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Correlation of Increased Mortality with the Suppression of Radiation-inducible Microsomal Epoxide Hydrolase and Glutathione S-Transferase Gene Expression by Dexamethasone: Effects on Vitamin C and E-Induced Radioprotection

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ABSTRACT. Previous studies in this laboratory have shown that γ-ray ionizing radiation in combination with oltipraz, a radioprotective agent, enhances hepatic microsomal epoxide hydrolase (mEH) and glutathione S-transferase (GST) expression. The present study was designed to investigate the effects of dexamethasone on the radiation-inducible expression of mEH and rGST genes and on the vitamin C and E-induced radioprotective effects in association with the expression of the genes. Treatment of rats with a single dose of dexamethasone (0.01–1 mg/kg, p.o.) caused a dose-dependent decrease in the constitutive mEH gene expression at 24 hr. The radiation-inducible mEH mRNA level (threefold increase after 3 Gy γ-irradiation) was decreased by 21% and 88% by dexamethasone at the doses of 0.1 and 1 mg/kg, respectively. Although dexamethasone alone caused 2- to 5-fold increases in the hepatic rGSTA2 mRNA level, rats treated with dexamethasone prior to 3 Gy irradiation exhibited 80%–93% suppression in the radiation-inducible increases in the rGSTA2 mRNA level. The inducible rGSTA3 and rGSTA5 mRNA levels were also significantly decreased by dexamethasone, whereas the rGSTM1 mRNA level was reduced to a lesser extent. Vitamin C and/or E, however, failed to enhance the radiation-inducible increases in hepatic mEH and rGST mRNA levels. Whereas rats exposed to 3 Gy irradiation with or without vitamin C treatment (30 or 200 mg/kg/day, p.o., 2 days) exhibited ~threefold increases in the mEH and rGSTA2/3/5 mRNA levels relative to untreated animals, dexamethasone treatment (1 mg/kg, p.o.) resulted in 64%–96% decreases in the mRNA levels at 24 hr. The inducible rGSTM1/2 mRNA levels in the vitamin C/E-treated rats were ~50% suppressed by dexamethasone. Although vitamin C and/or E treatment (200 mg/kg/day, p.o., 2 days) improved the 30-day survival rates of the 8 Gy γ-irradiated mice from 39% up to 74%, the improved survival rate of γ-irradiated animals was reduced to 30% by dexamethasone pretreatment (1 mg/kg/day, 2 days). The mean survival time of dexamethasone-treated animals was reduced to ~2 days from 14 days in the animals with total body irradiation alone. No significant hematologic changes were observed in mice at 10 days after dexamethasone plus γ-irradiation, as compared with irradiation alone. These results demonstrate that: dexamethasone substantially suppresses radiation-inducible mEH, rGSTA and rGSTM expression in the liver; vitamins C/E exhibit radioprotective effects without enhancing radiation-inducible mEH and GST gene expression; and inhibition of radiation-inducible mEH and rGST gene expression in the vitamin C- and E-treated animals by dexamethasone was highly correlated with reduction in the survival rate and the mean survival time of γ-irradiated animals. BIOCHEM PHARMACOL **56**;10:1295–1304, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. microsomal epoxide hydrolase; glutathione S-transferase; vitamin C; vitamin E; radioprotective effect; dexamethasone

Studies in this laboratory have shown that oltipraz induces phase II detoxification enzymes through transcriptional activation and that a radiation-inducible increase in the mRNA levels is enhanced by oltipraz [1, 2]. The expression of mEH§ and GST as well as increases in glutathione levels seem to be associated with protection of the liver against radiation-induced injury. Oltipraz also increased the survival rate of animals against lethal doses of irradiation [3]. Hence, the radioprotective effect of oltipraz, as evidenced by both improvement of liver function and enhancement in the survival of γ-irradiated animals, might be attributable to the enhanced expression of the phase II detoxification enzymes [3].

Glucocorticoids including dexamethasone have numerous pharmacological effects, endowing the organism with the capacity to resist many types of noxious stimuli [4]. It has been shown that glucocorticoids negatively regulate the

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§ *Abbreviations:* GST, glutathione S-transferase; mEH, microsomal epoxide hydrolase; SSC, standard saline citrate; TBI, total body irradiation.

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basal expression of the mEH gene through a glucocorticoid responsive element and that a high dose of dexamethasone increases the expression of rGSTA2 and rGSTM1/2 in the rat liver without increasing rGSTA1 or rGSTA3/5 mRNA levels [5, 6]. Nonetheless, the effects of a therapeutic dose of dexamethasone on mEH and rGST mRNA levels remain to be established. Given the wide pharmacological use and applications of dexamethasone, the present study was designed to determine the possible transcriptional negative regulatory effects of a therapeutic dose of dexamethasone on the radiation-inducible expression of mEH and rGST genes, and the implications of mEH and GST inhibition by dexamethasone in association with the alteration of the survival rate of γ-irradiated animals.

Antioxidant vitamins C and E play a role in the endogenous defenses against the peroxidation of membrane lipids [7–9]. Because ionizing radiation leads to the production of the activated oxygen species, increased glutathione levels in tissues by the vitamins may contribute to scavenging free radicals generated from irradiation and *in vivo* protection against radiation injury [10]. Radioprotective effects of vitamin C have been demonstrated in certain cells and animals, which would result from scavenging free radicals [11, 12]. Because the vitamins do not modify tumor growth delay induced by irradiation and concomitantly exhibit radioprotective effects in normal tissues [7, 13], these agents have great therapeutic potential as radioprotective agents. However, the molecular mechanism for the vitamin C and E-mediated radioprotective effects has not been elucidated yet. We were interested in determining whether vitamin C and vitamin E enhance radiation-induced mEH and GST mRNA levels in the liver and exhibit radioprotective effects against a lethal dose of ionizing radiation in animal models, and whether the vitamin C- and E-induced radioprotective effect could be altered by dexamethasone in conjunction with modulation of hepatic mEH and GST gene expression. This study provides evidence that vitamins C and E exhibit a bona fide radioprotective effect without increasing the expression of hepatic mEH and GST genes.

MATERIALS AND METHODS

Materials

[α-^{32}P]dCTP (>110TBq/mmol) and [γ-^{32}P]ATP (>110TBq/mmol) were purchased from New England Nuclear Research Products. Oltipraz was a gift from Rhone–Poulenc Rorer. Most of the reagents in the molecular studies were obtained from Sigma Chemical Co.

Animal Treatment

Male Sprague–Dawley rats (160–200 g) and ICR mice (20–25 g) were obtained from the Korean Food and Drug Administration (Seoul, Korea) and maintained at a temperature between 20 to 23° 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*.

Rats were placed in an acrylic chamber that allowed little movement. The chamber was designed to fill a radiation field sized at 42.5 cm × 42.5 cm, as described previously [1,2]. Rats were subjected to TBI at a dose rate of 13.65 cGy per minute from a ^{60}Co radiation source. Rats were exposed to a single dose of either 3 or 0.5 Gy irradiation. Vitamins C and E were administered by gavage at a daily dose of 30 or 200 mg/kg primarily at 0.5–1 hr after irradiation. Dexamethasone disodium phosphate in aqueous solution was administered to animals at the doses of 0.01, 0.1, and 1 mg/kg at the same time as the vitamins. Treatment of rats with the vitamins or dexamethasone 3 hr before irradiation resulted in comparable changes.

Mice were subjected to TBI at a dose rate of 108.4 cGy per minute from a ^{60}Co radiation source [3]. Mice were placed in an acrylic chamber which was designed to fill a radiation field sized at 31 cm × 31 cm. Mice were exposed to a single dose of 8 Gy radiation. Vitamins C and E were administered, as dissolved in aqueous solution and corn oil, respectively. The vitamins were gavaged at a dose of 200 mg/kg per day for 2 days prior to TBI. The second dose was administered at 3 hr before irradiation. Dexamethasone was administered to mice at the same time as the vitamins. Vitamins and/or dexamethasone were administered by gavage to mice before irradiation in order to minimize the physical stress of administration after the lethal dose of γ-ray exposure.

Isolation of Total RNA

Total RNA was isolated using the improved single-step method of thiocyanate-phenol-chloroform RNA extraction according to the methods of Chomczynski and Sacchi [14], as modified by Puissant and Houdebine [15].

Preparation of cDNA Probes for Major GST Subunits

cDNAs for major GSTs were prepared as described previously. Specific cDNA probes for GST genes rGSTA2 (287-684), rGSTA3 (122-488), rGSTA5 (122-530), rGSTM1 (643-963), and rGSTM2 (415-942) were amplified by reverse transcriptase-polymerase chain reaction (PCR) using the selective primers for each gene, as described previously [16–19]. PCR-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).

Northern Blot Hybridization

Northern blot was carried out according to the procedures described previously [1–3]. Briefly, total RNA isolated from rat livers 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° 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% BSA (Pentex Fraction V)], 0.1% SDS, 200 μg/mL of sonicated salmon sperm DNA and 5× standard saline/phosphate/EDTA (1× standard saline/phosphate/EDTA contains 0.15 M of NaCl, 10 mM of NaH_2PO_4, and 1 mM of 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 contains 0.15 M of NaCl and 0.015 M of sodium citrate, pH 7.4) 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°. After quantitation of mEH or 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, revision 1.0), followed by background subtraction.

Data 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 [20]. The Student's *t*-test was used to determine whether two population means differed significantly. The χ^2 test was employed to assess the statistical significance of the survival rates of mice.

RESULTS

Effect of Dexamethasone on Hepatic mEH Gene Expression

The enhancer region of the mEH gene includes a glucocorticoid responsive element, which has been shown to negatively regulate the expression of the genes [5, 21]. The effect of dexamethasone on mEH gene expression in conjunction with ionizing irradiation was assessed to further delineate the role of mEH expression in radioprotection (Fig. 1A and 1B). Treatment of rats with a single dose of dexamethasone at 0.01, 0.1, and 1 mg/kg caused a substantial dose-dependent reduction in constitutive mEH gene expression at 24 hr (i.e. 90%, 46% and 28% of untreated animals, respectively). Whereas exposure of rats to a 3 Gy of γ -irradiation resulted in threefold increase in the mEH mRNA level, rats exposed to 3 Gy irradiation in combination with dexamethasone at the doses of 0.1 and 1 mg/kg

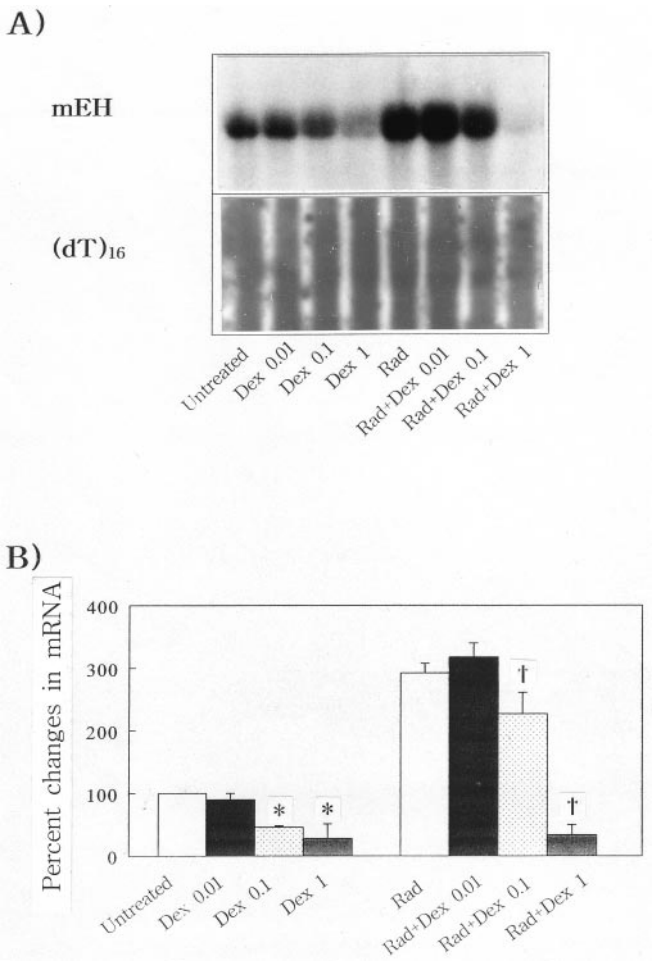

FIG. 1. RNA blot analysis of hepatic mEH gene expression after dexamethasone (Dex) treatment with or without 3 Gy of γ-irradiation (Rad). (A) Northern blot analysis was performed to examine mRNA levels for mEH in total RNA fractions (20 μg each) isolated from untreated rats or from rats at 24 hr after a single dose of dexamethasone at the dose of 0.01, 0.1, or 1 mg/kg in combination with or without γ-irradiation (Dex 0.01, 0.1, and 1, respectively). Amount of RNA loaded in each lane was assessed by rehybridization of the stripped membrane with ^{32}P-labeled poly(dT)$_{16}$. (B) Relative changes in mEH mRNA levels, as compared with untreated rats (untreated rats = 100%). Each point represents the mean ± SD of three experiments. Data were analyzed with one-way analysis of variance, followed by Newmann–Keuls test for comparison with untreated animals (*$P < 0.05$) or the animals exposed to radiation alone (†$P < 0.05$).

resulted in 21% and 88% decreases in the mRNA levels (Fig. 1A and 1B). Thus, dexamethasone was capable of inhibiting the constitutive and radiation-inducible expression of the hepatic mEH gene expression.

Effect of Dexamethasone on Hepatic rGSTA2 Gene Expression

The effect of dexamethasone on the hepatic rGSTA2 mRNA level was also assessed. The mRNA level of rGSTA2 was 4.5- and 3.7-fold increased 24 hr after dexamethasone treatment at the doses of 0.01 and 0.1

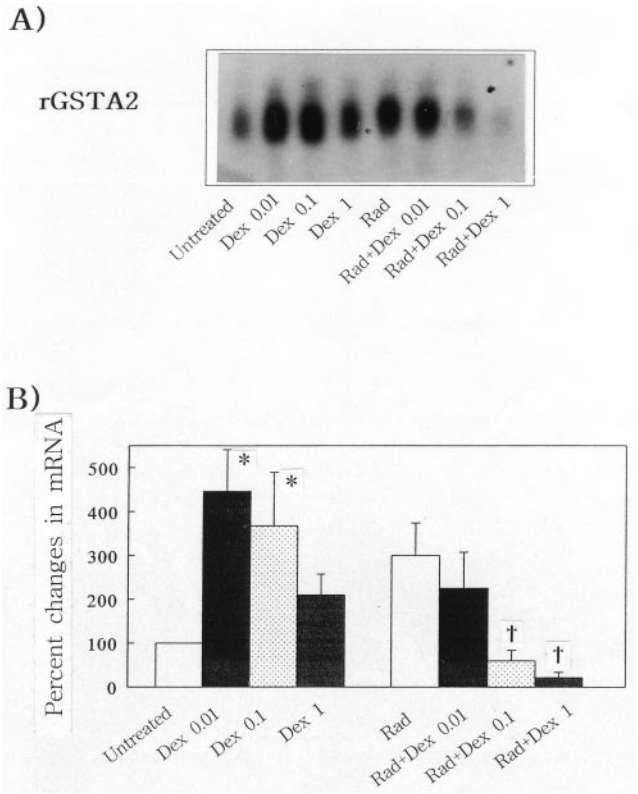

FIG. 2. Shown are RNA blot analyses of hepatic rGSTA2 mRNA level after dexamethasone (Dex) treatment with or without 3 Gy of γ-irradiation (Rad). (A) Northern blot analysis was performed to examine mRNA levels for rGSTA2. The lanes represent the same conditions as those shown in Fig. 1. (B) Relative changes in rGSTA2 mRNA levels. Each point represents the mean ± SD of three experiments. Data were analyzed with one-way analysis of variance, followed by Newmann–Keuls test for comparison with untreated animals (*$P < 0.05$) or the animals exposed to radiation alone (†$P < 0.05$).

mg/kg, respectively. Rats treated with 1 mg/kg of dexamethasone showed a 2.1-fold increase for the rGSTA2 mRNA level relative to that in untreated animals.

Although rats exposed to 3 Gy γ -irradiation exhibited a threefold increase in rGSTA2 mRNA relative to untreated animals, concomitant dexamethasone treatment at the doses of 0.01, 0.1, and 1 mg/kg resulted in 25%, 80%, and 93% decreases in the radiation-induced rGSTA2 mRNA level, respectively (Fig. 2A and 2B). These results showed that dexamethasone suppressed the radiation-inducible rGSTA2 expression in a dose-dependent manner, although dexamethasone alone increased the mRNA level at the relatively low doses, indicating that the mechanism for altering the rGSTA2 mRNA level by dexamethasone differs from that by γ-irradiation.

Effect of Dexamethasone on Hepatic rGSTA3/5 and rGSTM1/2

In contrast to the increases in the rGSTA2 mRNA level by dexamethasone, the mRNA levels for rGSTA3 and rGSTA5 were inhibited by dexamethasone in a dose-related manner. Dexamethasone at the doses of 0.01, 0.1, and 1 mg/kg caused 47%, 50%, and 67% decreases in the constitutive rGSTA3 mRNA 24 hr after treatment, respectively. The inducible rGSTA3 mRNA level by 3 Gy irradiation was also 37%, 80%, and 84% suppressed by concomitant dexamethasone treatment at the same doses, respectively (Fig. 3A and 3B). Similar changes were observed in the rGSTA5 mRNA levels in response to dexamethasone.

The constitutive expression of rGSTM1 and rGSTM2 mRNA failed to be significantly affected by dexamethasone at the doses of 0.01, 0.1, and 1 mg/kg, although dexamethasone at the dose of 1 mg/kg marginally increased the rGSTM1 mRNA level by 1.3-fold (Fig. 4A and 4B). The radiation-inducible increases in rGSTM1/2 were not significantly inhibited by dexamethasone treatment except for the decrease in rGSTM1 mRNA at the dose of 1 mg/kg (i.e. ~68%), showing that the inducible expression of rGSTA

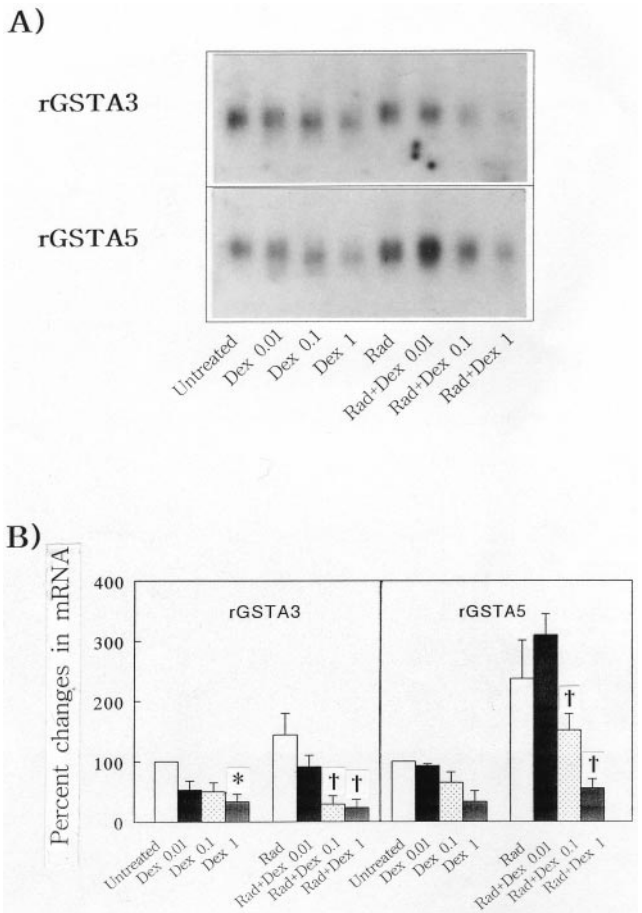

FIG. 3. Hepatic rGSTA3 and rGSTA5 mRNA levels in rats after dexamethasone (Dex) treatment with or without 3 Gy of γ-irradiation (Rad). (A) Northern blot analyses were performed to examine rGSTA3 and rGSTA5 mRNA levels. The lanes represent the same conditions as those shown in Fig. 1. (B) Relative rGSTA3 and rGSTA5 mRNA levels. Each point represents the mean ± SD of three experiments. Data were analyzed with one-way analysis of variance, followed by Newmann–Keuls test for comparison with untreated animals (*$P < 0.05$) or the animals exposed to radiation alone (†$P < 0.05$).

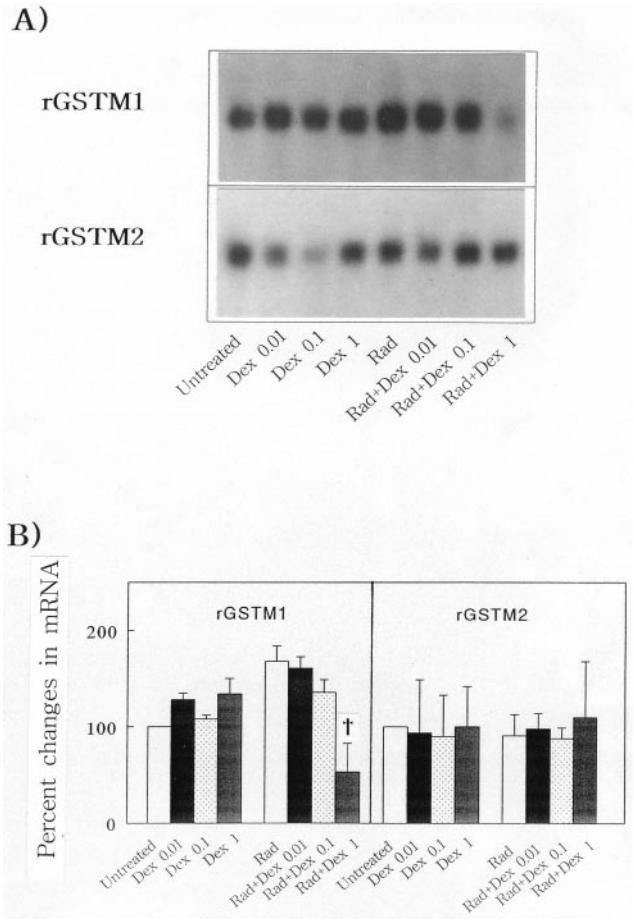

FIG. 4. RNA blot analysis of hepatic rGSTM1 and rGSTM2 mRNA levels after dexamethasone (Dex) treatment with or without 3 Gy of γ-irradiation (Rad). (A) Northern blot analyses for rGSTM1 and rGSTM2 mRNA levels were performed as described previously. The lanes represent the same conditions as those shown in Fig. 1. (B) Relative rGSTM1 and rGSTM2 mRNA levels. Each point represents the mean ± SD of three experiments. Data were analyzed with one-way analysis of variance, followed by Newmann–Keuls test for comparison with the animals exposed to radiation alone (†$P < 0.05$).

as well as mEH genes was particularly inhibited by dexamethasone.

Effect of Vitamins C and E on mEH and rGST mRNA Levels

Because previous studies have shown that oltipraz enhances radiation-inducible hepatic mEH and GST gene expression [1–3], the effect of vitamins C and E, nonsulfur-containing antioxidants, on the expression of the genes was assessed in rats for comparative purposes. Vitamin C and/or vitamin E alone failed to alter the mEH mRNA level (Fig. 5). TBI at the dose of 3 Gy resulted in a 3.3-fold increase in the mEH mRNA level at 24 hr post-treatment, as compared to that in untreated animals. Rats concomitantly exposed to vitamin C and/or vitamin E at the doses of 30 and 200 mg/kg in conjunction with 3 Gy irradiation failed to exhibit enhanced increases in the mEH mRNA level (Fig. 5) (Table 1).

The effects of vitamins C and E on the expression of major hepatic GSTs were determined. Treatment with vitamin C and/or E alone also failed to affect rGST gene expression. Rats treated with vitamin C and/or E (30 or 200 mg/kg, p.o.) in combination with 3 Gy of TBI exhibited 2- to 4-fold increases in rGSTA and rGSTM mRNA levels at 24 hr, as compared to that in untreated rats. Hence, the relative rGST mRNA levels in rats exposed to the vitamins

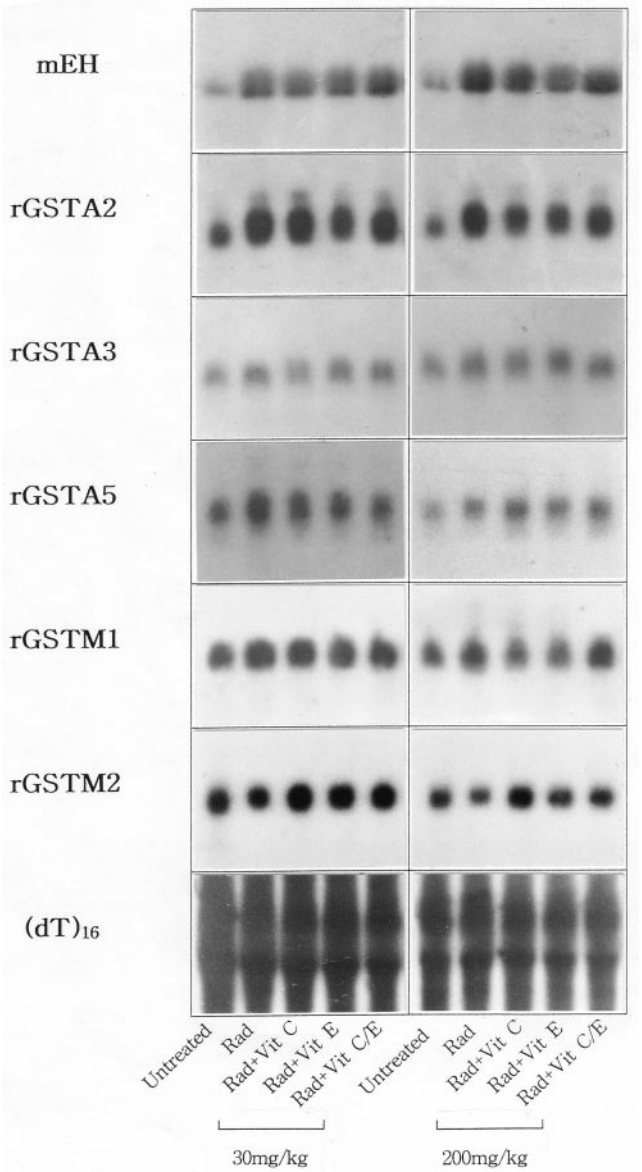

FIG. 5. Northern blot analyses of hepatic mEH and rGST mRNA levels in rats after 3 Gy of γ-ray irradiation in combination with vitamin C and/or E. The mRNA levels were determined in total RNA fractions (20 μg each) isolated from untreated rats or from rats at 24 hr after 3 Gy irradiation with or without vitamin C and/or E (30 or 200 mg/kg each, p.o.). The amount of RNA loaded in each lane was assessed by rehybridization of the stripped membrane with ^{32}P-labeled poly(dT)$_{16}$. Dex, dexamethasone; Rad, radiation.

TABLE 1. Percent changes in mEH and GST mRNA levels in rats after γ-ray irradiation in combination with vitamin C and/or E treatment

	Percent changes in mRNA levels						
		(30 mg/kg)			(200 mg/kg)		
	Rad	Rad + Vit C	Rad + Vit E	Rad + Vit C/E	Rad + Vit C	Rad + Vit E	Rad + Vit C/E
mEH	330 ± 100	400 ± 62	380 ± 44	440 ± 93	390 ± 47	330 ± 41	430 ± 142
rGSTA2	250 ± 125	380 ± 30	270 ± 69	370 ± 45	250 ± 61	230 ± 58	330 ± 86
rGSTA3	230 ± 85	290 ± 120	340 ± 125	310 ± 106	400 ± 123	340 ± 75	440 ± 141
rGSTA5	300 ± 65	350 ± 14	400 ± 50	340 ± 35	370 ± 184	300 ± 133	480 ± 147
rGSTM1	140 ± 63	290 ± 113	300 ± 151	310 ± 153	250 ± 154	180 ± 75	310 ± 26
rGSTM2	130 ± 34	220 ± 49	210 ± 100	150 ± 33	220 ± 65	150 ± 15	190 ± 34

Northern blot analyses were performed to determine mRNA levels in total RNA fractions isolated from untreated rats or from rats at 24 hr after 3 Gy irradiation with or without vitamin C and/or E (p.o.). Each point represents the mean ± SD of three experiments for the relative changes in mRNA levels (untreated rats = 100%). One-way analysis of variance showed no significant changes in the relative mRNA levels. Rad, radiation.

plus 3 Gy TBI were not significantly different from those caused by TBI alone (Table 1).

The effects of vitamins C and E at the doses of 30 and 200 mg/kg in combination with 0.5 Gy irradiation were also examined. The 0.5 Gy of ionizing radiation with the antioxidant vitamins resulted in no significant changes in mEH and GST gene expression (data not shown). These results demonstrated that antioxidant vitamins failed to enhance the radiation-induced expression of the mEH and GST genes.

Dexamethasone Suppression of mEH and GST mRNA Levels in Vitamin C and E-Treated Rats

We next examined the effects of dexamethasone on the radiation-induced expression of hepatic mEH and rGST genes in vitamin C/E-treated rats (Fig. 6). Although rats exposed to 3 Gy of TBI with or without vitamins C and E exhibited ~threefold increases in mEH and rGST mRNA levels, treatment of rats with dexamethasone at the dose of 1 mg/kg resulted in 84%–96% decreases in the inducible mEH and rGSTA2 mRNA levels (Table 2). Dexamethasone treatment also decreased the radiation-induced rGSTA3 and rGSTA5 mRNA levels in vitamin C/E-treated rats at 24 hr by 64% and 78%, respectively, while the mRNA levels for rGSTM1/2 were decreased to lesser extents (~50%).

In vivo *Radioprotective Effects of Vitamins C and E*

Previous studies have shown that pre- or post-treatment of mice with vitamins C and E in combination with γ-ray irradiation shared identical radioprotective effects [22]. ICR mice were administered vitamin C and/or vitamin E at the daily dose of 200 mg/kg each for 2 days by gavage prior to 8 Gy of irradiation in order to determine whether the vitamins were indeed effective against radiation-induced mortality (Fig. 7). Cumulative proportions of mice surviving after the lethal dose of TBI are shown in Fig. 7. Whereas 8 Gy of γ-ray irradiation resulted in 39% of the 30-day survival rate, vitamin C and vitamin E treatment of

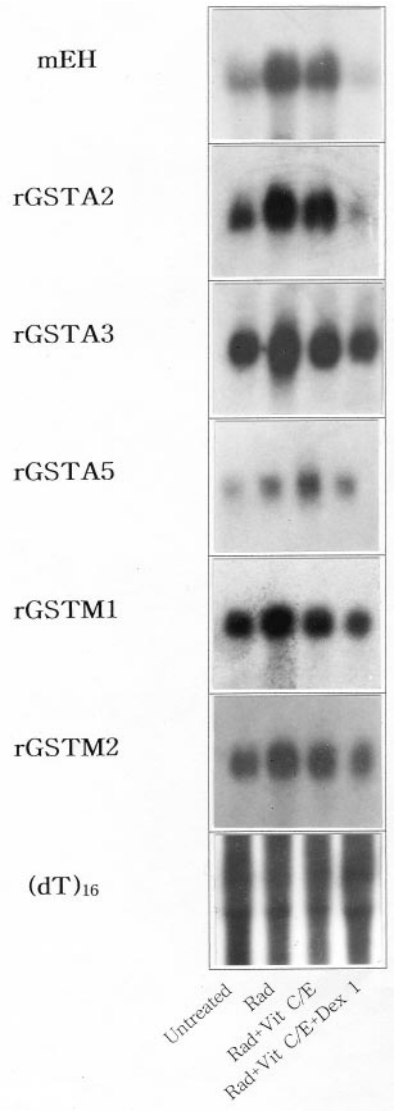

FIG. 6. Effects of dexamethasone (Dex) on mEH and rGST gene expression. The mRNA levels were assessed 24 hr after exposure of rats to 3 Gy of γ-irradiation (Rad). The mEH and rGST mRNA levels were determined in total RNA fractions (20 μg each) isolated from untreated animals or from rats 24 hr after a single dose of dexamethasone (1 mg/kg) in combination with vitamins C and E (200 mg/kg each, p.o.).

TABLE 2. Percent changes in mEH and GST mRNA levels in rats after γ-ray irradiation in combination with vitamin C/E plus dexamethasone treatment

	Percent changes in mRNA levels		
	Rad	Rad + Vit C/E	Rad + Vit C/E + Dex
mEH	330 ± 100	340 ± 130	56 ± 38*
rGSTA2	300 ± 74	330 ± 86	13 ± 11*
rGSTA3	250 ± 36	240 ± 78	87 ± 25*
rGSTA5	370 ± 95	340 ± 48	75 ± 23*
rGSTM1	178 ± 28	163 ± 67	87 ± 44
rGSTM2	140 ± 20	146 ± 48	73 ± 23

Northern blot analyses were carried out with total RNA fractions isolated from untreated rats or from rats 24 hr after 3 Gy irradiation. Vitamins C and E (200 mg/kg each, p.o.) were administered with or without a single dose of dexamethasone (1 mg/kg). Relative mRNA levels were compared to that of untreated rats (untreated = 100%). Each point represents the mean ± SD of three experiments. Data were analyzed with one-way analysis of variance followed by Newmann–Keuls test. Significantly different from those irradiated alone (*$P < 0.05$). Rad, radiation.

mice before γ-irradiation increased the survival rate to 57% and 52%, respectively. Mice treated with both vitamin C and vitamin E exhibited an additive increase in the survival rate to 74% (Fig. 7). These data provided evidence that the vitamins were effective *in vivo* as radioprotective agents against the lethal dose of γ-ray irradiation.

Effect of Dexamethasone on Vitamin C and E-Enhanced Radioprotection

The effect of dexamethasone (1 mg/kg/day, p.o., 2 days) was assessed in the ICR mice treated with the vitamins. The survival rate of 8 Gy-irradiated animals following treatment with vitamin C/E plus dexamethasone was substantially reduced to 30%, as compared to 74% in those irradiated with vitamin C/E treatment (Fig. 8). Most 8 Gy-irradiated mice treated with the vitamins plus dexamethasone died in the first 2 days. Thus, the mean survival time was substantially reduced to 2 days by dexamethasone from 14 days by TBI alone. These results provided evidence that dexamethasone-induced suppression in constitutive and inducible mEH and GST gene expression might be correlated with the increase in the mortality of γ-irradiated animals despite vitamin C- and vitamin E-elicited radioprotection.

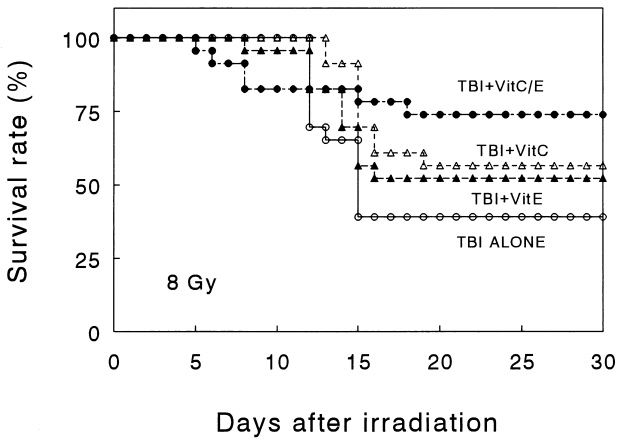

FIG. 7. Percentage survival as a function of time after 8 Gy of ^{60}Co γ-irradiation. Mice were administered by gavage with vitamin C (200 mg/kg/day, p.o., 2 days) and/or vitamin E (200 mg/kg/day, p.o., 2 days) before the lethal dose of irradiation. The last dose was given at 3 hr before irradiation. The lines represent animal survival rates for 25 mice per treatment group. The chi-square test revealed that the 30-day survival rate in the irradiated animals pretreated with vitamin C or vitamin C plus E was significantly increased, as compared with TBI ($P < 0.05$).

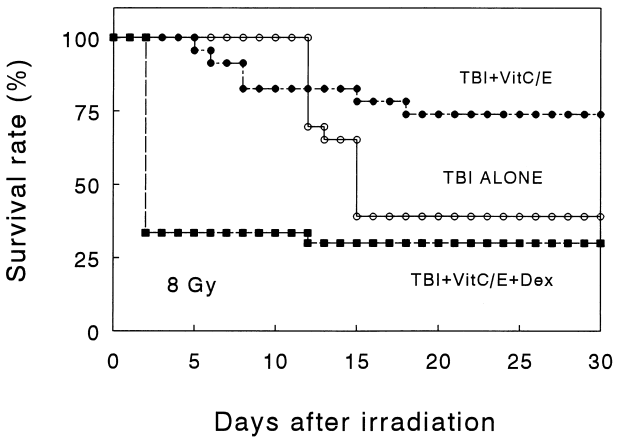

FIG. 8. Changes in the 30-day survival rate in mice irradiated at the dose of 8 Gy after treatment with dexamethasone (1 mg/kg/day, p.o., 2 days) in combination with vitamins C and E (200 mg/kg/day each, p.o., 2 days). The last dose was given at 3 hr before irradiation. The lines represent animal survival rates for 25 mice per treatment group. Concomitant dexamethasone treatment significantly decreased the survival rate and the mean survival time of the irradiated mice radioprotected with vitamins C and E, as assessed by chi-square test ($P < 0.05$). The survival rate and mean survival time of the animals irradiated with dexamethasone alone was fairly comparable to the result of TBI plus vitamin C/E and dexamethasone.

DISCUSSION

mEH and GSTs play an important role in the detoxification of electrophiles. Our previous studies showed that γ-ray ionizing radiation causes alterations in hepatic mEH and GST gene expression with the induction of the proteins and that expression of the genes is enhanced by oltipraz [1, 2]. Oltipraz treatment increases the cellular thiol levels and mEH and GSTs are potently induced by oltipraz [2, 23]. A further study in this laboratory revealed that dexamethasone antagonizes the radioprotective effect of oltipraz [3]. Thus, we were interested in the possible suppressive effects of dexamethasone on the γ-irradiation-inducible mEH and major GST expression at therapeutic doses. Because previous studies demonstrated the paralleled changes of the mEH and GST protein levels with those of mRNA [1, 2], the current study was primarily designed to determine the alterations of mRNA levels.

The present study clearly showed that dexamethasone differentially altered constitutive GST gene expression with concomitant suppression of mEH mRNA levels in the

rat liver. Waxman *et al.* showed that treatment of rats with dexamethasone at a relatively high dose (i.e. 100 mg/kg) for 4 days resulted in significant increases in rGSTA2, rGSTM1 and rGSTM2 mRNA levels without increasing rGSTA1 and rGSTA3/5 mRNA [6]. The relative changes in rGSTA2 mRNA in our study were in part consistent with those observed by Waxman *et al.* However, mEH, rGSTA3/5 and rGSTM1 mRNA levels were rather suppressed after treatment with dexamethasone in this study. This discrepancy would result from the different dose regimen used. In contrast to the differential changes in constitutive expression by dexamethasone alone in the present study, dexamethasone suppressed the radiation-inducible increases in both mEH and rGST mRNA levels. Dexamethasone at the dose of 1 mg/kg was highly effective in suppressing mEH and GST mRNA levels even in vitamin C and E-treated rats. This paralleled the increased mortality of irradiated animals. Whereas the animals irradiated at the dose of 3 Gy exhibited significant decreases in the white blood cell, red blood cell and platelet counts at day 10, dexamethasone (0.3 mg/kg) failed to significantly alter the hematologic values in the irradiated animals (data not shown). These results indicated that counts of peripheral blood cells including immune competent cells were not further suppressed by dexamethasone treatment in combination with 3 Gy irradiation.

Dexamethasone as well as γ-irradiation induce DNA fragmentation in lymphocytes [24]. Mathieu *et al.* put forth the hypothesis that DNA fragmentation in thymocytes induced by dexamethasone or by irradiation share some identical mechanisms [24]. Other studies, however, showed the beneficial effects of the clinical use of steroids in radiation therapy on the basis of the dexamethasone suppression of radiation-induced tumor necrosis factor (TNF)-α and interleukin-1 (IL-1) gene expression [25, 26]. In contrast, the present study clearly demonstrated that dexamethasone at the therapeutic doses partially or completely suppressed radiation-induced mEH and rGST gene expression and substantially reduced the survival rate and mean survival time of γ-irradiated animals radioprotected with vitamin C/E. Thus, dexamethasone even at low doses was capable of fully antagonizing the radioprotective effects of vitamins C and E.

Tocotrienol suppressed hepatic GST catalytic activities in vivo and reduced the increases in GST, GSH reductase and GSH peroxidase enzyme activities by xenobiotics [27–29]. The present research showed that vitamin C and/or E alone failed to increase mEH or GST expression. In contrast to the effects of oltipraz, the present study delineated no increase in radiation-inducible detoxifying gene expression by the vitamins. Although vitamins C and E were also effective in increasing the survival rate in the context of radiation-induced injury, radioprotective effects of vitamins C and E did not appear to be associated with the tissue-specific enhanced expression of the detoxifying enzymes.

Vitamin C easily undergoes autoxidation. Changes in levels of ascorbic acid and dehydroascorbic acid may cause a redox cycling of vitamin C with the oxidative stress induced by γ-ray irradiation [30]. The balance between pro- and antioxidant activity of vitamin C is dependent on the rate of free radical production as well as other antioxidant levels such as vitamin E within the lipid phase. Certain genes might be expressed in association with their anti- and pro-oxidant status and with the rate of production of reactive oxygen species. Nonetheless, the lack of enhancement in mEH and GST gene expression by vitamins C and E lends support to the hypothesis that vitamins C and E may not antagonize nor enhance the radiation-induced oxidative stress which stimulates a certain signal transduction pathway and thus activates the pathway for the expression of the detoxifying enzymes through modulation in the production of reactive oxygen species and supplementation of endogenous defense elements including GSH. Because protein expressed as part of adaptive responses may directly scavenge radicals and thus decrease the formation of superoxide, the radioprotective effects of vitamins C and E should be further studied in light of the activation of proto-oncogenes as well as cellular signaling.

Studies have shown that the effective dose of vitamin C against aberrations in chromosomes caused by 1 Gy of γ-ray was 25 mg/kg or greater in mice, whereas vitamin E rather exhibited the greatest protection at the same dose [22]. Vitamin C or E exerts significant reduction in the frequencies of micronuclei and chromosomal aberrations in bone marrow cells. Vitamin C exhibited radioprotection against iodine-131, as assessed by spermatogenesis in mice [31]. Sarma and Kesavan showed that radioprotection by vitamin E was appreciably greater than that afforded by vitamin C [22]. Administration of these antioxidant vitamins to mice immediately after irradiation was as effective as that at 2 hr before irradiation in the study.

A major disadvantage of potential organosulfur radioprotective agents (e.g. WR2721) involves inactivation of γ-glutamylcysteine synthetase, which may be responsible for the synergistic toxicity of the thiophosphate radio- and chemoprotection [32]. The vitamins are likely to be capable of providing radioprotection with less toxicity than the organosulfur radioprotective agents, although the effects of vitamins C and E on the activity of γ-glutamylcysteine synthetase require further study. Antioxidant vitamins may neutralize oxygen free radicals which are produced by γ-ray irradiation. Research carried out with vitamin E-deficient or supplemented diets indicated that pathological phenomena occurring as a consequence of GSH depletion depended on hepatic levels of vitamin E [33]. Although vitamin C and E-induced radioprotective effects did not appear to be related to enhanced expression of the mEH and GST enzymes in conjunction with irradiation, the expression of mEH and GST seemed to be crucial in protecting the vital organs against radiation-induced injury, which was supported in part by dexamethasone reversal of the vitamin C and E-induced radioprotective effects. The present study demonstrated that: dexamethasone at therapeutic doses

suppressed the radiation-inducible increases in both mEH and rGST mRNA levels, although the agent differentially altered the constitutive expression of the genes; dexamethasone-induced suppression in the detoxifying gene expression was associated with the increased mortality of the irradiated animals; and the antioxidant vitamins exhibited radioprotective effects *in vivo* in the absence of enhanced expression of the mEH and GST genes.

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