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Lipid peroxidation induced by carbon tetrachloride and its inhibition by antioxidant as evaluated by an oxidative stress marker, HODE

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Abstract

We have recently proposed total hydroxyoctadecadienoic acid (HODE) as a biomarker for oxidative stress in vivo. The biological samples such as plasma, urine, and tissues were first reduced and then saponified to convert the oxidation products of linoleate to HODE. In the present study, this method was applied to measure the oxidative damage induced by the administration of carbon tetrachloride to mice and also to evaluate the capacity of antioxidant to inhibit the above damage. α-Tocopherol transfer protein knock out (α-TTP$^{-/-}$) mice were used to evaluate antioxidant effect in the absence of α-tocopherol. The intraperitoneal administration of carbon tetrachloride to mice induced the increase in HODE in liver and plasma, which was followed by an increase in plasma glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT). F2-isoprostanes, another prevailing biomarker, were also increased similarly, but their concentration was approximately two to three orders of magnitude smaller than that of HODE. The lipophilic antioxidants such as γ-tocopherol, γ-tocotrienol and 2,3-dihydro-5-hydroxy-4,6-di-*tert*-butyl-2,2-dipentylbenzofuran (BO-653) were effective in suppressing the formation of HODE.

Keywords: Antioxidant; HODE; Isoprostanes; BO-653; α-Tocopherol transfer protein knockout mice; Carbon tetrachloride; Lipid peroxidation

Introduction

It is now generally accepted that lipid peroxidation is involved in the oxidative damage in vivo and pathogenesis of several disorders and diseases induced by reactive oxygen species. Lipid peroxidation may cause damage directly to biological molecules and membranes and may also induce the generation of toxic and signaling molecules (Leonarduzzi et al., 2000; Tang et al., 2002). Accordingly, lipid peroxidation products have received much attention as indices for oxidative stress. Lipid hydroperoxides are formed as the major primary product in the oxidation of polyunsaturated fatty acids and their esters, but hydroperoxides are the substrates of many enzymes such as glutathione peroxidases and phospholipases and they also undergo non-enzymatic secondary reactions (Girotti, 1998). Therefore, the amount of lipid hydroperoxides measured does not always reflect the extent of lipid peroxidation in vivo. The susceptibility of lipid hydroperoxides to secondary reactions depends on the lipid class. For example, phosphatidylcholine hydroperoxides are readily reduced to the corresponding hydroxides in plasma, while cholesteryl ester hydroperoxides are much stable than phosphatidylcholine hydroperoxides (Itoh et al., 2004).

Many biomarkers have been proposed and among them F2-isoprostanes have been reported as the gold standard for

Abbreviations: BSTFA, N,O-bis(trimethylsilyl)trifluoroacetamide; BO-653, 2,3-dihydro-5-hydroxy-4,6-di-*tert*-butyl-2,2-dipentylbenzofuran; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HODE, hydroxyoctadecadienoic acid; HPODE, hydroperoxyoctadecadienoic acid; 8-*iso*-PGF$_{2\alpha}$, 8-*iso*-prostaglandin F$_{2\alpha}$; PBS, phosphate-buffered saline; T, tocopherol; T3, tocotrienol; αTTP$^{-/-}$, α-tocopherol transfer protein knockout.

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the assessment of oxidative injury in vivo (Basu, 2004; Moore and Roberts, 1998). Numerous papers have shown that F2-isoprostanes are indeed good biomarker (Durand and Roberts, 2004). We have recently developed a method for the measurement of lipid peroxidation in vivo, where total hydroxyoctadecadienoic acid (HODE) is determined from physiological samples after reduction with sodium borohydride and saponification by potassium hydroxide (Yoshida and Niki, 2004). In this method, hydroperoxides and ketones as well as hydroxides of both free and ester forms of linoleic acid are measured as HODE. Linoleates are major polyunsaturated fatty acid in vivo and their oxidation proceeds by a straightforward mechanism to give 9- and 13-hydroperoxyoctadecadienotes (HPODE) as major product (Porter et al., 1995). Therefore, HODE thus measured may account for much of the lipid peroxidation in vivo. On the other hand, arachidonates, although more reactive toward oxygen radicals than linoleates, are contained in less amount than linoleates and their oxidation proceeds by complicated mechanisms to give versatile products, making F2-isoprostanes only a minor product.

In the present study, the oxidative damage induced by the administration of carbon tetrachloride, a well-established model for the oxidative damage in vivo, was evaluated by HODE and F2-isoprostanes. The liver damage induced by carbon tetrachloride has been studied extensively for more than 50 years (Hove and Hardin, 1951). In fact, this is the first example of the free radical-mediated, oxidative damage in vivo (Poli et al., 1985a; Slater, 1972). Carbon tetrachloride is metabolized through a cytochrome P450 enzyme in liver to give trichloromethyl radicals, which subsequently initiate free radical-mediated oxidations. Polyunsaturated fatty acids are quite reactive toward free radicals and readily undergo lipid peroxidation by chain mechanism. Thus, one molecule of free radical can oxidize many molecules of lipids. The formation of lipid peroxides induced by carbon tetrachloride has been observed in many studies (Basu, 2003). The hydroxylated derivatives of linoleic, arachidonic, and docosahexanoic acids formed in mouse liver following carbon tetrachloride administration were isolated and identified after treatment with phospholipase A2, diazomethane, triphenylphosphine, and TMS (Hughes et al., 1983, 1986). It has been also reported that the administration of carbon tetrachloride to rat increased the F2-isoprostanes (Morrow et al., 1992) and also that the plasma concentration of F2-isoprostanes was increased in patients with liver failure (Morrow et al., 1993). Since then, many papers have been published to show the correlation between F2-isoprostanes and liver diseases (Moore, 2004). Since the mechanism of hepatotoxicity induced by carbon tetrachloride has been well established, this system is appropriate to validate the usefulness of HODE and F2-isoprostanes and their relationship.

The biomarker for oxidative damage can be also used for the evaluation of in vivo activity of antioxidants. This is practically important for the assessment and evaluation of antioxidant capacity of natural and synthetic compounds, foods, beverages, supplements, and their components in vivo. It has been well established that vitamin E attenuates the hepatotoxicity induced by carbon tetrachloride (Corongju et al., 1985; el-Kateb et al., 1965; Poli et al., 1985b; Yoshikawa et al., 1982). The esters of vitamin E (Tirmenstein et al., 1999) and synthetic vitamin E-like antioxidant (Campo et al., 2001) have been also found to reduce carbon tetrachloride-induced liver injury. Vitamin E is a generic description for four isomers of tocopherol (T) and tocotrienol (T3) derivatives. α-T is the most abundant vitamin E in vivo, although γ-T is often contained in as much as, or even higher than, α-T in the diet and plant. α-Tocopherol transfer protein (α-TTP) in liver selectively transports α-T to the blood stream. The role of γ-T and tocotrienols has received much attention recently (Yoshida et al., 2003).

BO-653, 2,3-dihydro-5-hydroxy-2,2-dipentyl-4,6-di-*tert*-butylbenzofuran, is a synthetic antioxidant which has been designed for a potent radical-scavenging antioxidant (Noguchi et al., 1997a). It has been found that BO-653 scavenges free radicals as rapidly as α-T, the aryloxyl radical derived from BO-653 is much stable than α-tocopheroxyl radical (Watanabe et al., 2000), and that it inhibits the oxidation of low density lipoprotein (Noguchi et al., 1997b) and plasma (Itoh et al., 2004) more efficiently than α-T. In the present study, the protective effects of γ-T, γ-T3, and BO-653 against carbon tetrachloride-induced oxidative damage were assessed from HODE in comparison with F2-isoprostanes in the absence of α-T by using α-TTP knock out (α-TTP$^{-/-}$) mice which were fed vitamin E-deficient diet.

Methods

Reagents. BO-653 was prepared as described previously (Tamura et al., 2003). Natural forms of γ-tocopherol (γ-T) and γ-tocotrienol (γ-T3) were kindly supplied by Eisai Co. Carbon tetrachloride used as an acute stressor was obtained from Wako Pure Chemical Industries (Osaka, Japan). 8-*iso*-Prostaglandin $F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$), 8-*iso*-prostaglandin $F_{2\alpha}$-d_4 (8-*iso*-$PGF_{2\alpha}$-d_4), 13-hydroxy-9(Z), 11(E)-octadecadienoic acid (13-(Z,E)-HODE), 13-hydroxy-9(E), 11(E)-octadecadienoic acid (13-(E,E)-HODE), 9-hydroxy-10(E), 12(Z)-octadecadienoic acid (9-(E,Z)-HODE), 9-hydroxy-10(E), 12(E)-octadecadienoic acid (9-(E,E)-HODE) were from Cayman Chemical Company (MI, USA). Other materials were those of the highest grade available commercially.

Administration of carbon tetrachloride to mice. Male mice (specific pathogen-free, C57BL/6J (α-TTP$^{+/+}$ mice), weighing 19-24 g) were purchased from Nippon Clea Co. (Tokyo, Japan). Male α-TTP$^{-/-}$ mice (B6.129S7 α-Ttp^{tm1Csk} (Jishage et al., 2001)) from in-house colony were also used (weighing 19–24 g). Mice were fed a vitamin E-free diet (Funabashi Nojyo, Chiba, Japan) or a laboratory standard