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Effects of γ-Irradiated Pork Diet on Cytochrome P-450 System, Microsome Glucose 6-Phosphatase Activity and Antioxidative Defense Systems in Rat Hepatocarcinogenesis

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Abstract

This study investigated the effects of a γ-irradiated pork (0-30 kGy) diet on lipid peroxidation, cytochrome P-450 content, microsomal glucose 6-phosphatase (G-6-Pase) activity and antioxidative defense systems in diethylnitrosamine (DEN)-induced rat hepatocarcinogenesis. The body weight of rats fed irradiated diets did not change significantly. Liver weight was significantly increased by the administration of DEN, but not by irradiated diets at any dose level. There were no significant effects of gamma irradiation on the content of microsomal malondialdehyde (MDA), cytochrome P-450, or on the activity of G-6-Pase. However, with DEN treatment, cytochrome P-450 content was significantly increased while microsomal G-6-Pase activity was significantly decreased. The γ-irradiated diet supplement did not affect serum retinol or α-tocopherol concentrations. However, it did cause a significant decrease in hepatic retinol at 30 kGy. With DEN treatment, hepatic retinol content was even more significantly ($p < 0.05$) decreased compared to the non-irradiated control. The enzyme activities related to antioxidative defense systems, including glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx) and glutathione S-transferase (GST) were not affected by gamma irradiation. These results suggest that an irradiated pork diet up to 30 kGy may not cause a health hazard in experimental animals.

Key words: antioxidant enzyme, gamma irradiation, hepatocarcinogenesis

INTRODUCTION

The development of meat preservation techniques is essential for the maintenance of an adequate supply of fresh meat that is free of microbial contamination. Food poisoning from meat contaminated with pathogenic *E. Coli*, for an example, has become a serious health threat requiring rigorous measures to prevent outbreaks. Irradiation of meat as a type of pasteurization is known to be an effective technique (1) for sterilization from pathogenic bacteria and parasites. Recently, FDA allowed gamma irradiation up to 4.5 kGy for refrigerated beef, up to 7 kGy for frozen beef and up to 1.0 kGy for pork in order to secure microbial safety and to remove parasites from meat (2).

Although meat irradiation can resolve many problems occurring during storage and in spite of many reports that irradiation does not leave any residues in meat (3), consumers remain skeptical of the safety of irradiated foods. The long-term safety of food irradiation needs to be evaluated, and should include many different aspects including toxicity, nutrient destruction, mutation, carcinogenicity and radioactive residues.

Therefore, this study investigated the effects of a gamma irradiated pork diet on lipid peroxidation, membrane stability, antioxidant nutrients and antioxidative defense systems in rats with diethylnitrosamine (DEN)-induced hepatocarcinogenesis.

MATERIALS AND METHODS

Experimental design, animals and diet

Fresh pork samples were exposed to a 60-Co source of 100,000 Ci having a dose rate of 1 $kGyh^{-1}$. For gamma irradiation, lots (100 g) of the sample were aerobically packed in polyethylene containers (500 mm diameter × 800 mm long) and irradiated at doses up to 30 kGy on ice. Forty weanling Sprague-Dawley male rats weighing approximately 80~90 g were randomly divided into eight groups, including the control, and fed diets containing freeze-dried, irradiated diets containing irradiated pork (0 kGy, 3 kGy, 10 kGy and 30 kGy) supplying a 20% protein source for 8 weeks. Each experimental diet was isocaloric based on the AIN-76 formula with minor modifications

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(Table 1). One week after feeding, rats were intraperitoneally injected twice with 50 mg/kg body wt DEN in a physiological solution, and thereafter 0.05% phenobarbital was supplied via drinking water.

Collection of blood and tissues

Animals were fasted for 12 hrs and weighed before they were sacrificed by cervical dislocation. Blood taken from the heart was centrifuged at 3000 rpm for 20 min and the obtained serum was used for analysis. Livers were rinsed with ice-cold physiological saline and weighed. Cytosolic and mitochondrial fractions obtained by the method of Masmoudi et al. (4) were immediately frozen in liquid nitrogen and kept for enzyme assays at -80°C.

Measurement of microsomal lipid peroxide, cytochrome P-450 and glucose-6-phosphatase

Microsomal lipid peroxide was measured by the method of Bidlack and Tapple (5), a measurement of malondialdehyde (MDA) production.

Cytochrome P-450 was measured by the method of Omura and Sato (6). Microsomal fractions diluted with 0.1 M phosphate buffer (pH 7.0) were mixed with 4 mg of sodium dithionite and bubbled with CO_2 gas at a rate of 1 bubble/sec for 30 to 40 seconds. The content of cytochrome P-450 was calculated by the difference of absorbance at 450 nm and 490 nm.

Microsomal glucose-6-phosphatase (G-6-Pase) activity was measured by the method of Baginiski et al. (7).

Serum and hepatic retinol and α -tocopherol assays

α -Tocopherol and retinol were assayed by modified methods of Bieri et al. (8) and Hatam and Kayden (9), respectively. One hundred μL of serum or liver homogenate and 100 μL of α -tocopheryl acetate (50 μg/mL in ethyl alcohol) were mixed with 200 μL n-hexane for 10 min. After centrifuging at 1500 rpm for 5 min, the supernatants were evaporated with nitrogen gas. HPLC equipped with a Nava-pak C18 column (3.9×150 mm) was used for the determination of α -tocopherol and retinol.

Table 1. Diet composition

Ingredient	Amount (%)
Corn starch	63.2
Cellulose	5.0
Pork protein	20.0
Sucrose	4.975
DL-methionine	0.3
Corn oil	2.0
Salt mixture	3.5
Vitamin mixture[1)]	1.0
Vitamin E	0.015
BHT	0.01

[1)]Vitamin mixture was purchased from Oriental Yeast Company, Japan.

The mobile phase was methanol and H_2O (95:5) at a flow rate of 1.5 mL/min. α -Tocopheryl acetate was used as an internal standard.

Antioxidant enzyme activity

Cytosolic glutathione S-transferase (GST) was assayed by the method of Habig et al. (10). Cocktails including 2935 μL of 0.1 M phosphate buffer, 30 μL of 0.1 M glutathione, 25 μL of 0.12 M 2-4CNDB (1-chloro-2,4-dinitrobenzene) with 10 μL of sample were measured at 340 nm for 3 min at 25°C. Enzyme units were expressed as 2-4-dinitro benzene-glutathione produced/mg protein per min. Liver cytosolic glutathione peroxidase (GSH-Px) activity was measured by H_2O_2 coupled enzyme assay at 340 nm with modified method of Paglia and Valentine (11) and Deagen at al. (12). Glutathione reductase (GSH-Rx) was measured by the method of Carlberg and Mannervik (13) at 340 nm.

Statistical analysis

Data from individual experiments were analyzed by 2-way analysis of variance (ANOVA) and were expressed as the means ± standard deviation (SD). Statistical analysis was performed using SAS software (SAS Institute, Cary, NC USA), differences were separated by Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Body and liver weight of experimental animal

The body and organ weights of weanling Sprague-Dawley male rats were not affected by irradiated ingredients at any radiation dose (Table 2). However, liver weight was significantly increased by the administration of DEN, resulting in a significant increase in the weight ratio of liver to the body in DEN administered animals.

Table 2. Effect of γ -irradiated pork diet on body and liver weights of rats treated with DEN[1)]

Group[2)]	N[3)]	Body weight (g)	Liver weight (g)	Liver wt./ Body wt. ratio (%)
C	9	212.0±24.5[NS]	5.4±0.7[b]	2.5±0.1[b]
C-DEN	11	218.0±31.3	8.2±1.6[a]	3.8±0.3[a]
3I	9	225.4±19.4	5.8±0.6[b]	2.6±0.1[b]
3I-DEN	11	233.9±19.7	8.5±1.0[a]	3.6±0.3[a]
10I	9	226.8±12.5	5.7±0.5[b]	2.5±0.1[b]
10I-DEN	11	229.0±26.4	8.0±1.1[a]	3.5±0.2[a]
30I	9	229.6±20.2	5.9±0.8[b]	2.6±0.2[b]
30I-DEN	11	233.3±37.5	8.7±1.6[a]	3.7±0.3[a]

[1)]Values are mean ± SD. Means with different superscripts within a column are significantly different at $p<0.05$. NS=not significant.

[2)]C=not irradiation, 3I=3 kGy irradiation, 10I=10 kGy irradiation, 30I=30 kGy irradiation, DEN=diethylnitrosamine.

[3)]Number of animals.

Measurement of lipid peroxide and membrane stability

Neither irradiation per se nor the dose level affected the malondialdehyde (MDA) content. Although there was no statistically significant change, MDA content was prone to increase by administration of the carcinogen (Table 3). The increase of thiobarbituric acid reactive substance (TBARS) corresponded with that of the amount of carcinogen, which was consistent with the observations by Lankin et al. (14) that TBARS were increased at initiation or during the early stages of carcinogenicity. In general, lipid peroxidation is increased by drugs, toxic substances or carcinogens, as are the amounts of free radicals (15).

The contents of cytochrome P-450, which plays an essential role in the phase I reactions of drug metabolism, were increased significantly ($p<0.05$) by the administration of carcinogen (Table 3). In contrast, microsomal G-6-Pase activity, used as an index of membrane stability (7), was significantly ($p<0.05$) decreased by DEN in the 3 kGy and 30 kGy groups. None of the cytochrome P-450 and microsomal G-6-Pase enzymes were affected by irradiation itself. In general, microsomal membrane stability and mobility are inhibited by the increment of the synthesis of lipid peroxide and thus microsomal membrane-bound G-6-Pase enzyme is affected, resulting in hepatic damage and carcinogenesis (16).

Antioxidant vitamins

Irradiation did not affect serum retinol or α-tocopherol levels. However, it did cause a significant decrease in hepatic retinol at the 30 kGy dose. Serum retinol tended to increase with DEN treatment, while hepatic retinol content was significantly decreased compared to the non-irradiated control (Table 4). Serum retinol and α-tocopherol were increased by carcinogen administration. However, both serum retinol and α-tocopherol were unaffected by irradiation. A similar study (17) also observed the increase in serum α-tocopherol with carcinogen treatment, which was due to the defense mechanism of α-tocopherol from oxidative stress.

Carcinogen administration caused a significant decrease in liver retinol compared to the non-irradiated control, which might be due to the fact that hepatic storage of these antioxidant nutrients were depleted by the excessive cellular requirements. As a result, serum retinol and α-tocopherol were increased as hepatic antioxidants were mobilized into the blood pool.

Antioxidant enzyme activity

The results of the antioxidant enzyme assay are shown in Table 5. Though the activities of glutathione reductase (GSH-Rx), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) were not greatly influenced by the irradiated diets per se, those of GSH-Rx and GST were increased significantly while those of GSH-Px were significantly reduced by treatment with DEN. GSH-Px protects membrane components against lipid peroxides and peroxidative damage and is involved in detoxification

Table 3. Effect of γ-irradiated pork diet on hepatic microsomal malondialdehyde, cytochrome P-450 content and glucose 6-phosphotase activity in rats treated with DEN[1)]

Group[2)]	Malondialdehyde (nmoles MDA/mg protein)	Cytochrome P-450 (nmoles/mg protein)	G-6-Pase activity (nmole inorganic phosphate liberated/mg protein/min)
C	0.30 ± 0.11^{ab}	0.82 ± 0.18^{c}	582.8 ± 145.9^{abc}
C-DEN	0.30 ± 0.11^{ab}	1.71 ± 0.42^{ab}	406.5 ± 166.5^{bcd}
3I	0.27 ± 0.10^{ab}	0.85 ± 0.38^{c}	597.2 ± 313.7^{ab}
3I-DEN	0.32 ± 0.14^{ab}	1.80 ± 0.37^{ab}	390.8 ± 150.7^{cd}
10I	0.25 ± 0.07^{b}	0.88 ± 0.23^{c}	568.8 ± 129.4^{abc}
10I-DEN	0.27 ± 0.09^{ab}	1.55 ± 0.33^{b}	401.2 ± 149.3^{bcd}
30I	0.28 ± 0.10^{ab}	0.86 ± 0.16^{c}	622.3 ± 250.1^{a}
30I-DEN	0.36 ± 0.12^{a}	1.90 ± 0.20^{a}	371.8 ± 178.4^{d}

[1,2)]See footnote in Table 2.

Table 4. Effect of γ-irradiated pork diet on serum and hepatic retinol and α-tocopherol contents of rats treated with DEN[1)]

Group[2)]	Retinol (μg retinol/mL serum)	α-Tocopherol (μg tocopherol/mL serum)	Retinol (μg retinol/g liver)	α-Tocopherol (μg tocopherol/g liver)
C	0.29 ± 0.10^{NS}	11.27 ± 2.12^{c}	295.3 ± 97.3^{a}	20.9 ± 9.1^{ab}
C-DEN	0.44 ± 0.17	16.66 ± 2.91^{a}	158.0 ± 63.5^{cd}	20.1 ± 8.8^{ab}
3I	0.29 ± 0.19	11.27 ± 2.70^{c}	281.4 ± 57.7^{ab}	29.1 ± 7.4^{a}
3I-DEN	0.41 ± 0.15	15.56 ± 2.19^{ab}	119.2 ± 53.5^{d}	18.0 ± 7.7^{b}
10I	0.34 ± 0.15	11.44 ± 2.42^{c}	258.2 ± 57.2^{ab}	20.3 ± 5.3^{ab}
10I-DEN	0.44 ± 0.17	16.54 ± 3.15^{a}	119.2 ± 23.4^{d}	19.8 ± 6.1^{ab}
30I	0.29 ± 0.18	12.04 ± 1.98^{c}	208.8 ± 57.1^{bc}	20.6 ± 3.4^{ab}
30I-DEN	0.40 ± 0.16	13.45 ± 3.19^{bc}	114.4 ± 46.4^{d}	18.6 ± 6.9^{b}

[1,2)]See footnote in Table 2.

Table 5. Effect of γ -irradiated pork feeding on cytosolic glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx) and glutathione S-transferase (GST) activities in rats treated with DEN[1)]

Group[2)]	GSH-Px (nmole NADPH oxidized/ mg protein/min)	GSH-Rx (nmole NADPH oxidized/ mg protein/min)	GST (nmole CDNB conjugated/ mg protein/min)
C	891.7±116.2[a]	35.1± 8.2[b]	328.4± 65.6[b]
C-DEN	657.8±114.0[b]	51.2± 9.0[a]	757.2±107.5[a]
3I	859.2±110.0[a]	33.1± 8.9[b]	326.1± 58.5[b]
3I-DEN	644.8± 89.5[b]	51.9± 4.2[a]	795.9±116.7[a]
10I	835.4±136.9[a]	31.0± 4.5[b]	319.9± 43.6[b]
10I-DEN	621.2± 95.6[b]	49.7±10.0[a]	718.1±118.4[a]
30I	871.1±136.2[a]	30.0± 3.3[b]	315.7± 41.6[b]
30I-DEN	599.3± 42.4[b]	50.0±12.1[a]	775.6±143.3[a]

[1,2)]See footnote in Table 2.

process. Another study (18) also found inhibited GSH-Px activity in chemically induced hepatic mouse cancer cells. Carlberg et al. (19) reported an increase in cytosolic GST with hepatic carcinogen treatment, which was similar to the results of this study.

These results suggest that the consumption of pork irradiated at 3 kGy, 10 kGy and 30 kGy may not affect the formation of lipid peroxide or membrane stability, and further, may not promote chemically induced hepatocarcinogenesis. In conclusion, the consumption of pork irradiated up to 30 kGy appears to be safe in experimental animal diets.

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