Antioxidative Phytochemicals and Biomarkers

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ABSTRACT

Lipid peroxidation is known to be a free chain reaction which takes place both in vivo and in vitro, and forms lipid hydroperoxides and secondary products. These lipid peroxidation products are highly reactive and have been shown to interact with many biological components such as proteins, amino acids, amines, phospholipids and DNAs. As novel biomarkers for early stage of lipid peroxidation process, we have developed monoclonal antibodies specific to oxidatively modified lysines with linoleic acid-, arachidonic acid- eicosapentanoic acid- and docosahexaenoic acid-hydroperoxides. We have also succeeded in construction of novel biomarkers specific to overproduction of oxidative stress during inflammation process. Recently, we found more potent antioxidative phytochemicals such as sesame lignans, astaxanthin and curcuminoids, especially, tetrahydrocurcumin , one of the main metabolites and also produced by fermentation of yeast (D. hansenil) of curcumin. We have also succeeded in determining many functional activities including extention of ageing process.

Key words: oxidativw stress, lipid hydroperoxide, inflammation, sesame lignans, astaxanthin, curcumin, tetrahydrocurcumin

BIOMARKERS FOR LIPID PEROXIDATION

Recently we have been involved in developing novel type of evaluation systems for oxidative stress using "antibody chip" by application of monoclonal antibodies which are specific to the lipid hydroperoxide-modified proteins, phopholipids and nucleic acids. At an early stage of lipid peroxidation, the lipid hydroperoxide is formed and then decomposed into several aldehydes. These reactive aldehydes can easily react with proteins, nucleic acids, and amino-phospholipids, accompanied by stable and unstable adduct formation, however, our research has focused on the lipid hydroperoxide-derived protein modification. One of adducts, hexanoyl-lysine (HEL), has been detected in atherosclerotic plaques using polyclonal and monoclonal antibodies to HEL. Several amide-type adducts, azelayl-lysine (AZL), succinyl-lysine (SUL), glutaroyl-lysine (GLL), and propanoyl-lysine (PRL), have been also identified recently. Because these adducts are probably generated from oxidation products of n-3 and/or n-6 FAs with lysine residues, the simultaneous determination of all adducts may be a useful fingerprint to make clear what kinds of lipid are oxidized *in vivo*[1]. The HEL has been gradually used as one of the important biomarkers because the antibody to HEL and an ELISA kit are commercially available. More recently, we have succeeded in immunological and chemical detection of N-(hexanoyl) phosphatedylethanolamine and N-(hexanoyl)-phosphatedylserine in an oxidative model induced by carbon tetrachloride[2], and we just started to apply these markers for preparation of "Antibody chip".

On the other hand, PRL is a novel biomarker and therefore, we prepared the monoclonal antibody against PRL to evaluate the generation of PRL *in vivo*[3]. Then, we analyzed the relevance to aging and neurodegenerative disease using this antibody because ithas been reported that lipid peroxidation is promoted in the brain during aging and the aging was one of the most important risk factor of neurodegenerative diseases. Immunohistochemical detection using anti-PRL antibody suggested that propanoylation was generated in the hippocampus area and temporal lobe in the brain of Alzheimer's disease (AD) patients. Our recent data suggests that PRL has the capacity of not only the useful biomarker of AD but also trigger of pathological degradation to lead to AD etiology although the detailed examination is undergoing (Hisaka *et al*., in preparation).

BIOMARKERS FOR OXIDATIVE STRESS FORMED DURING INFLAMMATION

We have also succeeded in construction of novel biomarkers specific to oxidative stress formed during inflammation process. Neutrophil myeloperoxidase (MPO) generates hypochlorous acid (HOCl) and/or hypobromous acid (HOBr) using hydrogen peroxide and Br^-/Cl^-. The active halogenating species can modify proteins. The modification of protein by the reactive intermediates causes protein tyrosine halogenation forming chlorotyrosine (ClY) or bromotyrosine (BrY)[4]. MPO can catalyze dityrosine (DY) formation in the presence of H_2O_2, and suggesting that the DY may become one of universal protein oxidation markers[5]. We have immunochemically detected DY in lipofuscin of pyramidal neuron of aged human brains and the atherosclerotic lesion of apoE knockout mice. A potential role of DNA damage by leukocyte-derived reactive

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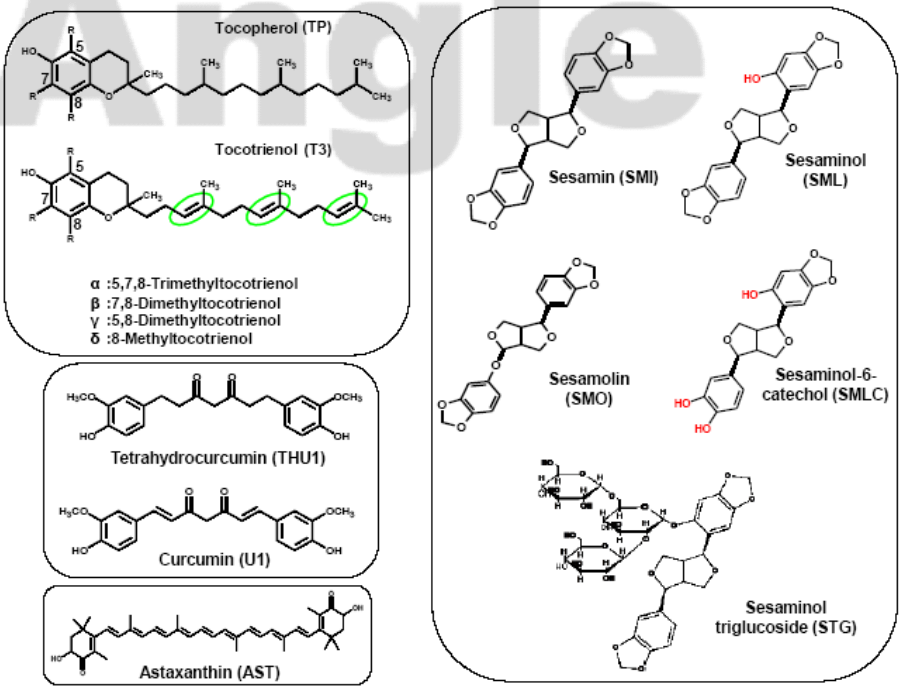

Figure 1. Structures of antioxidative food factors.

oxygen species (ROS) in carcinogenesis has also been suggested. Recently, we have succeeded in developing a novel monoclonal antibody that recognizes the hypohalous acid-modified DNA and found that the epitopes was identified as novel N^4,5-dihaloge- nated 2'-deoxycitidine residues[6]. We also succeeded to prepare the monoclonal antibody specific to 8-halogenated dGs, 8-cloro-deoxyguanosine (8-CldG) and 8-bromo-deoxyguanosine (8-BrdG)[7]. Using that antibody and liquid chromatography tandem mass spectrometry (LC-MS/MS), our results demonstrated facile formation of 8-halo-dGs in the liver and urine of rats administered LPS. Furthermore, with respect to the order of modification of dG, halogenation occurred prior to oxidation and nitration, which may be useful for evaluation of the progress of inflammatory diseases.

ANTIOXIDATIVE PHYTOCHEMICALS AND BRAIN AGING

Advances in understanding the neurodegenerative pathologies are creating new opportunities for the development of neuroprotective therapies, such as antioxidant food factors, lifestyle modification and drugs. However, the biomarker by which to determine the effect of the agent on neurodegeneration is limited. Recently, our research group focused on hexanoyl dopamine (HED), one of novel dopamine adducts derived from brain polyunsaturated acid, referring to its *in vitro* formation, potent toxicity to SH-SY5Y cells[8], and application to assess the neuroprotective effect of antioxidative food factors. Dopamine is a neurotransmitter and its deficiency is a characterized feature in Parkinson's disease (PD), thereby HED represents a new addition to understanding of dopamine biology and pathophysiology of PD and a novel biomarker for the assessment of neuroprotective therapies. We have established an analytical system using for the detection of HED and its toxicity to the neuroblstoma cell line, SH-SY5Y cells. We particularly report here on hexanoyl dopamine (HED), a dopamine modified adduct derived from arachidonic acid, referring to its formation, effect on SH-SY5Y cells and applications to investigate the neuroprotective effect of antioxidant foods, such as tocopherol, curcumin, sesame lignans and astaxanthin. We have demonstrated that HED was present in rat brain and toxic to the neuroblastoma SH-SY5Y cells, thereby representing a novel biomarker for the assessment of neuroprotective therapies against PD. By assessing the protective effect of antioxidant food factors on HED formation (Figure 1), sesaminol-6-catechol (SMLC) showed the most significant inhibitive effect on the *in vitro* HED formation[9](Figure 2). We also recently found that SMLC markedly prevented HED-induced ROS generation and cell death in SH-SY5Y cells. In addition, SMLC was detected in the brain of rats fed with SMLC-contained foods, suggesting that it could cross brain-blood barrier (BBB) and exhibit the neuroprotective effect *in vivo*[10]. SMLC is one of the main metabolites produced by p-450 during injestion of sesame lignans, in particular, sesaminol glucosides. We have investigated many biological activities of SMLC such as protection of colon carcinogenesis by meaqsuring modifying effect of dietary sesaminol glucosides on the formation of azoxymethane-induced premalignant lesions of rat colon[11], and also downreguration on the expression of cell adhesion molecules (CAMs) in human umbilical vein

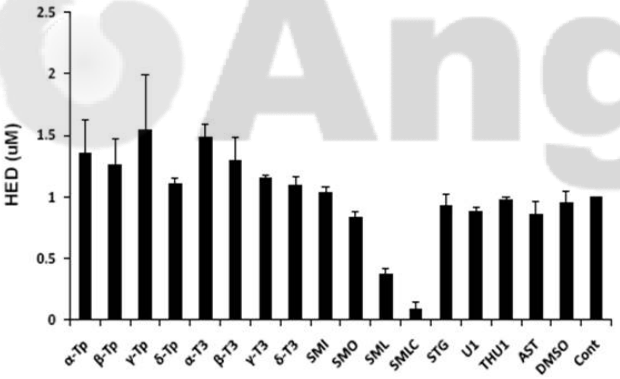

Figure 2. Inhibitory effects on HED Formation o by anti-oxidative food factors.

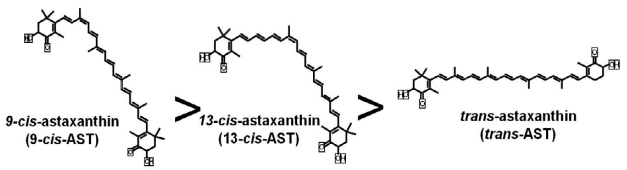

Figure 3. Biological activity of isomers of astaxan-

endothelial cells (HUVECs) induced by tumor necrosis factor-α (TNF-α)[12]. Our research group has also found that SMLC can be formed by fermentation using *Aspergillus saitoi*[13].

Recently, much attention has been focused on many biological activities of astaxanthin. It accumulates in the skeletal muscles of mammals after oral administration and may have a role in physiological muscle function. Our research group investigated the effect of astaxanthin on muscle metabolism during exercise and its effect on endurance. Astaxanthin treatment accelerated the decrease of body fat caused by training and the running time to exhaustion was prolonged in the trained mice by astaxanthin. Astaxanthin also decreased the oxygen uptake and respiratory quotient during recovery after running compared with mice on a normal diet. Elevation of the plasma lactate level and consumption of glycogen were inhibited by as-taxanthin when a single session of exercise was performed. Colocalization of fatty acid translocase with carnitine palmitoyltransferase I (CPT I) in skeletal muscle was increased by astaxanthin. We also found that hexanoyl-lysine (HEL) modification of CPT I was increased by exercise, while astaxanthin prevented this increase. These results suggested that astaxanthin promoted lipid metabolism rather than glucose utilization during exercise, which led to improvement of endurance[14]. We also investigated the effect and the mechanism of astaxanthin on reactive oxygen species (ROS)-mediated apoptosis in dopaminergic SH-SY5Y cells[15]. The treatment with DHA hydroperoxide (DHA-OOH), ROS-inducing neurotoxin, led to a significant decrease in viable dopaminergic SH-SY5Y cells by MTT assay[16], whereas a significant protection was shown while the cells were pretreated with astaxanthin. Moreover, astaxanthin pretreatment significantly inhibited apoptosis, mitochondrial abnormalities and intracellular ROS generation occurred in DHA-OOH-treated cells. The neuroprotective effect of astaxanthin is suggested to be dependent upon its antioxidant potential and mitochondria protection; therefore, it is suggested that astaxanthin may be an effective treatment for oxidative stress-associated neurodegeneration [17] .

Recently, we have been involved in investigation what is the real active form of astaxanchin, and we found that cis astaxanthin and especially 9-cis astaxanthin exhibits a higher antioxidant activity *in vitro* compared to the all-trans isomer.astaxanthin[18] (Figure 3). Until now, most of research showed that active form of astaxanthin is all-trans .astaxanthin, however, we speculated that 9-cis astaxanthin must be the active form of astaxanthin although the detailemore d examination must be carried out.

ANTIOXIDATIVE PHYTOCHEMICALS AND EXTENTION OF AGING

As other type of plant antioxidants, it was found that curcumin (U1), main yellow pigment of *Curcuma longa* (turmeric) has many interesting functional activities for prevention of life-style rlated diseases such as cancer, atherosclerosis and diabetes mellitus etc. Recently, we found that U1 was converted to tetrahydrocurcumin (THU1), one of the major metabolites produced during the absorption through the guts and THU1 was observed in the serum. We have also found that U1 can be metabolized to THU1 by fermentation of yeast (*D. hansenil*) used for food industry., and succeeded in commercial production of "Fermented Turmeric" which contains THU1 by collaboration between industrial and academic sectors, and we tried many biological activities of THU1 including protective role against atherosclerosis, carcinogenesis and diabetes mellitus using a wide variety of evaluation systems both *in vitro* and *in vivo*[19] .

However, the literature concerning the anti-aging mechanism of THU1 is limited to a single survival study in C57BL/J6 mice[20]. Recently, we found that THC regulated the nuclear localization of FOXO in cultured cells and inhibited phosphorylation of protein kinase B (PKB)/Akt kinase. Furthermore, genetic analyses in Drosophila revealed that foxo and Sir2 mediated the effects of THU1 on life span and the oxidative stress response. These results suggest that THC regulates the oxidative stress response and aging via an evolutionally conserved signaling pathway. The O-type forkhead domain transcription factor (FOXO) is involved in many biological processes such as aging, the oxidative stress response, and growth regulation. FOXO activity is tightly controlled within cells. In particular, growth factor signaling pathways and the oxidative stress response can both stimulate nuclear translocation of this transcription factor. Here, we show that THU1 regulates the oxidative stress response and aging via FOXO. In NIH3T3 cells, THC induced nuclear accumulation of FOXO4, a

member of the FOXO family of transcription factors, by inhibiting phosphorylation of protein kinase B (PKB)/Akt. In both of Yeast and Drosophila, THU1 attenuated the oxidative stress response, an effect that was blocked in a foxo mutant background. THU1 also extended the life span of Drosophila under normal conditions, and loss of either foxo or Sir2 activity eliminated this effect. Based on these results, THU1 may regulate the aging process via an evolutionarily conserved signaling pathway that includes both foxo and Sir2.

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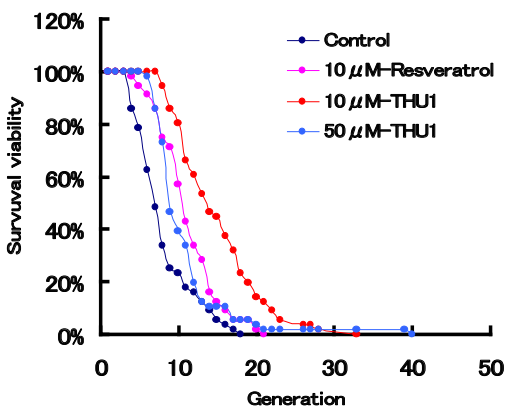

Figure 4. Extention of life span by resberatrol and THU1.

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