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In Vivo Radioprotective Effects of Oltipraz in γ-Irradiated Mice

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ABSTRACT. Previous studies in this laboratory have shown that oltipraz (Olt), a chemopreventive agent, enhances radiation(Rad)-inducible glutathione *S*-transferase (GST) and microsomal epoxide hydrolase (mEH) expression in the liver. The present study was designed to investigate the *in vivo* radioprotective effect of Olt in ICR mice exposed to a lethal dose of Rad. The 30-day survival rate of mice irradiated at the dose of 8 Gy was substantially increased to 91% by Olt pretreatment (100 mg/kg/day for 2 days), compared with 48% in animals irradiated alone. Light microscopic examinations revealed that exposure of mice to 8 Gy of γ-ray Rad resulted in hepatocyte degeneration in the surviving animals from Day 1 through Day 22 after irradiation with certain degrees of necrosis observed at early times, whereas Olt treatment provided protection of the liver against irradiation with no hepatic necrosis noted. Mice irradiated at the dose of 8 Gy exhibited time-related decreases in the white blood cell (WBC), red blood cell (RBC), and platelet counts with maximal reduction noted at Day 10. Animals irradiated with Olt treatment showed no difference in peripheral blood cell counts or in the ratio of myeloid to erythroid bone marrow cells, compared with those irradiated alone. Northern RNA blot analysis showed that treatment of mice with Olt at the dose of 100 mg/kg in combination with 8 Gy irradiation resulted in 12-fold increases in hepatic mEH and mGSTA3 mRNA levels at 24-hr post-treatment, whereas mGSTP1 mRNA levels were not altered. The mRNA levels for mEH and mGSTA3 were elevated after exposure of animals to both Olt and 8 Gy-γ ray to a greater extent than after irradiation alone. The enhanced survival rate (91%) in 8 Gy-irradiated animals after treatment with Olt (100 mg/kg/day for 2 days) was completely reversed by concomitant pretreatment with dexamethasone (Dexa) (0.1 and 1 mg/kg/day for 2 days), a known inhibitor of mEH and GST expression, resulting in a 42% and 28% survival rate, respectively. Mice irradiated after dexamethasone treatment at a dose of 1 mg/kg showed a reduced mean survival time compared with those treated with 0.1 mg/kg of dexamethasone (9 vs 14 days). The current study demonstrates that Olt is effective in increasing the survival rate of mice against ionizing Rad and that protective effects of Olt associated with enhanced expression of mEH and GST genes may represent its radioprotective efficacy. BIOCHEM PHARMACOL **55**;10:1585–1590, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. γ-ray radiation; gene expression; oltipraz; radioprotection; hepatoprotective effect; dexamethasone

Treatment of animals with Olt§ increases cellular thiol levels in mice. Of the dithiolthione compounds, Olt appeared to be a potent inducer of GST as well as other enzymes involved in maintaining levels of reduced glutathione [1]. GST induction is likely to be associated with protection against hepatotoxicants, including carbon tetrachloride and acetaminophen [2]. This compound has been studied as a chemopreventive agent which provides protection against aflatoxin B1-induced liver carcinogenesis [3–4].

It has been postulated that increases in nonprotein sulfhydryl within cells might be responsible for the radioprotective effects of dithiolesters. Increased glutathione levels in most organs following Olt treatment may contribute to the maintenance of cell homeostasis by scavenging free radicals produced from irradiation and/or xenobiotics as well as to *in vivo* protection of animals against Rad injury. This is supported by the observation that treating animals with reduced glutathione minimized acute and chronic Rad injury as a function of dose [5]. Radioprotective effects of Olt and other dithiolester analogs have been studied in EMT6 cells *in vitro* [6]. However, Olt was the least effective of the analogs examined [6]. In contrast, previous studies in this laboratory have shown that Olt was efficacious in protecting the liver against Rad-induced liver injury and that its hepatoprotective effect may be associated with the tissue-specific expression of antioxidant detoxifying enzymes [7, 8]. Enhanced induction of GST and mEH by Olt appeared to be related to the latter's protective effect against ionizing Rad. Given these conflicting observations

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§ *Abbreviations:* Dexa, dexamethasone; GST, glutathione S-transferase; mEH, microsomal epoxide hydrolase; Olt, oltipraz; Rad, radiation; RBC, red blood cell; SSC, standard saline citrate; TBI, total body irradiation; WBC, white blood cell.

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that Olt was minimally effective in protecting cells *in vitro* while protecting the liver against Rad-induced injury *in vivo*, we assessed the *in vivo* radioprotective efficacy of this agent against a lethal dose of Rad. Because we were interested in the role of hepatic mEH and GST expression in mice in terms of the radioprotective effects of Olt, the expression of the genes after irradiation and/or Olt treatment was monitored. To this end, the effects of Dexa, an inhibitor of mEH and certain GST gene expression, on the 30-day survival rate of irradiated mice were further assessed.

MATERIALS AND METHODS

Materials

[α-^{32}P]dCTP (110TBq, 3,000 Ci/mmol) and [γ-^{32}P]ATP (110TBq, 3,000 Ci/mmol) were purchased from DuPont/NEN. Most of the reagents in the molecular studies were obtained from Sigma. Olt was a gift from Rhone-Poulenc Rorer.

Animal Treatment

Male ICR mice were obtained from Daehan Laboratory Animals and maintained at a temperature between 20 to 23°C with a relative humidity of 50%. Animals were caged under the supply of filtered pathogen-free air and given food (Cheiljedang rodent chow, Korea) and water *ad lib*. Mice (25-30 g) were treated with a single dose of either 8 or 9 Gy Rad. Animals were subjected to TBI at a dose rate of 115.8 cGy/min from a ^{60}Co Rad source. Mice were placed in an acrylic chamber which allowed little movement. The chamber was designed to fill a Rad field sized at 31 × 31 cm^2. For histopathological examinations of the liver, surviving irradiated animals were killed at 1, 5, 10, and 22 days after irradiation. Animals were gavaged with Olt, suspended in 0.1% high viscosity carboxymethylcellulose (100 mg/kg/day) with or without Dexa sodium phosphate in aqueous solution (0.1-1.0 mg/kg/day for 2 days). The second dose was administered at 3 hr prior to irradiation.

Histopathology of the Liver

The effect of Olt in combination with ionizing irradiation on hepatic morphology was assessed by light microscopy. Livers were removed and fixed in 10% buffered formalin, processed, and embedded in paraffin. Sections 5 μm in thickness were stained with hematoxylin and eosin prior to examination. The samples were scored by a certified pathologist in a blinded fashion.

Hematology

Hematology was monitored using the Coulter® MAXM Hematology System.

Isolation of Total RNA

Total RNA was isolated using the improved single-step method of thiocyanate-phenol-chloroform RNA extraction according to the method of Chomczynski and Sacchi [9], as modified by Puissant and Houdebine [10].

Preparation of cDNA Probes for Major GST Subunits

cDNAs for mEH, mGSTA3 and mGSTP1 were prepared as described previously [7,8]. Specific cDNA probes for mGSTA3 (287-684) and mGSTP1 (643-963) were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) using the selective primers for each gene, as described previously [11,12].

Northern Blot Hybridization

Northern blotting was carried out according to the procedures described previously [7, 8]. Briefly, total RNA isolated from mouse liver was resolved by electrophoresis in a 1% agarose gel containing 2.2 M of formaldehyde and then transferred to supported nitrocellulose paper by capillary transfer. The nitrocellulose paper was baked in a vacuum oven at 80°C for 2 hr. The blot was incubated with hybridization buffer containing 50% deionized formamide, 5 × Denhardt's solution [0.1% Ficoll, 0.1% polyvinylpyrrolidine, and 0.1% bovine serum albumin (BSA) (Pentex Fraction V)], 0.1% SDS, 200 μg/mL of sonicated salmon sperm DNA and 5 × SSPE (1 × SSPE: 0.15 M of NaCl, 10 mM of NaH_2PO_4 and 1 mM of Na_2EDTA, pH 7.4) at 42°C for 1 hr without probe. Hybridization was performed at 42°C 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 and 0.1% SDS for 10 min at room temperature and twice in 0.1 × SSC and 0.1% SDS for 10 min at room temperature. Filters were finally washed in the solution containing 0.1 × SSC and 0.1% SDS for 60 min at 60°C. After quantitation of mEH and 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.

Scanning Densitometry

Scanning densitometry was performed with a Microcomputer Imaging Device, Model M1 (Imaging Research). The area of each lane was integrated using MCID software version 4.20, rev. 1.0, followed by background subtraction.

Statistical Analysis

Data were statistically analyzed by Newmann–Keuls test and the mean survival time was calculated by Litchfield and Wilcoxon analysis using computer programs for Pharmacological Calculations [13]. Student's *t*-test was used to determine whether two population means differed signifi-

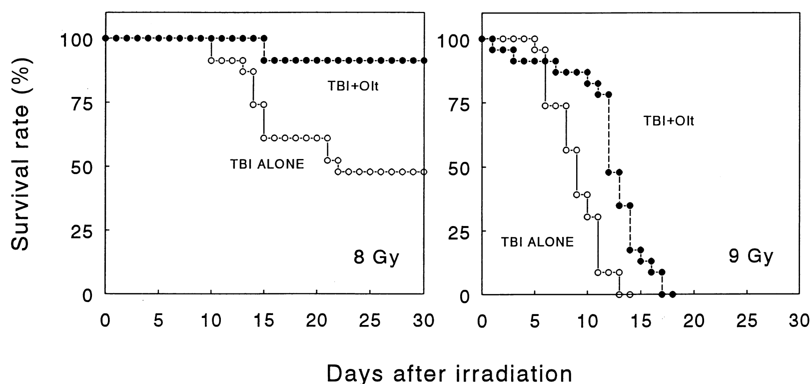

FIG. 1. Percentage survival as a function of time after ^{60}Co irradiation in mice treated with or without oltipraz (100 mg/kg/day, p.o., for 2 days) prior to either 8 Gy or 9 Gy irradiation. The lines represent animal survival rates for 23 mice per treatment group. Statistical analyisis revealed that oltipraz significantly improved the survival rate of the mice irradiated at 8 Gy, as assessed by the chi-square test ($P < 0.01$). However, the change in the mean survival time at 9 Gy was not statistically significant.

cantly. The χ-square test was employed to assess the statistical significance of the survival rate of mice.

RESULTS

Survival Rate of Mice after Irradiation

In order to determine whether Olt was effective in protecting animals against ionizing Rad, ICR mice pretreated with Olt for 2 days (100 mg/kg/day, p.o.) were exposed to lethal doses of Rad. Cumulative proportions of mice surviving after the lethal doses of TBI are shown in Fig. 1. The 30-day survival rate in mice irradiated at the dose of 8 Gy was 48%. In contrast, pretreatment of mice with Olt prior to irradiation caused the survival rate to increase to 91%. Mice irradiated at the dose of 9 Gy with Olt treatment exhibited improvement in the median survival time from 8 to 10 days, although the change was not statistically significant (Fig. 1). These results demonstrate that Olt was highly effective as a radioprotective agent against a lethal dose of Rad.

Histopathological Examinations of the Liver

We were interested in the possible hepatoprotective effect of Olt against 8 Gy of Rad. Histopathological changes occurred in mice after the lethal dose of irradiation. Light microscopic examinations revealed that exposure of mice to 8 Gy of γ-ray Rad resulted in hepatocyte degeneration in the surviving animals at Day 1 through Day 22 after irradiation with certain degrees of necrosis and vacuolization being observed (Table 1). The most prominent lesions observed after irradiation consisted of hepatic degeneration and necrosis around the area of large venules. In contrast, the animals irradiated with Olt treatment showed protection of the liver at Day 1 through Day 22 after irradiation (Table 1). Olt treatment significantly reduced the Rad-inducible hepatocyte degeneration and formation of vacuoles. No hepatic necrosis was observed in the animals irradiated with Olt pretreatment.

Hematologic Examinations

Hematologic examinations showed that irradiated mice at the dose of 8 Gy exhibited time-related decreases in the WBC, RBC, and platelet counts with maximal reduction in peripheral blood cells being noted at Day 10 after irradiation (Table 2). Hematologic values in the irradiated animals after Olt treatment failed to differ from those in animals with TBI alone. The ratio of myeloid to erythroid bone marrow cells in the irradiated mice was not altered by Olt, as determined by histopathological examinations (data not shown). These results indicate that Olt-induced radioprotective effects might be associated with improvement in the liver function, but not with modulation in bone marrow and peripheral blood cells.

TABLE 1. Morphological changes in the livers of surviving mice irradiated with or without oltipraz pretreatment

Parameters	Treatment	Day 1	Day 5	Day 10	Day 22
Degeneration	Rad	2.0 ± 0.7	1.2 ± 0.4	0.9 ± 0.7†	1.5 ± 0.7
	Rad + Olt	1.8 ± 0.4	0.7 ± 0.3†	0.9 ± 0.2†	0.5 ± 0.4*,‡
Necrosis	Rad	0.6 ± 0.9	0.2 ± 0.4	0	0
	Rad + Olt	0	0	0	0
Vacuolization	Rad	1.0 ± 1.0	0	0	0.1 ± 0.2‡
(fat degeneration)	Rad + Olt	0.2 ± 0.4**	0	0	0

ICR mice were irradiated at the dose of 8 Gy with or without oltipraz pretreatment (100 mg/kg/day, p.o. for 2 days). Living hepatocytes in the surviving animals were defined as the cells with well-demarcated nuclear membranes and with prominent round central nucleoli. Severity of liver lesions was classified as 0, no lesion; >0–1, mild; >1–2, moderate; and >2–3, severe, as assessed after hematoxylin and eosin staining (×100). Values represent mean ± SD ($N = 5$ animals). Data were analyzed with one-way ANOVA followed by Newmann–Keuls test for comparison with the animals irradiated alone at the respective time ($*P < 0.05$; $**P < 0.01$) or with the animals exposed to the respective treatment at day 1 ($^{†}P < 0.05$; $^{‡}P < 0.01$).

TABLE 2. Hematology results for surviving mice irradiated with or without oltipraz pretreatment

Hematology	Treatment	Day 1	Day 5	Day 10	Day 22
RBC	Rad	7.23 ± 0.16	6.30 ± 0.22†	5.21 ± 0.22‡	7.69 ± 0.41
(10^6/mm^3)	Olt + Rad	7.10 ± 0.31	5.99 ± 0.15	4.90 ± 0.27‡	7.78 ± 0.09
WBC	Rad	3.34 ± 1.67	1.52 ± 0.37	0.70 ± 0.40	7.56 ± 2.39†
(10^3/mm^3)	Olt + Rad	1.60 ± 0.54	0.46 ± 0.13	0.62 ± 0.30	5.06 ± 1.51
Hemoglobin	Rad	13.4 ± 0.2	11.8 ± 0.4†	9.7 ± 0.2‡	14.1 ± 0.8
(g/dL)	Olt + Rad	12.8 ± 0.7	11.2 ± 0.3†	9.0 ± 0.4‡	14.6 ± 0.2†
Hematocrit	Rad	41.5 ± 0.7	34.4 ± 1.0‡	28.6 ± 0.8‡	43.9 ± 2.0
(%)	Olt + Rad	39.9 ± 1.7	32.7 ± 0.5‡	26.7 ± 1.4‡	45.5 ± 0.7†
Platelet	Rad	1233 ± 98	709 ± 41	149 ± 25‡	1028 ± 63
(10^3/mm^3)	Olt + Rad	947 ± 286	392 ± 123†	159 ± 19‡	922 ± 74

Data represent means ± SE for five animals. RBC, red blood cell; WBC, white blood cell. Data were analyzed with one-way ANOVA followed by Newmann–Keuls test for comparison with the animals exposed to the respective treatment at day 1 ($^{\dagger}P < 0.05$; $^{\ddagger}P < 0.01$). Values in mice at day 1 after irradiation were comparable to those in untreated animals (RBC, 7.07 ± 0.21; WBC, 3.34 ± 0.61; hemoglobin, 13.1 ± 0.2; hematocrit, 39.0 ± 1.4).

Expression of Hepatic mEH and GST Genes

The expression in the hepatic mEH and GST genes in response to Olt and γ-ray irradiation was assessed by Northern blot analysis (Fig. 2A and B). RNA blot analyses showed that the surviving animals after 8 Gy irradiation exhibited 9- and 6-fold increases in mEH and mGSTA3 mRNA levels, respectively, whereas exposure of mice to Olt at the dose of 100 mg/kg in combination with 8 Gy irradiation resulted in 12-fold increases in hepatic mEH and mGSTA3 mRNA levels at 24 hr as compared with untreated animals. Thus, the mRNA levels were elevated to a greater extent after exposure of animals to both Olt and 8 Gy-γ rays than after irradiation alone. In contrast, mGSTP1 mRNA levels were not altered by irradiation and Olt.

Effects of Dexa on Olt-Enhanced Survival Rates

The radioprotective effect of Olt was assessed in combination with Dexa, which is known to suppress hepatic mEH and GST expression. The survival rate in 8 Gy-irradiated animals treated with both Olt (100 mg/kg/day) and Dexa at the doses of 0.1 and 1 mg/kg per day for 2 days was substantially reduced to 42% and 28%, respectively, which was in sharp contrast to 91% in irradiated animals with Olt (Fig. 3). Mice irradiated after Dexa treatment at a dose of 1 mg/kg showed a reduced mean survival time compared with those treated with 0.1 mg/kg of Dexa (9 vs 14 days) (Fig. 3). These results support the hypothesis that mEH and GST gene expression is highly correlated with hepatoprotective and Olt-induced radioprotective effects.

DISCUSSION

Cellular radioprotective mechanisms can provide protection against a subsequent exposure to Rad. Previous studies in this laboratory have shown both that γ-ray ionizing Rad causes alterations in hepatic GST and mEH gene expression with induction of the proteins and that expression of the genes is enhanced by Olt [7, 8]. It has also been proposed that significant elevation in GST expression in the liver by Olt is associated with the chemopreventive effects of Olt [3, 4, 14]. The present study was carried out to determine whether Olt improves the survival rate of animals and exhibits hepatoprotective effects against a lethal dose of γ-ray Rad. Olt was indeed effective as a radioprotective agent, as shown in this study.

The lethality is considered to result from cell death after irradiation, which is defined as the irreversible loss of reproductive capacity [15]. The cells in a given tissue have a certain probability of being killed after irradiation, and this probability increases with the dose and varies with the tissue and with the type of cell. With the doses we used, a proportion of the cells could very well lose their capacity for division or proliferation. Because reproductive cell death is related to the cell turnover rate of the tissue, damage in stem cells has serious consequences as they are programed to perform a large number of divisions. Our observation provided evidence that the increase in the death rate was in parallel with the changes in the hematopoietic cells, probably because the cells of the bone marrow divided or were able to divide to greater extents than other cell types. Nonetheless, cells in the other tissues could also contribute to the ultimate lethality of the animals. The increase in the survival rate of mice with improvement in liver morphology supports the possibility that protective effects of Olt in the tissues including the liver may represent enhancement in survival rate of γ-irradiated mice by Olt. The surviving animals pretreated with Olt prior to irradiation exhibited significant decreases in hepatocyte degeneration at Day 22. No hepatic necrosis and fat degeneration was observed in the animals exposed to Olt, which was in sharp contrast with those irradiated alone.

The current study demonstrated that Olt failed to modulate Rad-inducible changes in hematology. Although Olt treatment in combination with irradiation caused transient decreases in WBC and platelet counts as shown in Table 2, the changes were not statistically significant. That there

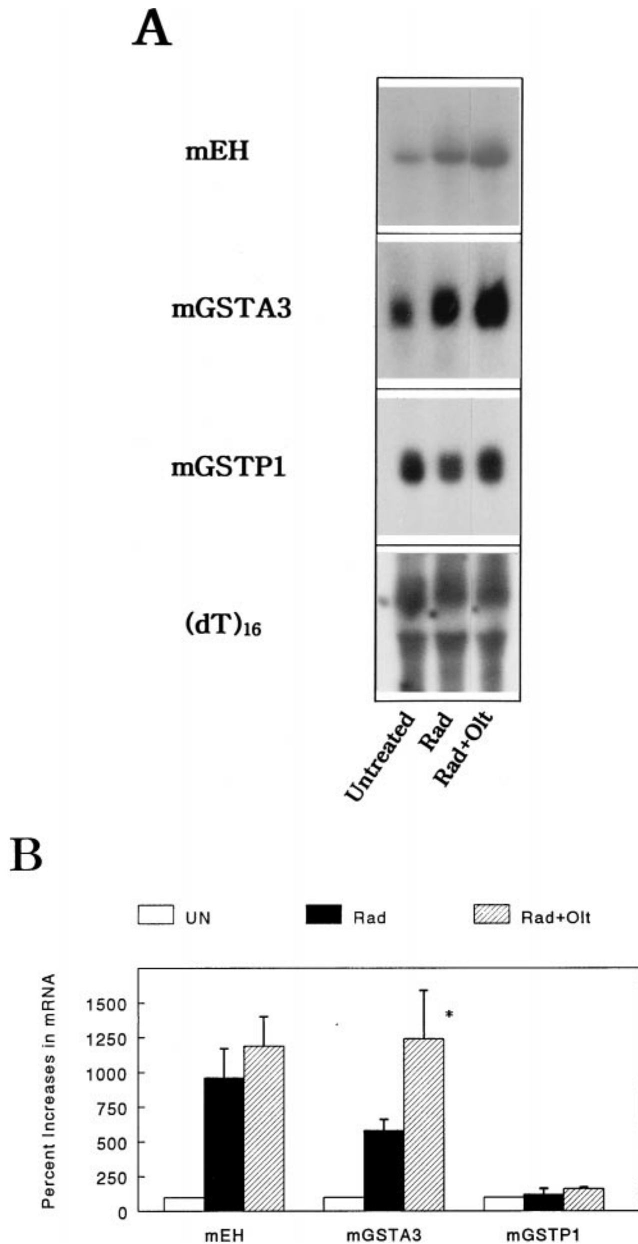

FIG. 2. The effects of 8 Gy γ-ray irradiation with oltipraz treatment in mice. Panel A) Representative Northern blots show hepatic mEH, mGSTA3 and mGSTP1 mRNA levels in untreated mice (UN) or in mice pretreated with or without oltipraz (100 mg/kg/day, p.o., for 2 days) prior to a single dose of 8 Gy irradiation. The stripped membrane was rehybridized with ^{32}P-labeled poly(dT)$_{16}$. Panel B) Relative changes in mEH, mGSTA3 and mGSTP1 mRNA levels as compared with untreated animals. Each point represents the mean ± SD with N = 4. *Significant when compared with those with radiation alone. The criterion for statistical significance as assessed by Student's *t*-test was set at $P < 0.05$.

was no improvement in hematology by Olt was in accordance with the results obtained at a lower dose of irradiation in rats [7]. The ratio of myeloid to erythroid bone marrow cells in mice exposed to TBI + Olt failed to differ from those with TBI alone, although the ratio in animals with TBI decreased in a time-related manner at Day 5 and Day 10 after irradiation (data not shown). Thus, the Olt-induced radioprotective effect is apparently associated with other protective effects including hepatoprotection, but not with changes in hematology.

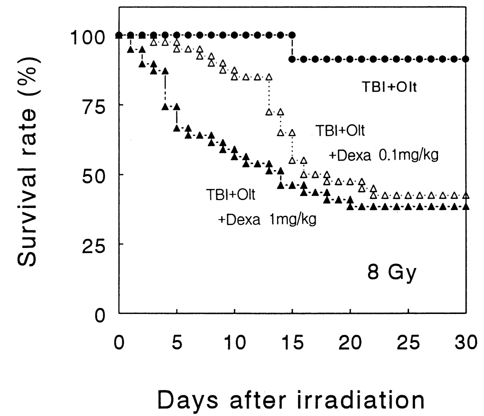

FIG. 3. Changes in the 30-day survival rate in mice irradiated at the dose of 8 Gy after treatment with Dexa (0.1 or 1 mg/kg/day, p.o., for 2 days) and/or oltipraz (100 mg/kg/day, p.o., for 2 days). The lines represent animal survival rates for 23 mice per treatment group. Dexa at the daily dose of 0.1 or 1 mg/kg significantly decreased the oltipraz-improved survival rate of the irradiated mice, as assessed by the χ-square test ($P < 0.01$).

Lymphoid cells and tissues are very radiosensitive and depleted of cells by small doses of Rad; total body irradiation leads to a rapid fall in the number of circulating B and T lymphocytes, the number of lymphocytes returning to normal only after several weeks. Thus, total body irradiation at the large dose that we used would inhibit the immune response [16]. Regeneration of the immune system results from proliferation and differentiation of bone marrow stem cells after total body irradiation. Although we only provided information on the immunocompetent cells in this study, it is highly likely that mechanisms of action of Olt as a radioprotective agent are different from those of known immunomodulators [16].

The radioprotective effect of Olt appeared to be associated at least in part with the enhanced expression of antioxidant detoxification enzymes. Ionizing Rad at the dose of 8 Gy in combination with Olt enhanced the expression of mEH and mGSTA3 genes in the liver compared with Rad alone, although mGSTP1 gene expression was not altered. It has been reported that Olt increases GST π levels at the dose of 1,000 mg/kg [14]. However, Olt minimally affected the mGSTP1 mRNA level at the dose employed in this study. The enhanced gene expression in mEH and rGSTA genes might be related to the hepatoprotective effects in mice, which was consistent with the previous results shown in rats [8].

The role of mEH and GST expression in protecting animals against Rad-induced injury was further supported by dramatic increases in the mortality rate in irradiated animals by Dexa. The enhancer region of mEH and certain GST gene includes a glucocorticoid responsive element, which negatively regulates the expression of the genes [17,

18]. Dexa caused decreases in constitutively expressed hepatic mEH and GST mRNA levels in a dose-related manner in rats[1]. Furthermore, Olt-inducible increases in hepatic mEH and GST mRNA levels were completely inhibited by pretreatment of rats with Dexa, which was consistent with the changes in survival rates of γ-irradiated animals in this study. Whereas most of the lethally irradiated animals died from Day 10 through 20, the survival rate of animals irradiated with Dexa treatment was rapidly decreased at early times in spite of Olt administration. This dramatic change in mean survival time may be correlated with the rapid changes in the expression of hepatic antioxidant enzymes including mEH and GST, although the possibility that Dexa suppresses immune cells as well as inflammatory responses and affects the animal survival rate against ionizing Rad was not excluded.

In summary, this study demonstrates that Olt was effective in increasing the survival rate of mice against ionizing Rad with no improvement in hematology, that hepatoprotective effects of Olt were strongly associated with its radioprotective efficacy and that the change in the expression of hepatic mEH and GST by Olt might be correlated with its radioprotective effects.

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References

1. Kensler TW, Egner PA, Dolan PM, Groopman JD and Roebuck BD, Mechanism of protection against aflatoxin tumorigenicity in rats fed 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (oltipraz) and related 1,2-dithiol-3-thiones and 1,2-dithiol-3-ones. *Cancer Res* **47:** 4271–4277, 1987.
2. Ansher SS, Dolan P and Bueding E, Chemoprotective effects of two dithiolthiones and butylhydroxyanisole against carbon tetrachloride and acetaminophen toxicity. *Hepatology* **3:** 932–935, 1983.
3. Primiano T, Egner PA, Sutter TR, Kelloff GJ, Roebuck BD and Kensler TW, Intermittent dosing with oltipraz: relationship between chemoprevention of aflatoxin-induced tumorigenesis and induction of glutathione S-transferases. *Cancer Res* **51:** 4319–4324, 1995.
4. Roebuck BD, Liu YL, Rogers AE, Groopman JD and Kensler TW, Protection against aflatoxin B1-induced hepatocarcinogenesis in F344 rats by 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione (oltipraz): predictive role for short-term molecular dosimetry. *Cancer Res* **51:** 5501–5506, 1991.
5. Arima R and Shiba R, Radioprotective effect of exogenous glutathione on rat parotid glands. *Int J Radiat Biol* **61:** 695–702, 1992.
6. Teicher BA, Stemwedel J, Herman TS, Ghoshal PK and Rosowsky A, 1,2-Dithiol-3-thione and dithioester analogues: potential radioprotectors. *Br J Cancer* **62:** 17–22, 1990.
7. Kim SG, Nam SY, Kim JH, Cho CK and Yoo SY, Enhancement of radiation-inducible expression of hepatic glutathione S-transferase genes by oltipraz: possible role in radioprotection. *Mol Pharmacol* **51:** 225–233, 1997.
8. Nam SY, Kim JH, Cho CK, Yoo SY and Kim SG, Oltipraz enhancement of radiation-induced hepatic microsomal epoxide hydrolase gene expression in rats. *Radiat Res* **147:**613–620, 1997.
9. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159, 1987.
10. Puissant C and Houdebine LM, An improvement of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Biotechniques* **8:** 148–149, 1990.
11. Hayes JD, Judah DJ, Neal GE and Nguyen T, Molecular cloning and heterologous expression of a cDNA encoding a mouse glutathione S-transferase Yc subunit possessing high catalytic activity for aflatoxin B1-8,9-epoxide. *Biochem J* **285:** 173–180, 1992.
12. Bammler TK, Smith CAD and Wolf CR, Isolation and characterization of two mouse Pi-class glutathione S-transferase genes. *Biochem J* **298:** 385–390, 1994.
13. Tallarida RJ and Murray RB, *Manual of Pharmacologic Calculations with Computer Programs*. Springer-Verlag, New York, 1987.
14. Clapper ML, Everley LC, Strobel LA, Townsend AJ and Engstrom PF, Coordinate induction of glutathione S-transferase α, μ, and π expression in murine liver after a single administration of oltipraz. *Mol Pharmacol* **45:** 469–474, 1994.
15. Tubiana M, Dutreix J and Wambersie A, *Introduction to Radiobiology*. Taylor Francis, New York, 1990.
16. Weiss JF, Kumar KS, Walden TL, Neta R, Landauer MR and Clark EP, Advances in radioprotection through the use of combined agent regimens. *Int J Radiat Biol* **7:** 709–722, 1990.
17. Bell PA, Falany CN, McQuiddy P and Kasper CB, Glucocorticoid repression and basal regulation of the epoxide hydrolase promoter. *Arch Biochem Biophys* **279:** 363–369, 1990.
18. Hayes JD and Pulford DJ, The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* **30:** 445–600, 1995.

[1] S. Y. Nam, C. K. Cho and S. G. Kim, Suppression of radiation-inducible microsomal epoxide hydrolase and glutathione S-transferases gene expression by dexamethasone correlates with the increased mortality: effects on vitamin C and E-induced radioprotection, manuscript submitted.