Chapter 30

Liver Carcinogenesis and 8-Hydroxy-deoxyguanosin Formation by Oxidized Lard and Dietary Oils

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Oxidized lard and dietary oils (soybean oil and sardine oil) were orally administered to C3H/HeN mice in order to examine the role of oxidized oils in cancer formation. After 12 months, oxidized sardine oil caused the highest tumor incidence among the oxidized dietary oils tested. The amounts of 8-hydroxy-deoxyguanosine (8-OH-dG) in the mice treated with oxidized sardine oil correlated with the rates of tumor incidence. The lard and sardine oil-fed groups exhibited significant increases of 8-oxo-dGTPase mRNA levels. The lard-fed group alone showed a slight increase of the expression levels of OGG1 mRNA, but generally the expression level was not changed by addition of oxidized fat or oils. Oxidized lard and dietary oils might be harmful for our health because of the increased risk of liver carcinogenesis related to 8-OH-dG formation. It is preferable to avoid re-used oils.

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Certain fats and dietary oils are used in large quantities in food preparation and consequently become components of foods and are ingested by people. Therefore, the toxicity of deteriorated or oxidized fats and oils has received much attention among not only food chemists but also consumers.

There have been numerous reports on the toxicity of oxidized fats and oils since the 1950s (*1*). In particular, heat-treated oils, such as oils used in cooking, have been known to cause various adverse effects, including organotoxicity of the internal organs (*2*) and reproductive toxicity (*3*). Dietary oxidized frying oil reportedly up-regulates the expression of PPARα in downstream genes and alters lipid metabolism in rats (*4*). High intakes of a mixture of lard and oxidized cod liver oil caused biological implications in rats (*5*). The toxicity of oxidized fats and oils is caused by oxidative secondary products because they are readily absorbed by the intestines (*6*). Among the many products formed in oxidized fats and oils, formaldehyde, acetaldehyde, acrolein, malonaldehyde (MA), glyoxal, and methylglyoxal have received much attention as chemicals implicated in various diseases (*7*).

In a previous study (*8*), oxidized dietary oils of lard, soybean, and particularly sardine were shown to increase spontaneous liver tumor development and the formation of 8-OH-dG in the liver DNA of C3H/HeN male mice. Therefore, it was suggested that the progression of liver tumors promoted by oxidized dietary oils was due to 8-OH-dG accumulated in the liver DNA and that the accumulation of 8-hydroxy-deoxyguanosine (8-OH-dG) is associated with DNA mutation and cancer promotion (*9*).

In the present study, oxidized lard and dietary oils (soybean and sardine) were orally administered to C3H/HeN mice. The formation of 8-OH-dG and mRNA of DNA repair enzymes (8-oxo guanine DNA glycosylase 1, OGG1, and 8-oxo dGTPase) in the livers was subsequently investigated.

Materials and Methods

Experimental Animals

C3H/HeN male mice (5 weeks of age) were purchased from Charles River Japan, Inc. (Kanagawa, Japan). Body weight and absence of infection were checked for 1 week. The C3H/HeN mice were divided into four groups (n = 8). The mice were fed four different diets (F-2, lard, soybean, and sardine) for 6 or 12 months by a previously reported method (*8*). Mice were housed in plastic cages lined with soft wood chips. The cages were placed in a conventional room, which was air conditioned at 23 °C and 55-70% humidity with a light/dark (12 h/12 h) cycle. The study adhered to the U.S. National Institutes of Health

guidelines for the use of experimental animals. The animal care method was approved by the Animal Care and Use Committee at Oita University of Nursing and Health Science in Oita, Japan.

Diets

The four diets used were commercial F-2 diet containing 4% general fats (Funabashi Farm Co., Ltd., Chiba, Japan); F-2 diet with 4% oxidized lard added; F-2 diet with 4% oxidized soybean oil added; and F-2 diet with 4% oxidized sardine oil added. All oxidized dietary oils tested contained various levels of secondary lipid peroxidation products as reported previously (*8*). Water was accessible *ad libitum*. The F-2 diet (34% of calories derived from fat) totaled 359 kcal/100 g. These pellet-diets were prepared by the Funabashi Farm Co., Ltd. (Chiba, Japan) and were vacuum packed and sealed in vinyl bags. They were stored at 4 °C until use. The special diet was changed once every two days.

Preparation of Oxidized Lard, Soybean Oil, and Sardine Oil

The dietary oils were oxidized by a previously reported method (*10*). Oils were placed in a round-bottom flask. The flask was rotated using a rotary evaporator at 37 °C for 15 days to allow the oils complete contact with the air. The oxidized oils were stored at 5 °C until used for animal studies and analysis for toxic dicarbonyl compounds.

Measurement of 8-OH-dG in Liver DNA, RNA in 8-Oxoquanine-DNA Glycosylase (OGGI), and 8-Oxo-dGTPase

Levels of 8-OH-dG in Liver DNA, RNA in 8-Oxoquanine-DNA glycosylase (OGGI), and 8-Oxo-dGTPase were measured using previously reported methods (*11*). Eight mice from each group were used for the measurement of 8-OH-dG levels. The total RNA was isolated from the livers of the 8 mice used for 8-OH-dG analysis.

Statistical Analysis

The statistical analysis of body weight, expression of repair enzymes mRNA, and amount of 8-OH-dG in each strain were performed using a one-way ANOVA with post-hoc Bonferroni/Dunn-test. The unpaired t-test was used for the statistical comparison with the results between C3H/HeN and C57BL/6.

Differences among the groups were considered statistically significant at a level of $p < 0.05$. Data are shown as mean ± SD.

Results and Discussion

Table I shows the spontaneous mortality rate of the experimental animals. After 6 months, the mortality rate in the soybean and sardine groups (3.3 and 6.6%, respectively) were higher than those in the F-2 (0%) and in lard (0%) groups. After 12 months, the spontaneous mortality rates increased significantly. Almost half of the mice in the lard group (45%) and the soybean group (43%) died after 12 months, whereas the mortality rates of the F-2 group (10%) and the sardine group (18%) were much lower.

Table I. Rate of Tumor Incidence in the Livers of Experimental Mice after 12 Months

	Number of mice with tumor and incidence rate (%)		
Diet	Total	Benign	Malignant
F-2	7 (17.5)	4 (10)	3 (7.5)
Lard	13 (32.5)	6 (15)	7 (17.5)
Soybean	10 (25.0)	3 (7.5)	7 (17.5)
Sardine	14 (35.0)	3 (7.5)	11 (27.5)[a]

[a] $p < 0.05$ vs F-2 group.

Table II shows the rate of tumor incidence in the livers of experimental mice after 12 months. The highest total tumor incidence was in the sardine (35%) group, followed by the lard (32.5%) group. However, neither of them was statistically significant relative to the F-2 control group. Malignant tumor incidence (7 – 11%) was higher than benign tumor incidence (3 – 6%) except in the case of the F-2 group. The results of the 6 months experiment are not shown, but benign tumors were found in all groups. The tumor incidence for the 6 months experiment was lower that that of the12 months experiment and the tumor sizes were smaller than those from 12 months.

The 8-OH-dG content in the liver DNA after 6 and 12 months is shown in Figure 1. The levels of 8-OH-dG increased significantly in the sardine-fed group ($p < 0.001$ vs F-2 group), which had the highest rate of malignant tumor

Table II. Mortality Rate of Experimental Mice

Diet	Mortality rate (%)	
	6 Months	12 Months
F-2	0	10
Lard	0	45[a]
Soybean	3.3	43[a]
Sardine	6.6	18

[a]$p < 0.001$ vs F-2 group.

incidence (Table II). The results indicate that there is a correlation between the level of 8-OH-dG formation and rates of tumor incidence. The soybean oil-fed group exhibited moderate increase ($p < 0.05$ vs F-2 group) after 12 months but did not show appreciable increase after 6 months. The lard-fed group showed over 30% increase both after 6 and 12 months ($p < 0.01$ vs F-2 group). Also, there has been clear evidence that the accumulation of 8-OH-dG in DNA is closely correlated with carcinogenesis (*12-14*).

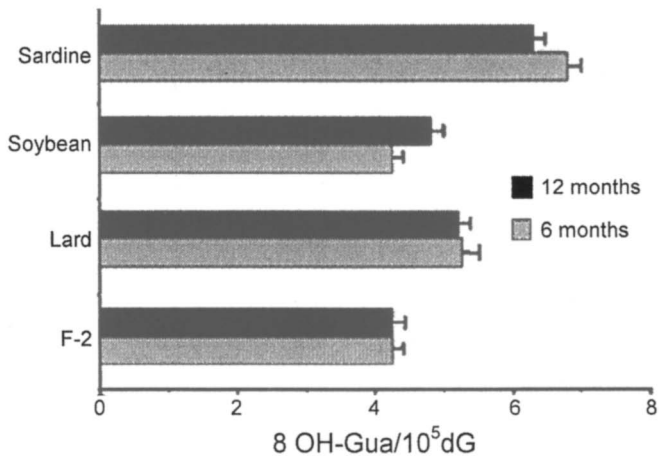

Figure 1. The level of 8-OH-dG in liver DNA from C3H/HeN mice fed with F-2 diet and three different oxidized oils. The values were mean ± SD (n = 8). The content of 8-OH-Gua in DNA was expressed as the ratio of 8-OHdG × 10^5 to total dG.

The expression levels of 8-oxo-dGTPase mRNA and OGG1 mRNA in the livers are shown in Figure 2.

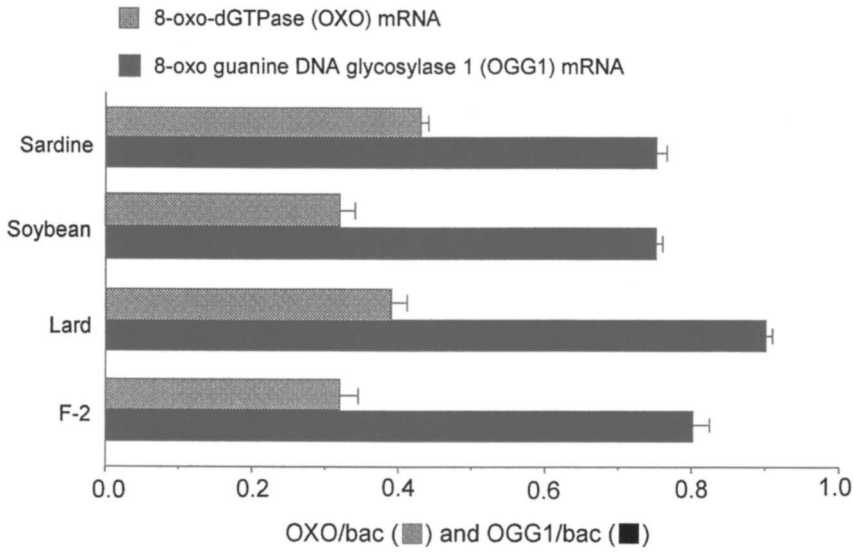

Figure 2. The expression of 8-oxo-guanine-DNA glycosylase 1 (OGG1) mRNA and 8-oxo-dGTPase mRNA in the livers from C3H/HeN mice fed with F-2 diet and three different oxidized oils. The values were mean ± SD (n = 8).

The expression levels of 8-oxo-dGTPase mRNA relative to the control (F-2-fed group) were 123% in the lard-fed group, 100% in the soybean oil-fed group, and 134% in the sardine oil-fed group. The lard and sardine oil-fed groups exhibited significant increases of 8-oxo-dGTPase mRNA levels. The expression levels of OGG1 mRNA relative to the control (F-2-fed group) were 113% in the lard-fed group, 94% in the soybean oil-fed group, and 94% in the sardine oil-fed group. Only the lard-fed group showed slight increase of the expression levels of OGG1 mRNA but generally the expression level was not changed by the addition of oxidized fat or oils. Decreasing mRNA in OGG1 and 8-oxo-dGTPase by oxidized lard and sardine oil caused the accumulation of 8-OH-dG in liver DNA, which might promote spontaneous liver tumorigenesis after 12 months (*8*). In the present study, the low response of the DNA repair enzyme system in C3H/HeN against oxidized dietary oils might promote accumulation of 8-OH-dG in DNA, and consequently cause a high susceptibility toward liver tumorigenesis in this strain.

References

1. Benes, V.; Sedlacek, B. A. J. *J. Hygi. Epid. Microbiol. Immun.* **1959**, *3*, 96-105.
2. Ebong, P. E.; Owu, D. U.; Isong, E. U. *Plant Foods Human Nutr.* **1999**, *53*, 209-222.
3. Isong, E. U.; Ebong, P. E.; Ifon, E. T.; Umoh, I. B.; Eka, O. U. *Plant Foods Human Nutr.* **1997**, *51*, 159-166.
4. Chao, P.-M.; Chao, C.-Y.; Lin, F.-J.; Huang, C.-J. *J. Nutr.* **2001**, *131*, 3166-3174.
5. Zidkova, J.; Sajdok, J.; Kontrava, K.; Kotrbova-Kozak, A.; Hanis, T.; Zidek, V.; Fucikova, A. *Czech. J. Food Sci.* **2004**, *22*, 108-120.
6. Frankel, E. N.; Neff, W.E. *Biochim. Biophys. Acta* **1983**, *754*, 264-270.
7. Okado-Matsumoto, A. I.; Fridovich, J. *Biol. Chem.* **2000**, *275*, 34853-34857.
8. Ichinose, T.; Nobuyuki, S.; Takano, H.; Abe, M.; Sadakane, K.; Yanagisawa, R.; Ochi, H.; Fujioka, K.; Lee, K.-G.; Shibamoto, T. *Food Chem. Toxicol.* **2004**, *42*, 1795-1803.
9. Wei, L.; Wei, H.; Frenkel, K. *Carcinogenesis* **1993**, *14*, 841-847.
10. Kaneda, T.; Sakai, H.; Ishii, S. *J. Biochem.* **1955**, *42*, 561-565.
11. Sadakane, K.; Ichinose, T.; Takano, H.; Abe, M.; Sera, N.; Yanagisawa, R.; Ochi, H.; Fujioka, K.; Lee, K.-G.; Shibamoto, T. *Food Chem. Toxicol.* **2006**, *44*, 1372-1376.
12. Takeuchi, T.; Morimoto, K. *Carcinogenesis* **1994**, *15*, 645-639.
13. Salim, E. I.; Wanibuchi, H.; Morimura, K.; Murai, T.; Makino, S.; Nomura, T.; Fukushima, S. *Carcinogenesis* **2002**, *23*, 1519-1529.
14. Schurkes, C.; Brock, W.; Abel, J.; Unfried, K. *Mutat. Res.* **2004**, *553*, 59-65.