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An in vivo Study of Lipid Peroxidation in Rats under Conditions of Oxidative Stress and the Antioxidant Effects of Probucol

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The purpose of this study was to investigate in vivo lipid peroxidation in rats under conditions of streptozotocin-induced oxidative stress and the antioxidant effects of probucol. In vivo lipid peroxidation was observed by measuring low molecular weight aldehydes and related carbonyl compounds in rat urine. Three groups of male Wistar rats weighing 165-190g were used: normal (N), streptozotocin-induced oxidative stress (OS) and oxidative stress plus probucol treatment (P). Following streptozotocin treatment of the rats, a variety of secondary lipid peroxidation products were increased. The levels of butanal, hexanal, hex-2-enal, hept-2-enal, octanal, non-2-enal, deca-2,4-dienal, 4-hydroxyhex-2-enal, 4-hydroxynon-2-enal, malondialdehyde(MDA), and unknown carbonyl compounds were significantly increased in the oxidative stress group compared to the control group. Treatment with probucol resulted in significant decreases in butnal, hexanal, hex-2-enal, octanal, deca-2,4-dienal, 4-hydroxyhex-2-enal, MDA and unknown carbonyl compounds. Hept-2-enal, hepta-2,4-dienal and non-2-enal appeared to have a tendency to decrease due to pobucol treatment.

Key words : oxidative stress, low molecular weight aldehydes, carbonyl compounds, probucol

INTRODUCTION

Free radical species that contain one or more unpaired electrons can be generated in cells and tissues by endogenous metabolic processes or exogenous compounds. Sub-cellular organelles are important contributors to intracellular production of a wide variety of free radicals and oxygen-derived radicals.[1,2] The accumulation of these species can become highly destructive to certain cells and tissues when there is an imbalance between the free radicals generated and the protective mechanisms that remove them. In addition, leakage of reactive oxygen species and related free radicals from the cell can cause oxidative damage to biological molecules such as lipids, proteins and DNA.[3,4]

The phospholipid component of cellular membranes is highly vulnerable to the activities of reactive free radicals. Oxidation of polyunsaturated fatty acids (PUFA) in the phospholipid bilayer can induce cellular injury and even cell death because of the modification and disintegration of biomembranes.[5] In addition, some oxidation products of PUFA have shown to possess high reactivity and cytotoxicity towards biomolecules. Recently, low molecular weight aldehydes and related carbonyl compounds have been identified in the urine of normal rats without the induction of oxidative stress.[6] These secondary lipid peroxidation products, in addition to malondialdehyde, appeared to be capable of damaging functional proteins, inhibiting enzyme acitivities, and causing cell lysis and loss of cellular reproductive integrity.[7,8] Some degenerative diseases and cellular aging processes have been suggested as outcomes associated with lipid peroxidation and the cytotoxic action of these aldehydes.[9,10]

The level of susceptibility to in vivo free radical-induced peroxidation or related damage to organ systems is associated with the overall balance between oxidative stress and the antioxidant potential of tissues. To cope with the injurious potential of free radicals including reactive oxygen species, biological systems possess several effective antioxidant defense systems which use enzymatic and nonenzymatic biological antioxidants.[11] Although no single antioxidant can be used for all kinds of diseases in which reactive oxygen species are involved, the possible involvement of free radicals in the pathogenesis of certain diseases has promoted a renewed interest in biological or synthetic antioxidants.

Probucol, a drug widely used in the treatment of hypercholesterolaemia, is known to possess antioxidant properties.[12] Its two phenolic hydroxyl groups appear to make probucol a powerful synthetic antioxidant. In vitro experiments suggested that probucol prevented the generation of lipid free radicals and the accumulation of reactive oxygen metabolites in oxidizing LDL[13] and renal tissues.[14] Other studies have reported that probucol

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appears to scavenge reactive free radicals by reacting with their phenolic hydrogens and preventing damage to vulnerable PUFA in biomembranes.[15] These characteristics enable probucol to demonstrate antioxidant activity and prevent lipid peroxidation in biological systems.

In the present animal experiment, the first objective was to evaluate the conditions of streptozotocin-induced oxidative stress by measuring urinary lipid peroxidation products such as low molecular weight aldehydes and related carbonyl compounds. Since streptozotocin is a strong oxidizing agent, this chemical may exert its damaging effects on pancreatic β-cells by generating reactive oxygen species.[16] The generation of these oxygen radicals may overwhelm antioxidant defenses and result in oxidative destruction of cellular membranes and serious tissue damage. The second objective was to investigate the protective effects of a phenolic antioxidant such as probucol by measuring in vivo formation of the secondary lipid peroxidation products. On a molar basis, studies have shown that probucol appeared at least as potent as α-tocopherol in preventing oxidative modification and lipid peroxidation in vitro.[17] Therefore, in the current study, probucol treatment may protect from streptozotocin-induced oxidative damage in rats. The low molecular weight aldehydes and related carbonyl compounds in rat urine were measured by the HPLC method.[6]

MATERIALS AND METHODS

Animals and diets

Three groups of male Wistar rats weighing 165-190g (7 animals/group) were used: normal (N), streptozotocin-induced oxidative stress (OS) and oxidative stress plus probucol treatment (P). All groups were fed the standard AIN-76 diet (Table 1). After adaptation to the diet for 4 days, animals in the OS and P groups were injected intraperitioneally with streptozotocin (40mg/kg body weight in 0.1 M citrate buffer, pH 5.5). The animals in the N group were given sham injections of citrate buffer only. After confirmation of diabetes by testing for glucose in the urine with glucose oxidase-impregnated strips, the P group was intraperitioneally injected with probucol on three consecutive days, and a fourth injection given two weeks later. Animals in the P group received 81mg probucol dissolved in 0.5ml of vacuum-distilled corn oil. At the end of the 7 week experimental period, urine was collected for 24 hours from each animal to detect any secondary lipid peroxidation products such as low molecular weight aldehydes and related carbonyl compounds.

Table 1. Composition of the standard AIN-76

Ingredients	%Composition
Corn starch[1]	45.0
Sucrose[2]	15.0
Cellulose[3]	5.0
Casein[3]	20.0
Corn oi[4,5]l	10.0
Mineral mixture[3,6]	3.5
Vitamin mixture[3,7]	1.0
DL-Methionine[3]	0.3
Choline bitartrate[3]	0.2

[1]CPC Food Service, Englewood Cliffs, NJ
[2]Crystal Sugar Corp., Moore head
[3]United States Biochemical Corp., Cleveland, OH
[4]Holsum Foods, Waukeshs, WI
[5]BHT was added as 0.02% of the corn oil
[6]AIN-76 Mineral mixture 10664. AIN-76 Vitamin mixture 10663.

Measurement of low molecular weight aldehydes and related carbonyl compounds

The low molecular weight aldehydes and related carbonyl compounds were measured as 2,4-dinitrophenyhydrazine(DNPH) derivatives in the urine of rats by the newly developed HPLC method.[6] The measurement of thiobarbituric acid reactive substances (TBARS) was performed by a modification of Yagi's method.[18] The amounts of TBARS were calculated as malondialdehyde (MDA) equivalents.

Statistical analysis

Data were presented as mean ± SEM. Comparisons between the N, OS and P groups were performed by ANOVA coupled with the Student-Newman-Keuls Method. A p-value of less than 0.05 was considered significant for all analyses.

RESULTS

In vivo formation of secondary lipid peroxidation products such as low molecular weight aldehydes and related carbonyl compounds was measured by the method of Kim et al.6) A number of alkanals (alk-2-enal, alk-2,4-dienal, and 4-hydroxyalk-2-enal), and related carbonyl compounds, were separated from rat urine and some of these were identified by HPLC. Various carbonyl compounds, measured as 2,4-DNPH derivatives at 378nm, from rats in the N, OS and P groups were expressed as mean area per day per 100g body weight.

Low molecular weight aldehydes and related nonpolar carbonyl compounds from rat urine were identified by co-chromatography in three different solvent systems and compared with pure standards, as indicated in Table 2. The identified compounds were butanal, hexanal, hex-2-enal, hept-2-enal, hepta-2,4-dienal, octanal, non-2-enal

Table 2. Production of low molecular weight aldehydes and related nonpolar carbonyl compounds generated from rat urine

Compounds / Groups	Normal	Oxidative stress	Probucol-treated oxidative stress
Butanal	1.784 ± 0.161[a]	15.689 ± 2.441[b]	5.253 ± 1.667[ac]
Hexanal	1.620 ± 0.648[a]	40.031 ± 17.671[b]	3.301 ± 1.319[ac]
Hex-2-enal	2.738 ± 1.535[a]	10.119 ± 2.301[b]	0.537 ± 0.161[ac]
Hept-2-enal	0.191 ± 0.029[a]	7.731 ± 3.812[b]	1.799 ± 0.328[ab]
Hepta-2,4-dienal	9.618 ± 2.943	17.795 ± 3.486	12.129 ± 2.583
Octanal	1.453 ± 0.465[a]	15.116 ± 3.561[b]	2.257 ± 0.551[ac]
Non-2-enal	0.508 ± 0.176[a]	2.358 ± 0.651[b]	1.167 ± 0.283[ab]
deca-2,4-dienal	0.289 ± 0.076[a]	140601 ± 3.482[b]	1.680 ± 0.483[ac]
Unknown compounds	3.611 ± 0.597[a]	33.137 ± 6.583[b]	6.683 ± 1.801[ac]

1) Values represent mean ± SEM of 6-7 animals per group.
2) The compounds are expressed as the peak area × 10^6 per day per 100g body weight.
3) Different letters denote significant differences($p < 0.05$) among group means by Student-Newman-Keuls method.

Table 3. Production of 4-hydroxyalk-2-enals and related polar carbonyl compounds generated from rat urine

Compounds / Groups	Normal	Oxidative stress	Probucol-treated oxidative stress
4-Hydroxyhex-2-enal	0.234 ± 0.025[a]	14.972 ± 5.344[b]	2.723 ± 1.319[ac]
4-Hydroxynon-2-enal	1.718 ± 0.387[a]	4.730 ± 0.948[b]	5.633 ± 1.090[b]
Unknown compounds	0.636 ± 0.131[a]	2.796 ± 0.470[b]	2.145 ± 0.380[b]

1) All values are mean ± SEM of 6-7 animals per group.
2) The compounds are expressed as the peak area × 10^7 per day per 100g body weight.
3) Different letters denote significant differences($p < 0.05$) among group means by Student-Newman-Keuls method.

Table 4. Production of total nonpolar and polar carbonyl compounds generated from rat urine

Compounds / Groups	Normal	Oxidative stress	Probucol-treated oxidative stress
Nonpolar compounds	0.613 ± 0.088a	4.965 ± 0.697b	1.553 ± 0.373ac
Polar compounds	0.985 ± 0.142a	66.640 ± 23.663b	17.383 ± 8.358ac

1) All values are mean ± SEM of 6-7 animals per group.
2) The compounds are expressed as the peak area × 10^8 per day per 100g body weight.
3) Different letters denote significant differences($p < 0.05$) among group means by Student-Newman-Keuls method.

and deca-2,4-dienal. Some of nonpolar carbonyl compounds were separated but not identified in the present experiments because of a lack of adequate standards. Further examination is needed for the identification of these compounds.

Various peroxidation products were detected in the normal rats without the induction of oxidative stress. Rats in the N group excreted higher levels of hepta-2,4-dienal than other compounds. Butanal, hexanal, hex-2-enal, hept-2-enal, octanal, non-2-enal, deca-2,4-dienal, and unknown compounds, were generated at higher levels in the OS group than in the N group. There was no statistical difference between the N and OS groups for the formation of hepta-2,4-dienal. However, rats in the OS group tended to excrete a higher amount of hepta-2,4-dienal than the animals in the N group. Probucol treatment of oxidatively-stressed rats resulted in significant decreases in butanal, hexanal, hex-2-enal, octanal, deca-2,4-dienal and other unknown compounds. Hept-2-enal, hepta-2,4-dienal and non-2-enal appeared to decrease in the P group when compared to the OS group, although there were no statistical differences in these compounds between the two groups.

Levels of 4-hydroxyalk-2-enals and related polar carbonyl compounds were elevated in the OS group compared to the N group, as shown in Table 3. 4-Hydroxyhex-2-enal(HHE),4-hydroxynon-2-enal(HNE) and related unknown compounds were significantly increased in the OS group compared to the N group. Probucol treatment resulted in significant decreases in HHE in the P group. Production of HNE and other unknown compounds appeared to decrease in the P group. However, no statistical differences in these carbonyl compounds were found between the OS and P groups.

The results for total nonpolar and polar carbonyl compounds in the N, OS and P groups are presented in Table 4. Total nonpolar and polar carbonyl compounds

were significantly increased in the OS group relative to the N group. Probucol treatment of rats under oxidative stress significantly reduced the levels of nonpolar and polar carbonyl compounds.

Urinary TBARS levels in the three groups were presented as MDA equivalents, as shown in Figure 1. The excretion of MDA was significantly increased in the OS group compared to that of the N group. Rats treated with probucol showed significantly decreased levels of MDA relative to the OS group.

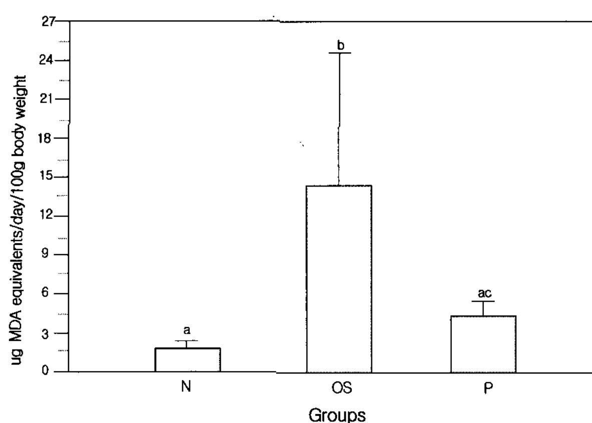

Fig. 1. Measurement of urinary MDA from rats in normal condition(N), oxidative stress(OS) and probucol-treated oxidative stress(P) Values represent mean + SEM of 6-7 ani mals per group. Different letters denote significant differences ($p<0.05$) between groups by Student-Newman Keuls method.

DISCUSSION

The present study found significant increases in low molecular weight aldehydes and related carbonyl compounds in the urine of rats under conditions of streptozotocin-induced oxidative stress. Since streptozo- tocin is a strong oxidizing agent, peroxidative damage to the membrane lipids of the rats is assumed to have occurred. A number of nonpolar and polar aldehydes found in this study were also found in microsomes peroxidized by ADP-Fe^{2+}.[19] This may indicate that the in vivo process of lipid peroxidation is similar to that induced in vitro. Similar short-chain carbonyl com-pounds, identified from secondary lipid peroxidation products, were separated and characterized by other investigators.[20,21]

Although the exact mechanisms for the formation of low molecular weight aldehydes have not been fully elucidated, the main mechanism is considered to be the so-called β-cleavage reaction of lipid alkoxyl radicals.[22] For instance, hexanal is the dismutation product of 13-OOH-linoleic-, 13-OOH-γ-linolenic- and 15-OOH-arachidonic acid.[23] 2,4-Decadienal is a breakdown product from the β-scission of 9-OOH-linoleic acid. Autoxidation of 2,4-decadienal can also produce hexanal.[24] The mechanisms of the formation of hydroxy aldehydes are more complex and have not been experimentally verified. However, it has been suggested that the major 4-hydroxyalkenal, HNE, can be formed from ω-6 PUFA.[25] HHE, an analogous product of HNE, was identified from the degradation of docosahexaenoic acid.[26,27] Considering the findings of previous studies, saturated and unsaturated aldehydes or hydroxyaldehydes seem to be produced from ω-6 and ω-3 PUFA. As indicated in previous studies, aldehyde formation is complex, and there might be many routes by which aldehydes can be formed from a great number of different PUFAs in biomembranes.

Low molecular weight aldehydes and related carbonyl compounds found in the current experiment have been recently suggested to be responsible for the damaging effects associated with free radical initiated peroxidation processes in biological systems. In particular, α,β-unsaturated aldehydes appear to inhibit several biological functions such as DNA and RNA synthesis, respiration and glycolysis.[28-30] HHE, HNE and deca-2,4-dienal, identified in this study, are known to be highly toxic and possess hepatotoxic, mutagenic and genotoxic pro-per- ties.[31,32] Among the 4-hydroxyalkenals, the cytotoxic HNE was reported to be in foodstuffs and oils at levels of 1-7 nmol/g.[33] Oral intake of a high dose of HNE can cause serious renal or hepatic damage because the low molecular weight aldehydes are more readily ab- sorbed by the intestine and incorporated into internal organs than are lipid peroxides and polymers.[34] Another cytotoxic aldehyde (4,5-dihydroxy-2,3-decenal, isolated from in vitro oxidized rat liver microsomes) resulted in the inhibition of microsomal glucose-6-phosphatase and reactivity towards cystein, glutathione, and other thiols comparable to that exhibited by HNE.[35] Therefore, the reactive low molecular weight aldehydes generated as part of lipid peroxidation processes can play a central role in causing cellular damage and some lipid peroxi-dation-related pathologi- cal conditions. The identification of aldehyde- modified LDLs in in vitro studies, and confirmation of their atherogenic potential, strongly supports the hypothesis of a pathogenic role for lipid peroxidation.[36] Thermal injury, liver diseases, diabetes, vascular disorders, cancer, cataracts, and other diseases are also thought to be related to lipid peroxidation and the cytotoxic action of low molecular weight al- dehydes.[37]

In the present study, probucol treatment in rats under conditions of oxidative stress resulted in a significantly-decreased generation of secondary lipid peroxidation compounds. This result indicates that probucol may act as an efficient antioxidant by scavenging lipid-related free radicals and thus preventing the propagation of lipid peroxidation. Pryor et al.[38] reported

that probucol scavenged peroxyl radicals from the linoleic acid micellar system, and that its antioxidant activity was comparable to α-tocopherol. Although the mechanism responsible for the free radical scavenging action of probucol is not yet completely understood, this may involve reactions with its phenolic groups because probucol is a dimer of the antioxidant, butylated hydroxytoluene, connected by a sulfur-carbon-sulfur bridge.[12] Evidence for the role of probucol as antioxidant was also provided by Siveski-Iliskovic et al[39], who found that the probucol treatment of rats with adriamycin-induced cardiomyopathy resulted in increased myocardial glutathione peroxidase and superoxide dismutase activity, with a concomitant decrease in lipid peroxidation as measured by evaluating the MDA content. This result suggested that probucol may improve cardiac function by acting as an antioxidant as well as by improving the endogenous antioxidant status of the heart. Probucol was also found to inhibit increases in macrophage-stimulated H_2O_2 in the pancreatic β-cells of probucol-treated NOD mice.[40] Based on the above studies, probucol's protective role against oxidative damage seems to be due to its free radical scavenger activities, thereby preventing PUFA oxidation in biomembranes. The current experiment also found that, due to the antioxidant action of probucol, levels of secondary lipid peroxidation products were decreased in rats subjected to streptozotocin-induced oxidative stress.

Literature Cited

1) Moslen MT. Reactive oxygen species in normal physiology, cell injury and phagocytosis. *Adv Exp Med Biol* 366 : 17-27, 1994
2) Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reac- tions, and methods for their quantification. *Toxicol Pathol* 30 : 620-650, 2002
3) Pacifici RE, Davies KJA. Protein, lipid and DNA repair systems in oxidative stress : The free-radical theory of aging revisited. *Gerontol* 37 : 166-180, 1991
4) Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. *Toxicol* 27 : 181-182, 2002
5) Farber JL, Kyle ME, Coleman JB. Biology of disease: Mechanisms of cell injury by activated oxygen species. *Lab Invest* 62 : 670-679, 1990
6) Kim SS, Gallaher DD, Csallany AS. Lipophilic aldehydes and related carbonyl compounds in rat and human urine. *Lipids* 34(5) : 489-496, 1999
7) Comporti M. Lipid peroxidation: biopathological significance. *Molec Aspects med* 1 : 199-207, 1993
8) Park JE, Yang JH, Yoon SJ, Lee JH, Yang ES, Park JW. Lipid peroxidation-mediated cytotoxicity and DNA damage in U937 cells. *Biochimie* 84 : 1198-1204, 2002
9) Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. *Proc Natl Acad Sci* 90 : 7915-7922, 1993
10) Cakatay U, Telci A, Kayali R, Tekeli F, Akcay T, Sivas A. Relation of aging with oxidative protein damage parameters in the rat skeletal muscle. *Clin Biochem* 36 : 51-55, 2003
11) Kehrer JP. Free radicals as mediators of tissue injury and disease. *Crit Rev Toxicol* 23 : 21-48, 1993
12) Barnhart JW, Sefranka JA, Mcintosh DD. Hypocholesterolemic effect of 4,4-(isopropyli-dene- dithio)-bis (2,6-di-t-butylphenol) (probucol). *Am J Clin Nutr* 23 : 1229-1233, 1970
13) Modi KS, Morrissey J, Shah SV, Schreiner GF, Klahr S. Effects of probucol on renal function in rats with bilateral ureteral obstruction. *Kidney Int* 38 : 843-850, 1990
14) Naito MN, Yamada K, Hayashi T, Asai K, Yoshi- mine N, Iguchi A. Comparative toxicity of oxi- datively modified low-density lipoprotein and lysophosphatidylcholine in cultured vascular en- dothelial cells. *Heart Vessels* 9 : 183-187, 1994
15) Marshall FN. Pharmacology and toxicology of pro- bucol. *Artery* 10 : 7-21, 1982
16) Wolff SP. Diabetes mellitus and free radicals. *British Med Bull* 49 : 642-652, 1993
17) Gotoh N, Shimizu K, Komuro E, Tsuchiya J, Noguchi N, Niki E. Antioxidant activities of probucol against lipid peroxidation. *Biochim Biophys Acta* 1128 : 147-154, 1992
18) Yagi K. Assay for blood plasma or serum. *Method in Enzymol* 105 : 328-331, 1984
19) Esterbauer H, Cheeseman, KH, Dianzani MU, Poli G, Slater TF. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe^{2+} in rat liver microsomes. *Biochem J* 208 : 129-140, 1982
20) Buffinton GD, Hunt NH, Cowdden WB, Clark IA. Detection of short-chain carbonyl products of lipid peroxidation from malaria parasite-infected red blood cells exposed to oxidative stress. *Biochem J* 249 : 63-68, 1988
21) Poli G, Dianzani MU, Cheeseman KH, Slater TF, Lang J, Esterbauer H. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by carbon tetrachloride or ADP-iron isolated rat hepatocytes and rat liver microsomal suspensions. *Biochem J* 227 : 629-638, 1985
22) Esterbauer H, Zollner H. Methods for determination of aldehydic lipid peroxidation products. *Free Rad Biol Med* 7 : 197-203, 1989
23) Esterbauer H, Cheeseman KH, Dianzani MU, Poli G and Slater TF. Separation and characterization of the aldehydic products of lipid peroxidation sti- mulated by ADP-Fe^{2+} in rat liver microsomes. *Biochem J* 208 : 129-140, 1982
24) Schieberle P, Grosch W. Model experiments about the formation of volatile carbonyl compounds. *J Am Oil Chem Soc* 58 : 602-607, 1981
25) Pryor W, Porter N. Suggested mechanisms for the production of 4-hydroxy-2-nonenal from the autoxi- dation of polyun- saturated fatty acids. *Free Rad Biol Med* 8 : 541-543, 1990
26) Esterbauer H, Schaur RJ and Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonalde- hyde and related aldehydes. *Free Rad Biol Med* 11 : 81-128, 1991
27) Van Kuijk FJGM, Holte LL, Dratz EA. 4-Hydro- xyhexenal: a lipid peroxidation product derived from oxidized docosahexaenoic acid. *Biochim Biophys Acta* 1043 : 116-118,

1990
28) Schauenstein E and Esterbauer H. Aldehydes in bio- logical systems, pp. 25-88, Pion Limited, Lodon, 1977
29) Bartsch H, Nair J. Potential role of lipid peroxidation derived DNA damage in human colon carcino- genesis: studies on exocyclic base adducts as stable oxidative stress markers. *Cancer Detect Prev* 26 : 308-312, 2002
30) Zhang WH, Liu J, Xu G, Yuan Q, Sayre LM. Model Studies on protein side chain modification by 4- Oxo-2-nonenal. *Chem Res Toxicol* 16 : 512-523, 2003
31) Uchida K. 4-Hydroxy-2-nonenal: a product and me- diator of oxidative stress. *Prog Lipid Res* 42 : 318-343, 2003
32) Esterbauer H. Cytotoxicity and genotoxicity of lipid- oxidation products. *Am J Clin Nutr* 57 : 779S-786S, 1993
33) Lang J, Celotto C, Esterbauer H. Quantitative determination of the lipid peroxidation product 4-hydroxynonenal by high-performance liquid chromatography. *Anal Biochem* 150 : 239-378, 1985
34) Nishikawa A, Sodum R, Chung FL. Acute toxicity of trans-5-hydroxy-2-nonenal in Fisher 344 rats. *Lipids* 27 : 54-58, 1992
35) Benedetti A, Comporti M, Fulceri R, Esterbauer H. Cytotoxic aldehydes originating from the peroxi- datioin of liver microsomal lipids. *Biochim Biophys Acta* 792 : 172-181, 1984
36) Esterbauer H, Dieber-Rotheneder M, Waeg G, Striegl G, Jurgens G. Biochemical, structural, and functional properties of oxidized low-density lipoprotein. *Chem Res Toxicol* 3 : 77-92, 1990
37) Yagi, K. Lipid peroxides and human diseases. Chem Phys Lipids 45 : 337-351, 1987
38) Pryor W, Strickland T, Church DF. Comparison of the efficiencies of several natural and synthetic antioxidants in aqueous sodium dodecyl sulfate micelle solutions. *J Am Chem Soc* 110 : 2224-2229, 1988
39) Siveski-Iliskoviv N, Kaul N, Singal PK. Probucol promotes endogenous antioxidants and provides pro- tection against adriamycin-induced cardiomyopathy in rats. *Circulation* 89 : 2829-2835, 1994
40) Fukuda M, Ikegami H, Kawaguchi Y, Sano T, Ogi- hara T. Antioxidant, probucol, can inhibit the gen- eration of hydrogen peroxide in islet cells induced by macrophages and prevent islet cell destruction in NOD mice. *Biochem Biophys Res Commun* 209 : 953-958, 1995