Effect of Catecholestrogens on Mortality and Liver Lipid Peroxide Level of Mice Irradiated with ^{60}Co γ -Ray

Akira TOMATSU,¹ Sadaaki KOMURA,² Nobuko OHISHI,²
Masami NAKANISHI,¹ and Kunio YAGI^{2,*}

¹*Department of Obstetrics and Gynecology, Aichi Medical University,
Nagakute, Aichi 480-11, Japan*

²*Institute of Applied Biochemistry, Yagi Memorial Park,
Mitake, Gifu 505-01, Japan*

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Summary When 10 weeks old male BALB/c mice received whole-body irradiation with an 8-Gy single dose of ^{60}Co γ -ray, the survival rate 30 days after the irradiation was only 5%, while the survival rate was increased to 70% when the mice were subcutaneously injected with catecholestrogen 2-hydroxyestradiol (2-OHE₂) 3 h before and 3 h after the irradiation. The survival rates of the mice given other test compounds were as follows: 2-hydroxyestrone, 20%; 2-hydroxyestriol, 20%; 4-hydroxyestradiol, 0%; 2-methoxyestrone, 0%; 2-methoxyestradiol, 0%; 2-methoxyestriol, 0%; estrone, 0%; estradiol, 5%; and estriol, 20%. Upon the irradiation, lipid peroxide levels in the liver of the animals markedly increased 4 days after the irradiation. This increase in the level was significantly suppressed by the subcutaneous injection of 2-OHE₂. These results indicate that 2-OHE₂ has a protective effect against radiation injury.

Key Words: catecholestrogens, radiation injury, mortality, lipid peroxide level

It has been accepted that lipid peroxides are causative of degenerative diseases such as atherosclerosis [1–3] and cataract [4–6] and aging itself [7]. We previously reported that the serum lipid peroxide level increases with age but that the rate of increase is different between women and men; that is, serum lipid peroxide levels of 30–50 years old women are significantly lower than those of men in the same age category [8]. As to the reason for this difference, we suspected the effect of female hormones and found in *in vitro* experiments that estrogens have an antioxidant activity as expected from the presence of a phenolic hydroxyl group in

*To whom correspondence should be addressed.

their structure [9]. We also reported from *in vivo* experiments that subcutaneous injection of estrogen or its metabolite catecholestrogen brought about a decrease in lipid peroxide levels in the serum and liver of mice [10]. The antioxidant activity of catecholestrogens was recognized by Wollenberg *et al.* [11] and Nakano *et al.* [12] in their *in vitro* experiments. In addition, we found that serum and liver lipid peroxide levels of ovariectomized mice were significantly increased and that these increases were suppressed by the administration of estrogen or catecholestrogen [13]. Our clinical study also revealed that the serum lipid peroxide level of women was significantly increased after bilateral ovariectomy [14]. These results support the idea that estrogens and catecholestrogens may play a role as an endogenous antioxidant.

As is well known, lipid peroxides are generated by radiation and have deleterious effects on organs and tissues. Since catecholestrogens were found to possess a potent antioxidant activity, one would expect that catecholestrogens would have a protective activity against radiation injury. The present study was undertaken to test this assumption in mice irradiated with ^{60}Co γ -ray.

MATERIALS AND METHODS

Animals. Male mice of the BALB/c strain (10 weeks old, 25 ± 2 g) were used. They were fed a commercial laboratory chow (CE-2, Clea Japan, Inc., Tokyo) and given water *ad libitum*.

Chemicals. Estrone (E_1) and 17β -estradiol (E_2) were purchased from Nacalai Tesque Inc., Kyoto; estriol (E_3), 2-hydroxyestrone (2-OHE $_1$), 2-hydroxyestradiol (2-OHE $_2$), 2-hydroxyestriol (2-OHE $_3$), 4-hydroxyestradiol (4-OHE $_2$), 2-methoxyestrone (2-MeOE $_1$), 2-methoxyestradiol (2-MeOE $_2$), and 2-methoxyestriol (2-MeOE $_3$), from Sigma Chemical Co., St. Louis, MO; and carboxymethyl cellulose sodium salt (CMC), glutathione, and *dl*- α -tocopheryl acetate, from Wako Pure Chemical Industries, Ltd., Osaka.

Each of these drugs was suspended in 1% CMC and injected subcutaneously 3 h before and 3 h after the irradiation. The mice of the control group were given the same volume of 1% CMC.

^{60}Co γ -ray irradiation. Mice were placed in a restrainer and given whole-body irradiation at a dose rate of 0.84–0.96 Gy/min with a ^{60}Co irradiation apparatus (RTGS-2DM, Shimadzu, Kyoto). The total dose was 8 Gy.

Measurement of lipid peroxide level and lipids. Lipid peroxide level in the liver was measured by the method of Ohkawa *et al.* [15] and expressed in terms of malondialdehyde. Lipids in the liver were extracted by the method of Folch *et al.* [16] from 20% liver homogenates in physiological saline, and the amounts of total cholesterol, triglycerides, and phospholipids were measured by use of respective reagent kits purchased from Kyowa Medex Co., Ltd., Tokyo.

Statistical analysis. Experimental values were analyzed by the Student's *t*-test and chi-square test.

RESULTS

Effect of interval between 2-OHE₂ injection and radiation on survival rate of lethally irradiated BALB/c mice

From our previous observation that subcutaneous injection of 2-OHE₂ was effective to suppress an increase in the lipid peroxide level of ovariectomized mice [13], we adopted the subcutaneous injection route for the administration of 2-OHE₂ to check its potency. First, we examined the effect of the interval between the injection and the irradiation on the survival rate. 2-OHE₂ was injected into male BALB/c mice at a dose of 2 mg/kg body weight either 15 min, 1 h, 3 h, or 6 h before and after the irradiation. We found that the survival rate of mice 30 days after the irradiation was 30% when 2-OHE₂ was given 15 min before and 15 min after the irradiation, and that the rate reached maximum (ca. 70%) when the drug was injected 1–6 h before and after the irradiation.

Then, 2-OHE₂ was given to the mice according to the protocol shown in Table 1; viz, 3 h before and 3 h after the irradiation (group 1); once 3 h before the irradiation (group 2); and once 3 h after the irradiation (group 3). The maximal survival rate was observed in the mice of group 1. The rate of groups 2 and 3 was lower than that of group 1, but higher as compared with that of the control (group 4). These results indicate that the treatment both before and after the irradiation is necessary to obtain the maximum effect of 2-OHE₂.

Radioprotective effect of catecholestrogens on lethally irradiated BALB/c mice

Under the optimum conditions for the radioprotection of 2-OHE₂ described above, comparison among the three catecholestrogens was made. Each catecholestrogen was given at a dose of 2 mg/kg body weight 3 h before and 3 h after the whole-body irradiation with ⁶⁰Co γ -ray, and the survival of the mice was followed for 30 days (Fig. 1). In the mice of control group, the first death occurred on the 10th day after the irradiation, and the percent survival of the mice after 30 days was

Table 1. Effect of different protocol of 2-OHE₂ administration on survival of mice after ⁶⁰Co γ -irradiation.

Group	Administration at		Survival	%
	3 h before	3 h after		
1	2-OHE ₂	2-OHE ₂	13/20	65**
2	2-OHE ₂	—	8/20	40*
3	—	2-OHE ₂	5/20	25
4	—	—	1/20	5

Mice received whole-body irradiation with an 8-Gy single dose of ⁶⁰Co γ -ray. 2-OHE₂ was subcutaneously injected at a dose of 2 mg/kg body weight 3 h before and 3 h after or once either before or after the irradiation as described under "Materials and Methods." Number of mice in each group was 20. Significant difference from the control (group 4) as analyzed by the chi-square test: * $p < 0.05$; ** $p < 0.01$.

only 5%. When 2-OHE₂ was injected, the onset of the first death was delayed to the 15th day after the irradiation, and the survival rate after 30 days remarkably increased to 70%. In the case of mice injected with 2-OHE₁ or 2-OHE₃, their survival rate was always higher than that of the control animals. The injection with *dl*- α -tocopheryl acetate also resulted in a significant protection until the 21st day.

Table 2 shows the survival rate 30 days after the irradiation of mice given each of various test drugs. Among them, 2-OHE₂ alone remarkably protected mice from death. The injection with 2-OHE₁, 2-OHE₃, E₃, or *dl*- α -tocopheryl acetate brought about lower degrees of protection.

Effect of dosage of 2-OHE₂ on the survival rate of lethally irradiated BALB/c mice

As shown in Table 3, the effect of 2-OHE₂ against radiation injury increased in a dose-dependent manner. The effective dose (ED₅₀), which gives 50% protection, was calculated to be about 0.8 mg/kg body weight.

Effect of 2-OHE₂ on liver lipid peroxide level of lethally irradiated BALB/c mice

Casarett [17] described that there are three phases of the radiation syndrome in the rat after an 8-Gy single dose of whole-body γ -ray irradiation: *viz.*, 1) an initial phase up to 4 days after the irradiation, in which prostration, fasting, and

Fig. 1. Effect of catecholestrogens on the survival rate of mice after ⁶⁰Co γ -ray irradiation. Mice received whole-body irradiation with an 8-Gy single dose of ⁶⁰Co γ -ray. Each catecholestrogen was injected 3 h before and 3 h after the irradiation as described under "Materials and Methods." ○, 2-OHE₁; ●, 2-OHE₂; △, 2-OHE₃; ▲, *dl*- α -tocopheryl acetate; □, control. Number of mice in each group was 20. Significant difference from the control as analyzed by the chi-square test: **p* < 0.05; ***p* < 0.01.

Table 2. Effect of catecholestrogens, their monomethyl ethers, and estrogens on survival of mice after ^{60}Co γ -irradiation.

Test sample	Survival	%
2-OHE ₁	4/20	20
2-OHE ₂	14/20	70*
2-OHE ₃	4/20	20
4-OHE ₂	0/20	0
2-MeOE ₁	0/20	0
2-MeOE ₂	0/20	0
2-MeOE ₃	0/20	0
E ₁	0/20	0
E ₂	1/20	5
E ₃	4/20	20
<i>dl</i> - α -Tocopheryl acetate	3/20	15
Glutathione	1/20	5
Control	1/20	5

Mice received whole-body irradiation with an 8-Gy single dose of ^{60}Co γ -ray. Each test compound was subcutaneously injected at a dose of 2 mg/kg body weight 3 h before and 3 h after the irradiation as described under "Materials and Methods." Number of mice in each group was 20. Significant difference from the control as analyzed by the chi-square test:

* $p < 0.01$.

Table 3. Effect of dose of 2-OHE₂ on survival of mice after ^{60}Co γ -irradiation.

Dose of 2-OHE ₂ (mg/kg)	Survival	%
4	14/20	70**
2	13/20	65**
1	11/20	55**
0.5	9/20	45*
0.25	0/20	0
0	1/20	5

Mice received whole-body irradiation with an 8-Gy single dose of ^{60}Co γ -ray. 2-OHE₂ was subcutaneously injected 3 h before and 3 h after the irradiation as described under "Materials and Methods." Number of mice in each group was 20. Significant difference from the control as analyzed by the chi-square test: * $p < 0.05$; ** $p < 0.01$.

diarrhoea occur; 2) a remission phase from 5 to 7 days, in which the animals regain normal feeding and mobility; and 3) a final phase from 8 days, in which hemorrhage occurs, which leads to death. Since a marked increase in the lipid peroxide level in the liver was observed in the initial phase [18], we examined the change in the lipid peroxide level in the liver of mice 4 days after the irradiation. As shown in Table 4, the level was increased by the irradiation to one over twice that of the control. This increase was significantly suppressed by the injection of 2-OHE₂. When the lipid peroxide level was expressed per mg of lipids, the same results were obtained.

Table 4. Effect of 2-OHE₂ on liver lipid peroxide level in mice after ⁶⁰Co γ-irradiation.

Group	⁶⁰ Co	2-OHE ₂	Lipid peroxide level	
			(nmol/100 mg liver)	(nmol/mg lipids)
1	—	—	51.9±5.4	21.7±2.1
2	+	—	101.6±8.5*	41.1±2.2*
3	+	+	43.0±6.9	21.6±2.5

Mice received whole-body irradiation with an 8-Gy single dose of ⁶⁰Co γ-ray. 2-OHE₂ was subcutaneously injected at a dose of 2 mg/kg body weight as described under "Materials and Methods." Lipid peroxide level was measured 4 days after the irradiation. Mean±SE is given. Number of mice in each group was 9. Significant difference from the values of group 1 as analyzed by the Student's *t*-test: **p*<0.01.

DISCUSSION

The present data indicate for the first time that catecholestrogens have a considerable radioprotective effect *in vivo*. Although the details of the mechanism for the radioprotective effect of catecholestrogens remain to be elucidated, their antioxidant activity is principally postulated to explain the effect. Among the various estrogens, catecholestrogens, and methoxy derivatives of catecholestrogens examined in the present study, 2-OHE₂ was most effective in protecting mice from radiation death. As to the antioxidant activity *in vitro*, it was reported that the potency of 2-OHE₂ is almost the same as that of 2-OHE₁ [12]. However, the present results indicate that the radioprotective effect of 2-OHE₁ was lower than that of 2-OHE₂. This result might be ascribable to the fact that the plasma metabolic clearance rate of 2-OHE₁ is higher than that of 2-OHE₂ [19]. 2-OHE₃ was ineffective in the present *in vivo* radioprotection experiments, probably reflecting that *in vitro* antioxidant activity of 2-OHE₃ is lower than that of 2-OHE₂ [12]. Further study is needed to compare the bioavailability of these catecholestrogens for a better understanding of the reason(s) for their different potency *in vivo*.

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