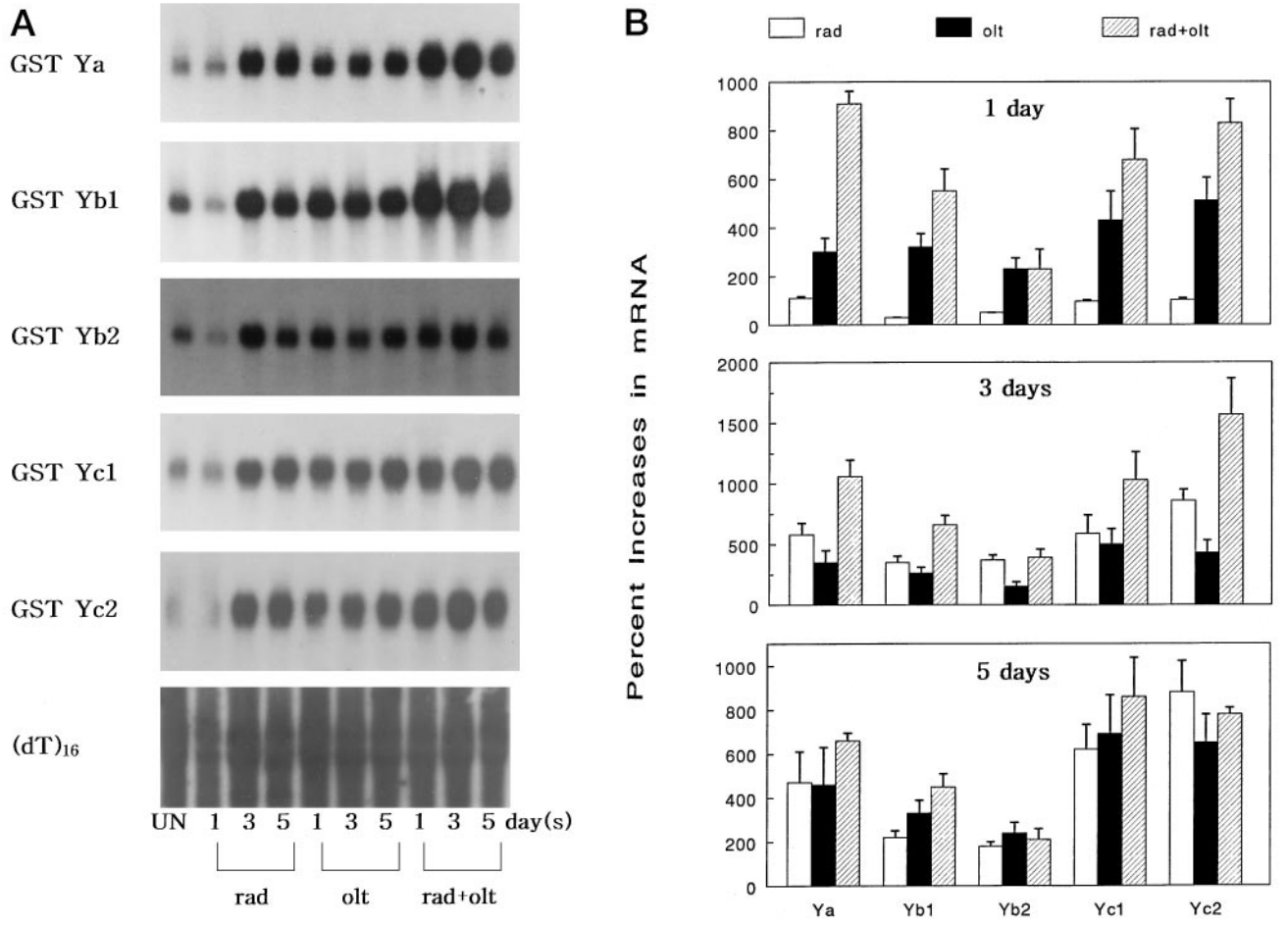

Fig. 5. The effects of 0.5 Gy γ-ray radiation with oltipraz treatment. A, Representative Northern blots show hepatic GST mRNA levels in rats that were untreated (*UN*) or from rats that were exposed to 0.5 Gy of radiation/days for 1, 3 or 5 days (*rad*); that received oltipraz (30 mg/kg/day) for 1, 3, or 5 days (*olt*); or that received both (*rad+olt*) for 1, 3, or 5 days. The stripped membrane was rehybridized with ^{32}P-labeled poly(dT)$_{16}$. B, Percent increases in GST mRNA levels at day 1, 3, or 5 after exposure of rats to daily 0.5 Gy of radiation (*rad*), to oltipraz treatment (30 mg/kg orally) (*olt*), or to both (*rad+olt*). Each value is the mean ± standard deviation from four determinations.

5-day treatments, respectively. Thus, increases in GST Ya mRNA reached plateau at day 3 after treatment (Fig. 5, A and B).

Although Yb1 mRNA levels were minimally changed after 0.5 Gy of radiation alone, oltipraz treatment at the daily dose of 30 mg/kg at 3 hr before 0.5 Gy of radiation caused much greater increases in Yb1 mRNA levels (i.e., a ~6-fold relative increase) than did the treatment of rats with either oltipraz or 0.5 Gy of radiation (Fig. 5, A and B). The levels of GST Yb2 mRNA were minimally affected by radiation and oltipraz (i.e., ~2-fold). GST Yc1/2 mRNA levels failed to be altered in response to a single dose of 0.5 Gy of radiation, whereas oltipraz treatment with radiation caused 7–8-fold increases in Yc1 or Yc2 mRNA compared with control animals, which was greater than those caused by oltipraz alone (i.e., ~4-fold). The relative increase in Yc2 mRNA in response to 0.5 Gy of ionizing radiation was greater than the increase in GST Yc1 mRNA, probably because *Yc2* is an inducible GST subunit.

To confirm whether the expression of hepatic GST mRNA levels parallels the induction of the proteins, immunoblot analyses for GST subunits Ya, Yb1, Yb2, and Yc were carried out with hepatic cytosolic proteins. Fig. 6 shows the representative immunoblots. Hepatic cytosol produced from rats irradiated with 0.5 Gy of γ-ray radiation/day for 5 days resulted in 1.4-, 2.9-, and 1.3-fold inductions of GST Ya, Yb1, and Yc forms, respectively, relative to control animals (Table 1). Rats exposed to daily 0.5-Gy doses of γ-ray radiation in combination with oltipraz exhibited 2.1-, 3.6-, and 1.5-fold increases in the expression of GST Ya, Yb1, and Yc forms at 5 days after treatment, respectively, compared with control animals (Table 1). Changes in GST Yb2 were less notable than those in other forms (i.e., <1.5-fold). These immunoblot analyses provide evidence that irradiation induces GST proteins and that increases in hepatic GST mRNA levels caused by radiation and oltipraz were associated with *bona fide* elevation of the protein levels.

Oltipraz effects against radiation-induced liver injury

Possible oltipraz protection of the liver against radiation-induced injury was examined through light microscopic analysis. Exposure of rats to 0.5 Gy of γ-ray radiation/day for 3–5 days resulted in mild-to-moderate degrees of hepatocyte degeneration with sinusoidal congestion (Fig. 7). Animals treated with oltipraz (30 mg/kg/day) for 3–5 days with daily 0.5-Gy doses of radiation exhibited a low degree of liver

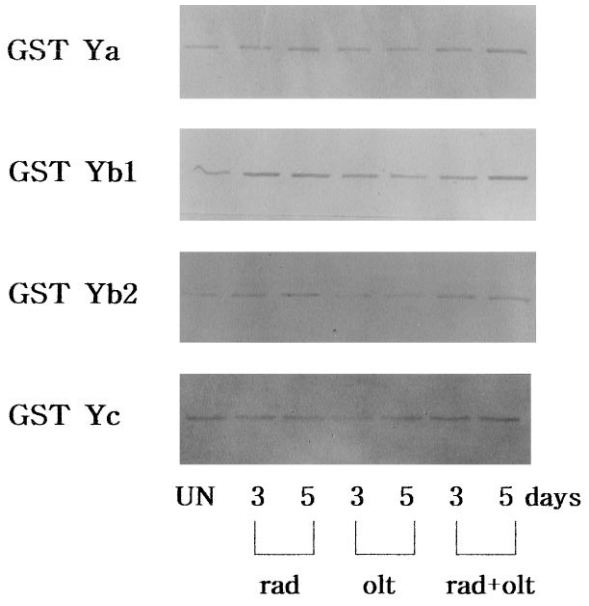

Fig. 6. Representative immunoblot analysis of rat hepatic cytosolic proteins with rabbit anti-rat GST Ya, Yb1, Yb2, or Yc antibodies. Each lane was loaded with 1.5 μg of cytosolic proteins. Western immunoblots show GST protein levels in the hepatic cytosol isolated from rats that were untreated (*UN*) or from rats that were exposed to 0.5 Gy of radiation/day for 3 or 5 days (*rad*); that received oltipraz (30 mg/kg/day) for 3, or 5 days (*olt*); or that received both (*rad+olt*) for 3 or 5 days. The relative fold increases were determined from multiple immunoblottings using different groups of animals (four determinations).

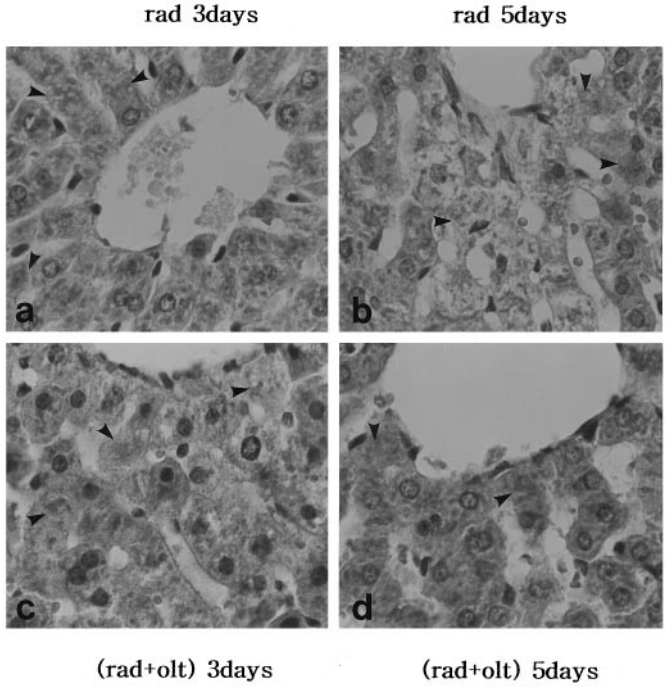

Fig. 7. Protective effects of oltipraz against radiation-induced liver injury. Animals were irradiated at the daily dose of 0.5 Gy for 3 or 5 days with (*rad+olt*) or without (*rad*) oltipraz administration (30 mg/kg/day orally). Hematoxylin and eosin-stained sections of livers were photographed at 40×. *Arrows*, representative degenerated hepatocytes. a, Liver from a rat irradiated with 0.5 Gy/day for 3 days. Photomicrograph shows a mild degree of hepatocyte degeneration. b, Liver from a rat irradiated with 0.5 Gy/day for 5 days. c, Liver from a rat irradiated with 0.5 Gy/day for 3 days that also received oltipraz (30 mg/kg/day orally). Photomicrograph shows moderate hepatocyte degeneration. d, Liver from a rat irradiated with 0.5 Gy/day for 5 days that also received oltipraz (30 mg/kg/day orally). Photomicrograph shows minimal hepatocyte degeneration and mild sinusoidal congestion.

injury (Fig. 7). Living hepatocytes were also counted in an oil immersion field. Whole-body irradiation of rats at the daily dose of 0.5 Gy for 3 and 5 days caused 68% and 75% hepatocyte degeneration, respectively (Table 2). Interestingly, however, daily treatment of animals with oltipraz at the dosage of 30 mg/kg/day for 3 and 5 days caused substantial protection of hepatocytes against radiation-induced injury, resulting in 48% and 6% degeneration in liver cells at 3 and 5 days after treatment, respectively.

Discussion

Oltipraz protects the liver against toxicant-induced hepatotoxicity (26, 27). Significant elevation in GST activity in the liver may be associated with the reduced hepatotoxicity of acetaminophen or carbon tetrachloride after oltipraz treatment (27). GST genes, including *Ya2*, *Yb1*, *Yp*, and *Yc2*, are coordinately regulated by oltipraz (28–31). The results of comprehensive mechanistic studies on oltipraz support the hypothesis that GST induction by oltipraz represents chemopreventive effects of this agent against aflatoxin B1-induced chemical carcinogenesis (26, 32). It has also been shown that mRNA levels for GST α, μ, and π were increased in mice at day 1 and peaked at days 2 and 4 after treatment with a large dose of oltipraz (28). Transcription factors must be produced to initiate a cascade of molecular events. An ARE has been shown to be involved in the regulation of the rat GST *Ya* gene. Recent studies have shown that transcriptional activation of the GST *Ya* gene through ARE in response to oxidative stress is mediated by an unique signal transduction pathway and not by known members of the activator protein-1 family

TABLE 1

Major GST protein expression after irradiation in combination with oltipraz treatment

Animals were irradiated at the daily dose of 0.5 Gy for 3 or 5 days with or without oltipraz (30 mg/kg/day orally). Band intensities in immunoblots were assessed by loading different amounts of cytosolic proteins to avoid saturation, followed by scanning densitometry. The relative fold increases were determined from multiple immunoblottings using different groups of animals (four groups). Values represent mean ± standard deviation (four determinations).

Subunit	Radiation		Oltipraz		Radiation + oltipraz	
	3 day	5 day	3 day	5 day	3 day	5 day
GST Ya	1.0 ± 0.1	1.4 ± 0.2[b]	1.1 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	2.1 ± 0.4[a,c]
GST Yb1	2.9 ± 0.2[a]	2.9 ± 0.2[a]	1.6 ± 0.3	1.0 ± 0.2	2.3 ± 0.4[a]	3.6 ± 0.5[a,c]
GST Yb2	1.2 ± 0.5	1.2 ± 0.3	0.9 ± 0.2	0.7 ± 0.2	1.1 ± 0.1	1.4 ± 0.1
GST Yc	1.1 ± 0.2	1.3 ± 0.2	1.1 ± 0.3	0.7 ± 0.2	1.2 ± 0.1	1.5 ± 0.3[b]

Data were analyzed with one-way analysis of variance followed by Newman-Keuls test for comparison with untreated animals ([a] $p < 0.01$; [b] $p < 0.05$) or with those irradiated for 5 days ([c] $p < 0.01$).

TABLE 2

Effects of whole-body irradiation in conjunction with oltipraz on the viability of hepatocytes

Animals were irradiated at the daily dose of 0.5 Gy for 3 or 5 days with or without oltipraz (30 mg/kg/day orally). Living hepatocytes were counted in oil immersion field (100×). Living hepatocytes are defined as the cells with well-demarcated nuclear membranes and with prominent round central nucleoli. There were 75–85 (mean, 80) normal cells in untreated animals in oil-immersion field on light microscopic examination. Percentage of degenerated cells was calculated using the following formula: (80 − countable living hepatocytes)/80. Animals were killed at 24 hr after the last treatment. Values represent mean ± standard error (number of determinations from five animals = 10).

Treatment	Countable living cells	Degenerated hepatocytes
		%
Radiation 3 days	25.3 ± 3.4	68.4
Radiation 5 days	20.4 ± 2.7	74.6
Radiation 3 days + oltipraz 3 days	42.0 ± 6.0[a]	47.5
Radiation 5 days + oltipraz 5 days	75.7 ± 6.8[b]	5.7

Data were analyzed with one-way analysis of variance followed by Newman-Keuls test for comparison with animals irradiated for 3 days ([a] $p < 0.01$) or 5 days ([b] $p < 0.01$).

(33, 34). The redox-labile species produced from oltipraz by cytochrome P450 may serve as a stimulant for phase II enzyme expression.

The modulation in GST μ expression may represent the susceptibility of individuals to cancer, which is partly supported by a significant decrease in the GST μ levels in patients with lung or bladder cancer (35, 36). The levels of GST class μ forms might be also correlated with the susceptibility of smokers to lung cancer development. Oltipraz is effective in modulating GST μ expression in rats (29).

Cellular radioprotective mechanisms can provide protection against a subsequent exposure to radiation. The current study was designed to establish whether ionizing radiation causes induction of hepatic GSTs potentially as part of adaptive responses and whether oltipraz modulates radiation-induced alterations in GST gene expression and protects the liver from radiation-induced injury. The effects of toxic or subtoxic doses of ionizing radiation on the expression of GST mRNA and proteins were comparatively assessed to study whether radiation-induced change in the gene expression has a common molecular basis in gene regulation with that caused by oltipraz. The results of a previous study showed that γ-ray ionizing radiation causes alterations in hepatic mEH gene expression with the induction of the protein and that the mEH gene expression is enhanced by oltipraz treatment (36a). The results of the current study clearly demonstrate that the expression of major GST genes is increased as a function of time after ionizing radiation, that major GST subunits are coordinately expressed after exposure of animals to γ-ray irradiation, and that radiation-induced elevations in GST expression are further enhanced by oltipraz treatment. Exposure of rats to 0.5 Gy of γ-ray radiation/day resulted in greater alterations in GST mRNA expression in combination with oltipraz than did exposure to 3 Gy of radiation/day. This was probably due to the difference in the extent of radiation-induced liver cell injury. Rats exposed to a single dose of 0.5 Gy of γ-ray radiation in combination with oltipraz (i.e., 24 hr) exhibited greater enhanced increases in the expression of GST Ya and Yc1/2 mRNA levels than did those that received 3–5-day multiple treatments. Instead, multiple treatment caused a plateau in the elevation of mRNA levels, especially in class α mRNA levels. This was also observed in the expression of mEH.[1] These results suggest that the mechanism of increases in GST gene expression by irradiation may be shared with that of oltipraz (e.g., transcriptional activation). The immediate early gene products, including Jun, EGR, Fos, and Jun/Fos heterodimers, regulate transcription by binding to activator protein-1 sites in certain genes (11). Alteration in the early gene transcription by radiation is being studied in our laboratory.

Common decreases in GST mRNA levels observed at the early times after radiation exposure suggest that a common regulator of the gene expression may exist. This suppression in GST expression at early times after ionizing radiation may represent cell cycle-specific growth arrest and repair of damaged DNA. The time course in the number of hypoxic cells after irradiation is also consistent with the change in the GST mRNA levels at the early time points (1). The rapid decreases in GST Ya mRNA at early times after irradiation (i.e., 3–8 hr) may also be associated with oxygen level in the cells.

A limited number of reports are available on the induction of gene expression by ionizing radiation. Woloschak *et al.* examined the expression of proto-oncogenes in mice liver after 3 Gy of γ-ray radiation (9). According to their study, c-fos and c-myc mRNA levels were not affected by γ-ray exposure, whereas c-*src* and c-H-*ras* transcription levels were elevated 2–3-fold within 1 hr after treatment. Dose-dependent increases in EGR1 and Jun mRNA expression in human cell lines have also been demonstrated, although Fos mRNA level was not increased after irradiation in the cells (37). *TNF-α* is the only mammalian gene found to be increased after exposure to ionizing radiation (38). Recently, genes inducible by irradiation in radioresistant human melanoma cells were cloned through the use of differential hybridization (39). These cDNA clones include quinone reductase, thymidine kinase, and tissue plasminogen activator. Quinone reductase is associated with cellular defense among the genes. The transcriptional regulation of quinone reductase gene in response to xenobiotics is similar to that of certain GST genes (40).

The results of the current study demonstrate that oltipraz at a relatively low dose is effective in protecting the liver against radiation-induced injury. The radiation-inducible alterations in GST (and mEH) gene expression are likely to be cellular adaptive responses. Thus, the enhanced GST gene expression by oltipraz may represent its cytoprotective effect against radiation injury. The use of oltipraz failed to protect the peripheral blood cells from radiation-induced injury (data not shown). The lack of enhanced GST gene expression by oltipraz in the blood cells (e.g., erythrocytes and platelets) may account for its failure in cytoprotection, indicating that oltipraz hepatoprotective effects may be related to tissue-specific GST expression. Further mechanistic studies remain to establish whether the expressions in hepatic GST (and mEH) are virtually associated with the hepatoprotective effects of oltipraz against radiation-induced liver injury.

In summary, the current study shows that ionizing radiation stimulates the expression of hepatic GST genes in rats and that the expression of the genes is enhanced by oltipraz treatment, which may represent a protective role and implicate the host defense mechanism.

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