Chapter 10
Immunochemical Detection of Lipid Hydroperoxide- and Aldehyde-Modified Proteins in Diseases

Akihiko Sugiyama and Jing Sun

Abstract Polyunsaturated fatty acid (PUFA) is easily peroxidized by free radicals and enzymes. When this occurs, it results in the compromised integrity of cellular membranes and leads to lipid hydroperoxide as a major reaction product, which is decomposed into aldehyde. Lipid hydroperoxide-modified lysine is known to be an early product of the lipid peroxidation process, suggesting that it might be a PUFA-oxidative stress marker during the initial stage of oxidative stress. Lipid hydroperoxides cause or enhance ROS-mediated DNA fragmentation. The α,β-unsaturated aldehydes are end products of PUFA peroxidation. They are highly reactive and readily attack and modify the protein amino acid residues into aldehyde-modified proteins. Lipid peroxidation-derived α,β-unsaturated aldehydes are capable of inducing cellular stress-responsive processes such as cell signaling and apoptosis. The lipid hydroperoxide- and aldehyde-modified proteins have been immunohistochemically detected in diverse pathological situations such as atherosclerosis, Alzheimer's disease, Parkinson's disease, and chemical material-induced liver injury and renal tubular injury in humans and experimental animals. These findings suggest that the expression of the lipid hydroperoxide- and aldehyde-modified proteins is closely associated with the pathogenesis of these diseases in humans and experimental animals.

Keywords Aldehyde • Immunohistochemistry • Lipid hydroperoxide • Oxidation • Polyunsaturated fatty acid

A. Sugiyama (✉) • J. Sun
Course of Veterinary Laboratory Medicine, School of Veterinary Medicine, Faculty of Agriculture, Tottori University, Minami 4-101 m Koyama-cho, Tottori 680-8553, Japan
e-mail: sugiyama@muses.tottori-u.ac.jp

Y. Kato (ed.), *Lipid Hydroperoxide-Derived Modification of Biomolecules*,
Subcellular Biochemistry 77, DOI 10.1007/978-94-007-7920-4_10,

1 Lipid Hydroperoxide- and Aldehyde-Modified Proteins

Polyunsaturated fatty acid (PUFA) is easily peroxidized by free radicals and enzymes. When this occurs, it results in compromised integrity of cellular membranes and leads to lipid hydroperoxide as a major reaction product, which is then decomposed into aldehyde (Kato and Osawa 2010).

Lipid hydroperoxides such as N^{ε}-hexanoyl, N^{ε}-propanoyl, N^{ε}-azelayl, N^{ε}-glutaroyl and N^{ε}-Succinyl can be generated from PUFA peroxidized by free radicals (Kato and Osawa 2010). These lipid hydroperoxides react with lysine to form lipid hydroperoxide-modified proteins such as N^{ε}-hexanoyl-modified lysine (HEL), N^{ε}-propanoyl-modified lysine (PRL), N^{ε}-azelayl-modified lysine (AZL), N^{ε}-glutaroyl-modified lysine (GLL) or N^{ε}-Succinyl-modified lysine (SUL) (Kato and Osawa 2010). Lipid hydroperoxide-modified lysine is an early product of the lipid peroxidation process, suggesting that it might be a PUFA-oxidative stress marker during the initial stage of oxidative stress (Kato and Osawa 2010). HEL and PRL are classified as a group of alkylamide-type adducts, whereas AZL, GLL and SUL are classified as carboxyalkylamide-type adducts (Kato and Osawa 2010). HEL is formed by the reaction of lysine with lipid hydroperoxide derived from linoleic acid or arachidonic acid (Kato et al. 1999). PRL is formed by the reaction of lysine with lipid hydroperoxide derived from docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) or α-linolenic acid (Hisaka et al. 2009). SUL is an N-acyl carboxylic adduct composed of a C4 unit from the COOH terminus of DHA (Kawai et al. 2006). AZL is formed by oxidized linoleic acid and α-linolenic acid (Kato and Osawa 2010), while GLL is formed by oxidized arachidonic acid and EPA (Kato and Osawa 2010). Lipid hydroperoxides cause or enhance ROS-mediated DNA fragmentation (Higuchi 2003).

The aldehydes such as acrolein, 4-hydroxy-2-nonenal (HNE), malondialdehyde (MDA), 4-hydroxyhexenal (HHE), crotonaldehyde (CRA), 4-oxo-2-nonenal (ONE), and glyoxal are end products of the peroxidation of PUFA (Kato and Osawa 2010). They are classified as α,β-unsaturated aldehydes, which are highly reactive and readily attack and modify the protein amino acid residues to protein-bound aldehydes such as acrolein-modified lysine, HHE-modified histidine, CRA-modified lysine and MDA-modified lysine (Kato and Osawa 2010; Uchida 2000). Lipid peroxidation-derived α,β-unsaturated aldehydes are capable of inducing cellular stress-responsive processes such as cell signaling and apoptosis (Lee et al. 2004; Liu et al. 2010). Acrolein is a potent alkylating agent that reacts with matrix tissue or cell surface proteins and alters the structure and function of matrix proteins (Uchida 1999). It also reduces the intracellular GSH level (Uchida 1999). These events activate stress-signaling pathways via protein phosphorylations (Uchida 1999). HNE demonstrates a wide range of biologic activities, including inhibition of protein and DNA synthesis, inactivation of enzymes, stimulation of phospholipase C, reduction of gap junction communication, and stimulation of neutrophil migration (Uchida 1999). HHE induces apoptosis of endothelial cells, which is mediated by the enhancement of apoptotic Bax and the suppression of anti-apoptotic Bcl-2 by peroxynitrite generation (Lee et al. 2004). HHE induces mitochondrial permeability transition (MPT), which

leads to the breakdown of the mitochondrial membrane potential, the inability to synthesize ATP, and finally cell death (Kim et al. 2003; Kristal et al. 1996). HHE also depletes neuronal glutathione (GSH) content and neuronal reactive oxygen species (ROS) in rat cerebral cortical neurons (Long et al. 2008). CRA can penetrate through the cell membrane and bind to GSH without any metabolic activation (Liu et al. 2010). A reduced GSH level leads to imbalance of cellular redox and causes increases of ROS and apoptosis (Liu et al. 2010). CRA-induced apoptosis is mediated via cytochrome c release and caspase cascade (Liu et al. 2010). CRA causes both apoptosis and necrosis, and there is a transition from apoptosis to necrosis corresponding with increasing the CRA concentration (Liu et al. 2010).

These lipid hydroperoxide- and aldehyde-modified proteins have been immunohistochemically detected in diverse diseases in both humans and experimental animals.

2 Atherosclerosis

In humans, atherosclerosis and its complications, i.e., myocardial infarction, stroke, and peripheral vascular diseases, are major causes of morbidity and mortality in the Western world (Grant Maxie and Robinson 2007). Atherosclerosis affects the large elastic arteries (aorta and iliac) and the large and medium muscular arteries (carotid, coronary and femoral). The essential lesion is the atheroma or fibrofatty plaque, which is a focal, raised, intimal plaque with a lipid core (mainly cholesterol and its esters) covered by a fibrous cap (Grant Maxie and Robinson 2007).

Multiple pathogenetic influences can contribute to the development of atherosclerosis (Grant Maxie and Robinson 2007). Oxidised low-density lipoprotein-cholesterol (ox-LDL) plays a major role in the initiation and progression of atherosclerosis (Mitra et al. 2011). Formed by oxidative stress, ox-LDL also triggers the generation of reactive oxygen species (ROS) from a variety of cell types, and contributes to oxidative stress (Mitra et al. 2011). Previous studies have demonstrated many risk factors for atherosclerosis that induce oxidative stress in the vessel wall, including smoking (Bernhard and Wang 2007), diabetes mellitus (Nicolls et al. 2007), dyslipidemia (Mügge et al. 1994), hypertension (Huang et al. 1998), and periodontitis (Ekuni et al. 2009). Lipid peroxidation is involved in the development of atherosclerosis. There is an increase in the levels of serum malondialdehyde in human patients with atherosclerosis and in the rat atherosclerosis model, compared to those of controls (Tamer et al. 2002; Ekuni et al. 2009). There is immunohistochemical evidence of HEL presence in atherosclerotic lesions of humans (Kato et al. 1999), cholesterol-fed rabbits (Fukuchi et al. 2008) and periodontitis model rats (Ekuni et al. 2009). PRL has been immunohistochemically detected in atherosclerotic lesions of hypercholesterolemic rabbits (Hisaka et al. 2009). The presence of AZL was also immunohistochemically detected in the atherosclerotic lesions of humans and rabbits (Kawai et al. 2003, 2004). Immunoexpression of acrolein-modified keyhole limpet hemocyanin (KLH) have

been detected in atherosclerotic lesions from a human aorta (Uchida et al. 1998). It is speculated that HEL, PRL, AZL and acrolein-modified protein may be involved in the pathogenesis of atherosclerosis.

3 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurologic disorder characterized clinically as a cognitive impairment that includes memory impairment and at least one of the following cognitive disturbances: aphasia, apraxia, agnosia, or disturbance in executive functioning (Zarkovic 2003). The pathology of AD is dominated by neuronal loss and the formation of amyloid-containing neuritic (senile) plaque and neurofibrillary tangles in the frontal cortex and hippocampus (Zarkovic 2003). Immunoreactive intensity of the HNE-histidine adduct in CA2, CA3 and CA4 sectors in the hippocampi was significantly higher in AD patients than in the controls (Fukuda et al. 2009). Strong immunoexpression of acrolein-modified KLH occurred in more than half of the neurofibrillary tangles in AD patients (Calingasan et al. 1999). These results show that pyramidal neurons in these sectors of hippocampi and the neurofibrillary of AD patients are prone to undergo lipid peroxidation. The production of cytotoxic products such as HNE and acrolein may be responsible for the pathogenesis of AD (Fukuda et al. 2009).

4 Parkinson's Disease

Parkinson's disease (PD) is a chronic progressive neurodegenerative movement disorder characterized by a profound and selective loss of nigrostriatal dopaminergic neurons in humans (Jenner 2003). Clinical signs of PD include motor impairments involving resting tremor, a slowing of physical movement, postural instability, gait difficulty, and rigidity (Jomova et al. 2010). The most striking pathological feature of PD is a progressive loss of dopaminergic neurons in the substantia nigra, leading to dopamine deficit in the striatum (Jomova et al. 2010). One of the pathological hallmarks of PD is the presence of intracellular inclusions of Lewy bodies that consist of aggregates of α-synuclein (Jomova et al. 2010). The toxic effects of α-synuclein include impaired endoplasmic reticulum (ER) to Golgi vesicular trafficking and ER stress, Golgi fragmentation, sequestration of anti-apoptotic proteins into aggregates, and the formation of pores on cellular membranes (Cooper et al. 2006).

Oxidative stress has been implicated as one of the important contributors to nigral cell death in PD (Yoritaka et al. 1996). In the previous study, a significantly higher proportion of nigral melanized neurons was positively immunostained for HNE-modified protein in PD than in the control patients (Yoritaka et al. 1996). Immunolocalization of HNE-adduct was demonstrated in Lewy bodies of PD and in

diffuse Lewy body disease (Castellani et al. 2002). The previous study revealed that in the dopamine neurons of the substantia nigra containing neuromelanin obtained from PD patients, acrolein-adduct co-localized with α-synuclein, which was then modified by acrolein with inhibition of proteasome activity (Shamamoto-Nagai et al. 2007). Those previous studies indicate that α,β-unsaturated aldehyde such as HNE and acrolein may be related to the pathogenesis of PD.

5 Carbon Tetrachloride-Induced Liver Injury

Carbon tetrachloride (CCl_4) once was widely used as a solvent, cleaner, and degreaser both for industrial and home use (Weber et al. 2003). Today CCl_4 proves itself useful in experimental models and induces liver injury in many species, including non-human primates (Yoshida et al. 1999), and humans (Weber et al. 2003). The liver is the principal site for CCl_4-induced effects to manifest themselves (Weber et al. 2003). Within hours after the administration of CCl_4, hepatic steatosis and central lobular necrosis are induced (Hartley et al. 1999). Endoplasmic reticulum, plasma membrane, mitochondria, and Golgi apparatus are the main subcellular structures of hepatocytes affected by CCl_4 (Reynolds 1963). CCl_4 metabolism begins with the formation of the trichloromethyl free radical, CCl_3^*, through the action of the mixed function cytochrome P450 oxygenase system of the endoplasmic reticulum (Recknagel et al. 1989; McCay et al. 1984). The CCl_3^* radical reacts with various biologically important substances such as amino acids, nucleotides and fatty acids, as well as proteins, nucleic acids and lipids (Castro 1984). In the presence of oxygen, the CCl_3 radical is converted to the trichloromethyl peroxy radical, CCl_3OO^*. CCl_3OO^* is far more likely than CCl_3^* to abstract a hydrogen from polyunsaturated fatty acids (PUFA) (Forni et al. 1983), thereby initiating the process of lipid peroxidation (Cheeseman et al. 1985). The abstraction of a hydrogen from fatty acid initiates a complex series of reactions that terminate in the complete disintegration of the PUFA molecule with the formation of aldehydes, other carbonyles, and alkanes (Weber et al. 2003). Lipid peroxidation may damage cellular functions in two ways: by compromising membrane function and by covalent binding of reactive intermediates (Weber et al. 2003).

The formation of SUL has been immunohistochemically observed around the portal vein in the liver of mice fed with DHA followed by an intraperitoneal injection of CCl_4, whereas the immunoexpression of SUL was scarcely observed in the control, DHA-alone, and CCl_4-alone groups, using monoclonal antibody (mAb2B12) raised against the SUL adduct (Kawai et al. 2006).

As early as 6 h after oral administration of CCl_4 (1.0 ml/kg), MDA-amine and 4-HNE-sulfhydryl adducts were immunohistochemically detected in hepatocytes localized to zone 2 of the hepatic lobule (Hartley et al. 1999). The density of MDA-adducts-positive hepatocytes and HNE-adducts-positive hepatocytes increased with time up to 36 h post-CCl_4 administration. MDA-adducts- or HNE-adducts-positive hepatocytes were no longer detected by 36–72 h post CCl4 administration

(Hartley et al. 1999). These results demonstrate that HNE-adducts and MDA-adducts in a time-dependent manner, appear to be associated with liver injury-induced CCl_4.

6 Acetaminophen-Induced Liver Injury

Although an overdose of acetaminophen (*N*-acetyl-*p*-aminophenol; APAP) causes liver injury in humans (Larson et al. 2005) and experimental animals (Chen et al. 2009; Saito et al. 2010), APAP is a safe and effective analgesic and antipyretic drug when used at therapeutic levels (Rumack 2004). The initial step in toxicity is the cytochrome P450 metabolism to N-acetyl-p-benzoquinone imine (NAPQI), a highly reactive metabolite (Jaeschke and Bajt 2006; Masubuchi et al. 2005; Nelson 1990). NAPQI depletes the intracellular storage of glutathione (GSH) in the hepatocyte (Bessems and Vermeulen 2001; Jaeschke and Bajt 2006; Nelson 1990). Since GSH is the cofactor for GSH-peroxidase detoxification of peroxides, a main mechanism of peroxide detoxification is compromised in APAP-induced liver injury (Hinson et al. 2010). Thus, GSH depletion could lead to increased intracellular peroxide, and increase oxidative stress via mechanisms of the Fenton reaction (Hinson et al. 2010). This mechanism involves the reduction of peroxide by ferrous ions forming the hydroxyl radical, which leads to oxidation of lipids, proteins and nucleic acids (Hinson et al. 2010).

In the previous study (Sun et al. 2012), the immunoexpression of PUFA oxidation markers PRL, HEL, 4-HHE-histidine and CRA-lysine was examined up to 24 h post-APAP intraperitoneal injection in rats (1 g/kg body weight). Three hr after the post-intraperitoneal injection of APAP in rats, vacuolated hepatocytes were observed in the centrilobular region of the hepatic lobule (Fig. 10.1a); and at 6 h, they increased in number and expanded their distribution to the medzonal region. Apoptotic cells were occasionally observed in hepatocytes in the centrilobular region. Then, at 12 h, coagulative necrosis, single cell necrosis and apoptosis were observed in all regions. At 24 h, necrotic and apoptotic changes became more prominent. Three hour after the post-intraperitoneal injection of APAP, a few vacuolated hepatocytes were positive for TUNEL stain in the centrilobular region (Fig. 10.1b). The TUNEL-positive rate tended to increase at 6 h and showed significant increases at 12 and 24 h. Hepatocytes in the control group were negative for PRL, HEL, HHE-histidine and CRA-lysine. Immunohistochemical expression of these oxidation markers was first detected in the cytoplasm of degenerative hepatocytes in the centrilobular region of the hepatic lobule at 3 h after the injection, earlier than the occurrence of hepatocyte apoptosis (Fig. 10.1b–f). Immunohistochemical expression of these oxidation markers was observed in almost all degenerative hepatocytes 6–24 h post-APAP injection. The results thus suggest that the generation of PRL, HEL, 4-HHE-histidine and CRA-lysine may be the sign of early events preceding the induction of hepatocyte apoptosis, and thus may prove useful for the early detection of oxidative stress-related liver cell injury, and for the detection of PUFA oxidations that may be involved in the pathogenesis of APAP-induced liver injury.

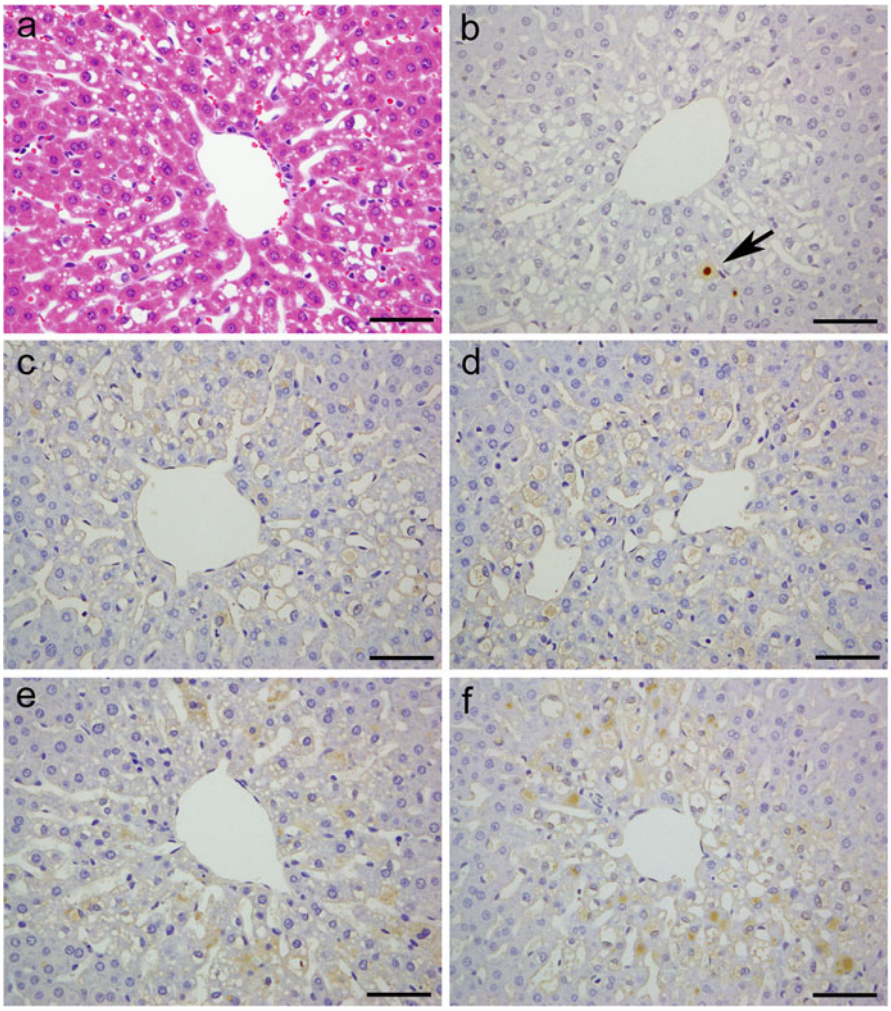

Fig. 10.1 Centrilobular lesions 3 h post-acetaminophen intraperitoneal injection. Bar = 50 μm. (**a**) Vacuolated hepatocytes were observed in the centrilobular region of the hepatic lobule. HE stain. (**b**) A few vacuolated hepatocytes were positive for TUNEL stain in the centrilobular region. (**c**–**f**) Immunohistochemical expression of PRL (**c**), HEL (**d**), HHE (**e**), CRA (**f**) was detected in the cytoplasms of almost all degenerative hepatocytes in the centrilobular region (These figures have been reprinted from the Journal of Veterinary Medical Science, 74, 141–147, 2012. A part of these figures has been revised and used.)

7 Cisplatin-Induced Renal Tubular Injury

Cisplatin [*cis*-diamminedichloroplatinum (II)] is a major anticancer drug used for the treatment of solid tumors in the testis, ovary, head and neck, and elsewhere (Pabla and Dong 2008; Yao et al. 2007). Though the mechanism behind the

anticancer activity of cisplatin is not completely understood, a widely-held view is that cisplatin binds to DNA, leading to the formation inter- and intrastrand cross-links (Pabla and Dong 2008). Cross-linking results in defective DNA templates and arrest of DNA synthesis and replication (Pabla and Dong 2008). The cross-linking can further induce DNA damage in rapidly dividing cells such as neoplastic cells (Pabla and Dong 2008). The chief dose-limiting side effect of cisplatin is nephrotoxicity (Fillastre and Raguenez-Viotte 1989; Pabla and Dong 2008; Yao et al. 2007; Zhou et al. 2004). The kidney accumulates cisplatin to a greater degree than other organs, and the disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity (Aray and Safirstein 2003). Cisplatin-induced nephrotoxicity is characterized by proximal tubular injury and decreased glomerular filtration (Jones et al. 1985; Pabla and Dong 2008). Cisplatin causes tubular injury through multiple mechanisms, including oxidative stress, DNA damage, apoptosis and inflammation (Pabla and Dong 2008; Yao et al. 2007).

Cisplatin causes the generation of oxygen free radicals, such as hydrogen peroxide, superoxide anions and hydroxyl radicals (Kruidering et al. 1997; Masuda et al. 1994). In particular, the hydroxyl radical is highly reactive among oxygen radicals. Once excessive hydroxyl radicals are released, lipid peroxidation, which causes changes in the fluidity and permeability of membranes, is induced (Schmidley 1990). Cisplatin-induced nephrotoxicity is closely associated with increased lipid oxidation markers such as malondialdehyde and 4-HNE in kidney tissue (Chirino et al. 2008; Greggi Antunes et al. 2000; Zhou et al. 2006). In the previous study (Sugiyama et al. 2011), immunoexpression of HEL and acrolein in rat kidneys was examined up to 4 days after cisplatin injection. Cisplatin-induced tubular injury was observed histopathologically on days 2–4 after injection, and became more severe time-dependently. Thus, few histopathological changes were observed in rats at day 1. At days 2 and 3, degenerative changes seen in the S3 segment of the proximal tubule consisted of hydropic degeneration, cytoplasmic vacuolization and tubular dilation. These histopathological changes were more severe at day 3 than day 2. At day 4, many pyknotic nuclei, and the widespread desquamation and necrosis of tubular epithelial cells of the S3 segment of the proximal tubule were observed. There was a significant increase in the number of TUNEL-positive cells at days 3 and 4. Tubular epithelial cells in saline-treated control rats and cisplatin-treated rats at day 1 were negative for HEL or acrolein. The immunohistochemical expression of these oxidation markers was first detected in the cytoplasm of degenerative tubular cells at day 2, preceding the induction of tubular cell apoptosis. On days 3–4, the cytoplasms of damaged proximal tubular cells were immunostained for these oxidation markers. These findings suggest that expression of HEL and acrolein in the S3 segment of the proximal tubule associate closely with pathogenesis of cisplatin-induced renal tubular injury.

8 Conclusion

Lipid hydroperoxide- and aldehyde-modified proteins have been immunohistochemically detected in diverse pathological situations such as atherosclerosis, neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease, carbon tetrachloride- or acetaminophen-induced hepatoxicity and cisplatin-induced nephrotoxicity. These findings suggest that the expression of the lipid hydroperoxide- and aldehyde-modified proteins associate closely with the pathogenesis of these diseases in humans and experimental animals.

References

Aray I, Safirstein RL (2003) Cisplatin nephrotoxicity. Semin Nephrol 23:460–464

Bernhard D, Wang XL (2007) Smoking, oxidative stress and cardiovascular diseases-do anti-oxidative therapies fail? Curr Med Chem 14:1703–1712

Bessems JG, Vermeulen NP (2001) Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol 31:55–138

Calingasan NY, Uchida K, Gibson GE (1999) Protein-bound acrolein: a novel marker of oxidative stress in Alzheimer's disease. J Neurochem 72:751–756

Castellani RJ, Perry G, Siedlak SL et al (2002) Hydroxynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem Lewy bodies in humans. Neurosci Lett 319:25–28

Castro JA (1984) Mechanistic studies and prevention of free radical cell injury. In: Paton W, Mitchell J, Turner P (eds) Proceedings of IUPHAR 9th International Congress Pharmacol, MacMillan

Cheeseman KH, Albono EF, Tomasi A et al (1985) Biochemical studies on the metabolic activity of halogenated alkanes. Environ Health Perspect 64:85–101

Chen YH, Lin FY, Liu PL et al (2009) Antioxidative and hepatoprotective effects of magnolol on acetaminophen-induced liver damage in rats. Arch Pharm Res 32:221–228

Chirino YI, Sánchez-González DJ, Martínez-Martínez CM et al (2008) Protective effects of apocynin against cisplatin-induced oxidative stress and nephrotoxicity. Toxicology 245:18–23

Cooper AA, Gitler AD, Cashikar A et al (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. Science 313:324–328

Ekuni D, Tomofuji T, Sanbe T et al (2009) Periodontitis-induced lipid peroxidation in rat descending aorta is involved in the initiation of atherosclerosis. J Periodontal Res 44:434–442

Fillastre JP, Raguenez-Viotte G (1989) Cisplatin nephrotoxicity. Toxicol Lett 46:163–175

Forni LG, Packer JE, Slater TF et al (1983) Reaction of the trichloromethyl and halothane-derived peroxy radicals with unsaturated fatty acids: a pulse radiolysis study. Chem Biol Interact 45:171–177

Fukuchi Y, Miura Y, Nabeno Y et al (2008) Immunohistochemical detection of oxidative stress biomarkers, dityrosine and Nε-(hexanoyl)lysine, and c-reactive protein in rabbit atherosclerotic lesions. J Atheroscler Thromb 15:185–192

Fukuda M, Kanou F, Shimada N et al (2009) Elevated levels of 4-hydroxynonenal-histidine Michael adduct in the hippocampi of patients with Alzheimer's disease. Biomed Res 30:227–233

Grant Maxie M, Robinson WF (2007) Cardiovascular system. In: Grant Maxie M (ed) Ubb, Kennedy, and Palmer's pathology of domestic animals, vol 3, 5th edn. Sanders, New York

Greggi Antunes LM, Darin JDC, Biancii MLP (2000) Protective effects of vitamin C against cisplatin-induced nephrotoxicity and lipid peroxidation in adult rats: a dose-dependent study. Pharmacol Res 41:405–411

Hartley DP, Kolaja KL, Reichard J et al (1999) 4-Hydroxynoneal and malondialdehyde hepatic protein adducts in rats treated with carbon tetrachloride: immunohistochemical detection and lobular localization. Toxicol Appl Pharmacol 161:23–33

Higuchi Y (2003) Chromosomal DNA fragmentation in apoptosis and necrosis induced by oxidative stress. Biochem Pharmacol 66:1527–1535

Hinson JA, Roberts DW, James LP (2010) Mechanisms of acetaminophen-induced liver necrosis. Handb Exp Pharmacol 196:369–405

Hisaka S, Kato Y, Kitamoto N et al (2009) Chemical and immunochemical identification of propanoyllysine derived from oxidized n-3 polyunsaturated fatty acid. Free Radic Biol Med 46:1463–1471

Huang A, Sun D, Kaley G et al (1998) Superoxide released to high intra-arteriolar pressure reduces nitric oxide-mediated shear stress-and agonist-induced dilations. Circ Res 83:960–965

Jaeschke H, Bajt ML (2006) Intracellular signaling mechanisms of acetaminophen-induced liver cell death. Toxicol Sci 89:31–41

Jenner P (2003) Oxidative stress in Parkinson's disease. Ann Neurol 53:S26–S36

Jomova K, Vondrakova D, Lawson M et al (2010) Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem 345:91–104

Jones TW, Chopra S, Kaufman JS et al (1985) Cis-diamminedichloroplatinum (II)-induced acute renal failure in the rat. Correlation of structural and functional alterations. Lab Invest 52:363–374

Kato Y, Osawa T (2010) Detection of lipid-lysine amide-type adduct as a marker of PUFA oxidation and its applications. Arch Biochem Biophs 501:182–187

Kato Y, Mori Y, Makino Y et al (1999) Formation of Nepsilon-(hexanonyl)lysine in protein exposed to lipid hydroperoxide. A plausible marker for lipid hydroperoxide-derived protein modification. J Biol Chem 274:20406–20414

Kawai Y, Kato Y, Fujii H et al (2003) Immunochemical detection of a novel lysine adduct using an antibody to linoleic acid hydroperoxide-modified protein. J Lipid Res 44:1124–1131

Kawai Y, Fujii H, Kato Y et al (2004) Esterified lipid hydroperoxide-derived modification of protein: formation of a carboxyalkylamide-type lysine adduct in human atherosclerotic lesions. Biochem Biophys Res Commun 313:271–276

Kawai Y, Fujii H, Okada M et al (2006) Formation of N^{ε}-(succinyl)lysine in vivo: a novel marker for docosahexaenoic acid-derived protein modification. J Lipid Res 47:1386–1398

Kim JS, He L, Lemasters JJ (2003) Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem Biophys Res Commun 304:463–470

Kristal BS, Park BK, Yu BP (1996) 4-Hydroxyhexenal is a potent inducer of the mitochondrial permeability transition. J Biol Chem 271:6033–6038

Kruidering M, Van de Water B, de Heer E (1997) Cisplatin-induced nephrotoxicity in porcine proximal tubular cells: mitochondrial dysfunction by inhibition of complex I to IV of the respiratory chain. J Pharmacol Exp Ther 280:638–649

Larson AM, Polson J, Fontana RJ et al (2005) Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 42:1364–1372

Lee JY, Je JH, Kim DH et al (2004) Induction of endothelial apoptosis by 4-hydroxyhexenal. Eur J Biochem 271:1339–1347

Liu XY, Yang ZH, Pan XJ et al (2010) Crotonaldehyde induces oxidative stress and caspase-dependent apoptosis in human bronchial epithelial cells. Toxicol Lett 195:90–98

Long EK, Murphy TC, Leiphon LJ et al (2008) Trans-4-hydroxy-2-hexenal is a neurotoxic product of docosahexaenoic (22:6; n-3) acid oxidation. J Neurochem 105:714–724

Masubuchi Y, Suda C, Horie T (2005) Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. J Hepatol 42:110–116

Masuda H, Tanaka T, Takahama U (1994) Cisplatin generates superoxide anion by interaction with DNA in a cell-free system. Biochem Biophys Res Commun 203:1175–1180
McCay PB, Lai EK, Poyer JL et al (1984) Oxygen and carbon-centered free radical formation during carbon tetrachloride metabolism. J Biol Chem 259:2135–2143
Mitra S, Goyal T, Mehta JL (2011) Oxidized LDL, LOX-1 and atherosclerosis. Cardiovasc Drug Ther 25:419–429
Mügge A, Brandes RP, Böger RH et al (1994) Vascular release of superoxide radicals is enhanced in hypercholesterolemic rabbits. J Cardiovasc Pharmacol 24:994–998
Nelson SD (1990) Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Semin Liver Dis 10:267–278
Nicolls MR, Haskins K, Flores SC (2007) Oxidant stress, immune dysregulation, and vascular function in type I diabetes. Antioxid Redox Signal 9:879–889
Pabla N, Dong Z (2008) Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int 73:994–1007
Recknagel RO, Glende EA Jr, Dolak JK et al (1989) Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 43:139–154
Reynolds ES (1963) Liver parenchymal cell injury. I. Initial alterations of the cell following poisoning with carbon tetrachloride. J Cell Biol 19:139–157
Rumack BH (2004) Acetaminophen misconceptions. Hepatology 40:10–15
Saito C, Yan HM, Artigues A et al (2010) Mechanism of protection by metallothionein against acetaminophen hepatotoxicity. Toxicol Appl Pharmacol 242:182–190
Schmidley JW (1990) Free radicals in central nervous system ischemia. Stroke 21:1086–1090
Shamamoto-Nagai M, Maruyama W, Hashizume Y et al (2007) In Parkinsonian substantia nigra, alpha-synuclein is modified by acrolein, a lipid-peroxidation product, and accumulates in the dopamine neurons with inhibition of proteasome activity. J Neural Transm 114:1559–1567
Sugiyama A, Sun J, Nishinohara M et al (2011) Expressions of lipid oxidation markers, N^{ε}-hexanoyl lysine and acrolein in cisplatin-induced nephrotoxicity in rats. J Vet Med Sci 73:821–826
Sun J, Sugiyama A, Hisaka S et al (2012) Immunohistochemical detection of polyunsaturated fatty acid oxidation markers in acetaminophen-induced liver injury in rats. J Vet Med Sci 74:141–147
Tamer L, Sucu N, Polat G et al (2002) Decreased serum total antioxidant status and erythrocyte-reduced glutathione levels are associated with increased serum malondialdehyde in atherosclerotic patients. Arch Med Res 33:257–260
Uchida K (1999) Current status of acrolein as a lipid peroxidation product. Trends Cardiovasc Med 9:109–113
Uchida K (2000) Role of reactive aldehyde in cardiovascular disease. Free Radic Biol Med 28:1685–1696
Uchida K, Kanematsu M, Sakai K et al (1998) Protein-bound acrolein: potential markers for oxidative stress. Proc Natl Acad Sci U S A 95:4882–4887
Weber LW, Boll M, Stampfl A (2003) Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. Crit Rev Toxicol 33:105–136
Yao X, Panichpisal K, Kurtzman N et al (2007) Cisplatin nephrotoxicity: a review. Am J Med Sci 334:115–124
Yoritaka A, Hattori N, Uchida K et al (1996) Immunohistochemical detection of 4-hydroxynoneal protein adducts in Parkinson disease. Proc Natl Acad Sci U S A 93:2696–2701
Yoshida T, Adachi E, Nigi H et al (1999) Changes of sinusoidal basement membrane collagens in early hepatic fibrosis induced with CCl_4 in cynomolgus monkeys. Pathology 31:29–35
Zarkovic K (2003) 4-Hydroxynoneal and neurodegenerative diseases. Mol Aspect Med 24:293–303
Zhou H, Kato A, Yasuda H et al (2004) The induction of cell cycle regulatory and DNA repair proteins in cisplatin-induced acute renal failure. Toxicol Appl Pharmacol 200:111–120
Zhou H, Kato A, Miyaji T et al (2006) Urinary marker for oxidative stress in kidneys in cisplatin-induced acute renal failure in rats. Nephrol Dial Transplant 21:616–623