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Effect of X-ray Irradiation on Lipid Peroxide Levels in the Rat Submandibular Gland

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We examined the effect of local x-ray irradiation on the changes in the lipid peroxide level in submandibular gland, liver, and blood plasma. Rats five weeks of age received a single low dose of 3 Gy x-ray irradiation to their neck regions. The lipid peroxide level in the submandibular gland was significantly enhanced at seven days after irradiation, as was the level in the blood plasma at two hours, seven and 14 days after irradiation. The lipid peroxide level in the liver decreased, as compared with levels in the controls. There was a slight tendency for acinar cells of the submandibular gland to show pyknosis and anomalous nuclei within three days after irradiation. These results suggest that radiation injury results in an elevation of the lipid peroxides in the submandibular gland, and an increased level in the blood.

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Introduction.

Salivary glands exposed to the radiation field for treatment of oral malignancy are frequently injured, resulting in so-called radiation-sialoadenitis.

Extensive studies have suggested a relationship between radiation-induced lipid peroxidation and damage to living cell membranes (Wills and Wilkinson, 1967; Yukawa and Nakazawa, 1980). Hiramitsu *et al.* (1974) demonstrated that the remarkably elevated value of lipid peroxide in the retina prior to reduction of the electro-retinogram amplitude was observed after local irradiation to the heads of rabbits, and histological changes in the irradiated eyes were quite similar to those of eyes injected intravitreally with linoleic peroxide. This report suggests the involvement of lipid peroxidation in the toxicity of local x-ray irradiation.

El-Mofty and Kahn (1981) reported that as early as two hours after irradiation, signs of cell membrane injury were demonstrable in the parotid acinar cell by electron microscopy, and they also suggested that the peroxidation of membrane lipids was related to the lethal effects of radiation on salivary gland cells. Furthermore, Akita *et al.* (1984) reported that a relatively high dose of γ -irradiation to the neck regions of mice induced an elevation in the lipid peroxide level in the submandibular gland. Consequently, the lipid peroxide might play an important role in the mechanism of radiation toxicity in the salivary gland.

On the above basis, we examined changes in the lipid peroxide levels in the submandibular gland, blood, and liver after low-dose x-ray irradiation of rats.

Materials and methods.

The animals used were 5-to-7-week-old male rats of the Wistar strain. The diet consisted of rat-rabbit pellets (CE-1,

Oriental Yeast Co., Tokyo) and water *ad libitum*. The experimental animals were irradiated with x-rays (NR-3436F, Shimadzu Seisakusho Co., Kyoto), and the exposure factors were as follows: 180 kV x-ray at 15 mA, 0.7 mm Cu + 0.5 mm Al filtration, 30 cm focal distance, 1.15 mm Cu half-value layer, 1.5 \times 2.5 cm field size, and exposure rate of 0.55 Gy/min. Animals were anesthetized intraperitoneally with pentobarbital, and were put into a dome shield made from Pb about 3 mm thick, so that only the neck region over the right submandibular gland would be exposed. Each rat was administered a calculated skin dose of 3 Gy.

Experimental animals were divided into five groups of from 8 to 16 rats each. Two hours, one, three, seven, and 14 days after irradiation, animals were killed by cervical dislocation. Seventy-nine rats were used as non-irradiated controls, which were of the same strain, sex, and age and which were fed in the same manner.

The lipid peroxide level in the blood plasma was determined according to Yagi (1976), with the thiobarbituric acid (TBA) method using tetramethoxypropane (1, 1, 3, 3-tetramethoxypropane, Tokyo Kasei Kogyo Co., Tokyo) as an external standard. Blood was drawn directly from the heart, and 0.1 mL was put into 2.0 mL of 0.9% NaCl in a centrifuge tube and shaken gently. After centrifugation, 4.0 mL of 0.167 mol/L H₂SO₄ was added to 0.5 mL of supernatant, and 0.5 mL of 10% phosphotungstic acid was added prior to mixing. After it had stood at room temperature for five min, the mixture was centrifuged. The sediment was mixed with 2.0 mL of 0.167 mol/L H₂SO₄ and 0.3 mL of 10% phosphotungstic acid and was centrifuged. To the sediment, 4.0 mL of distilled water and 1.0 mL of TBA reagent (0.67% aqueous TBA (BDH Chemicals Co., Poole, England) + glacial acetic acid 1:1, v/v) were added. The reaction mixture was heated at 95°C for 60 min in an oil bath. After being cooled with tap water, 5 mL of n-butanol was added, and the mixture was shaken vigorously and then centrifuged. The n-butanol layer was taken for fluorometric measurement (515 nm excitation and 553 nm emission).

The lipid peroxide level in tissues was determined according to Ohkawa *et al.* (1979), with slight modification. Submandibular glands were weighed and homogenized in 4.0 mL of 0.9% w/v NaCl with a Potter-Elvehjem homogenizer. To 0.5 mL of homogenate, 3.5 mL of distilled water and 1.0 mL TBA reagent were added. Livers were perfused with ice-cold 0.9% NaCl *via* the portal vein, and then homogenized in 0.9% NaCl with an ultra-trax homogenizer. The homogenate was adjusted to a tissue concentration of 10%. To 0.2 mL of homogenate, 3.8 mL of distilled water and 1.0 mL of the TBA reagent were added. These reaction mixtures were heated at 95°C for 60 min in an oil bath. After being cooled with tap water, 5.0 mL of n-butanol was added, the mixtures were shaken vigorously and then centrifuged. The absorbance at 532 nm of the n-butanol layer was measured. The lipid peroxide concentration was calculated in terms of nmol malondialdehyde/mL of blood or g of tissue wet weight.

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TABLE
CHANGES IN LIPID PEROXIDE LEVEL AFTER X-RAY IRRADIATION

Tissue	Lipid Peroxide Level ^a				
	2 Hrs	1st Day	3rd Day	7th Day	14th Day
Submandibular Gland	116.7 ± 9.0	103.1 ± 9.4	108.9 ± 5.8	116.3 ± 5.0 ^b	107.2 ± 10.5
Liver	108.9 ± 10.1	105.2 ± 20.6	89.9 ± 13.7	80.7 ± 7.8 ^b	96.2 ± 18.2
Blood Plasma	140.1 ± 14.7 ^b	99.6 ± 12.0	121.9 ± 12.3	124.5 ± 9.6 ^b	131.7 ± 10.8 ^c

The dose of x-ray irradiation was 3 Gy. Values are means ± SE for from nine to 16 rats.

^aLipid peroxide levels are expressed as the percentage of those of age-matched rats without irradiation.

^bp<0.05; ^cp<0.01.

Histological observations on the irradiated submandibular gland were performed by light microscope after H&E staining.

Significance of differences in the lipid peroxide level between irradiated group and age-matched control was determined by Student's *t* test.

Results.

The Table shows lipid peroxide levels in the irradiated rats as a percentage of the age-matched controls.

The lipid peroxide levels in the irradiated submandibular glands were higher than the control values. However, there was a significant difference between the irradiated group and age-matched controls only at seven days after irradiation.

The lipid peroxide levels in the livers of irradiated animals tended to be lower than those in the controls. A significant difference was observed only at seven days after irradiation.

The lipid peroxide levels in the blood plasma of the irradiated group were generally higher than those of controls. Significant differences were seen at two hours, seven days, and 14 days after irradiation.

Neither degeneration, as cellular atrophy or vacuolization, nor inflammation was observed in any irradiated submandibular gland during the experimental periods. However, slight pyknosis and anomalous nuclei in both serous and mucous acinar cells were seen within three days after irradiation.

Discussion.

The present results demonstrate that a low dose of x-ray irradiation to the neck regions of rats caused an elevation in the lipid peroxide level in the submandibular gland and in the blood.

Sholley *et al.* (1974) and Miyoshi (1973) reported that the onset of radiation injury in the salivary gland was seen immediately or (at the latest) within seven days following irradiation. Although marked histological changes, such as atrophy or vacuolization of acinar cells, in the irradiated submandibular gland are considered to require relatively high doses of x-ray irradiation, a low dose of x-ray irradiation was applied in the present study compared with other experimental studies for radiation effects on the submandibular gland. For this reason, histological changes were slight; however, they did appear within three days after irradiation. Likewise, the lipid peroxide levels were elevated simultaneously in the submandibular gland and blood soon after irradiation. These results suggest that the radiation injury develops concomitantly with the elevation of lipid peroxide level in the submandibular gland, and it affects the increased level in the blood by leakage from the damaged gland. This is essentially in agreement with the study of Yagi *et al.* (1977), who showed that the elevation of lipid peroxide

in the retina coincided with the elevation of lipid peroxide in the blood in model experiments for the retinopathy of prematurity. Consequently, the lipid peroxide level in the blood is thought to indicate damage in the irradiated site.

On the other hand, the lipid peroxide levels in the liver were lower than control values. It is difficult to provide the appropriate explanation of why the lipid peroxide in the liver is decreased after irradiation. Wills (1970) suggested that enhancement of the rate of peroxidation was explained not only on the basis of a direct free-radical attack, but also by a possibility for protective anti-oxidant being destroyed by irradiation. Since the neck region was irradiated locally in our experiments, the protective functions for lipid peroxidation in the liver should not have been affected by irradiation. A postulate may be made that the catabolizing process for lipid peroxide (*i.e.*, glutathione, superoxide dismutase, or vitamin E system) is enhanced by the high level of lipid peroxide in the blood, and therefore the lipid peroxide level in the liver may be decreased after irradiation. Further studies into this problem are necessary.

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