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Role of transition metals, Fe(II), Cr(II), Pb(II), and Cd(II) in lipid peroxidation

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Abstract

Cod liver oil was oxidized with Fenton-like systems containing transition metals Fe(II), Cr(II), Pb(II), and Cd(II). Malonaldehyde (MA) formed from 10 μl cod liver oil oxidized by a Fenton-like system containing each metal at levels of 0.25, 0.5, 1 and 4 μmol was analyzed by a gas chromatograph equipped with a nitrogen phosphorus detector. The MA production exhibited dose response and the greatest amount (837.0 ± 19.1 nmol) was obtained by the Fe(II) system at the level of 1 μmol. Generally, higher MA formation is observed in the lower the third ionization potential of the metal. The decreasing order of MA formed in the metal systems at the level of 1 μmol is Fe(II) > Cr(II)(274.1 ± 20.1 nmol) > Pb(II)(150.7 ± 13.0 nmol) > Cd(II)(95.4 ± 6.7 nmol). The amounts of MA formed in Cr(II), Pb(II), and Cd(II) systems were considerably lower than those in the Fe(II) system. The relative formations of MA in the Cr(II) and Pb(II) systems were similar to those in the Fe(II) system. The results suggest that trace amounts of metals contribute oxidative effects to lipid peroxidation followed by various diseases.
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Keywords: Cadmium; Chromium; Cod liver oil; Fenton reagent; Iron; Lead; Lipid peroxidation; Malonaldehyde

1. Introduction

The association between lipid peroxidation and various diseases including atherosclerosis, diabetes, Alzheimer's, cancer and AIDS is well known (Uchiyama, Matsuo, & Sagai, 1985). The oxidative degradation of lipids has, therefore, been studied widely in order to investigate the mechanisms of diseases associated with lipid peroxidation (Shibamoto, 2006). In order to initiate lipid peroxidation, a lipid molecule must be activated by an initiator. The most common initiators are reactive oxygen species (ROS). They are superoxide ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), triplet oxygen ($^3O_2$), hydroxyl radical ($^{\cdot}OH$), alkoxyl radical ($RO^{\cdot}$), and peroxy radical ($ROO^{\cdot}$). ROS play an important role in lipid peroxidation. There have been many reports on proposed mechanisms of lipid peroxidation. One of the most well known mechanisms is the autoxidation of unsaturated fatty acids such as linoleic acid, linolenic acid, arachidonic acid, various ω-3 fatty acids, and cod liver oil (Barclay & Ingold, 1981; Chan & Levett, 1977; Porter, Wolf, Yarbro, & Weenen, 1979; Roozen, Frankel, & Kinsella, 1994).

The autoxidation of unsaturated fatty acids occurs slowly, initiated by a triplet oxygen ($^3O_2$) (Simic & Karel, 1979). ROS abstracts a hydrogen atom from a methylene group of an unsaturated fatty acid and subsequently forms free radicals such as a peroxy radical (Matsuo, 1985). Once these free radicals are formed, lipid peroxidation progresses and, consequently, many secondary toxic oxidation products are formed. These products, such as formaldehyde, acetaldehyde, malonaldehyde, and glyoxal, subsequently promote adverse effects in the biological system (Shibamoto, 2006).

The two major components involved in lipid peroxidation are unsaturated fatty acids, such as ω-3 fatty acids, and oxygen. The lipid peroxidation in polyunsaturated fatty acids is most commonly a radical-chain process

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involving free radicals (Potter, 1968; Sikorski & Kolakowska, 2002). The key step in the initiation of lipid peroxidation is hydrogen abstraction from the allylic bond of polyunsaturated fatty acids. The hydroxyl radical (·OH) is the most efficient ROS to abstract hydrogen and initiate a chain reaction of lipid peroxidation.

Autocatalysis of the chain reaction process occurs via formation of ·OH by the action of a metal ion, such as Fe^{2+}. Once ·OH is produced, the chain reaction of lipid peroxidation occurs. Therefore, metals present in biological systems, such as Fe(II), Cr(II), Pb(II), and Cd(II), may play an important role in *in vivo* lipid peroxidation. In the present study, oxidation of ω-3 fatty acid rich, cod liver oil was induced by these metals to investigate their roles in lipid peroxidation.

2. Materials and methods

2.1. Chemicals

Cod liver oil fatty acid methyl esters, ferrous chloride tetrahydrate, lead chloride, chromium chloride, cadmium chloride, trizma HCl, trizma base, *N*-methylhydrazine (NMH), 1-methylpyrazole (1-MP), and 2-methylpyrazine (2-MP) were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Butylated hydroxy toluene (BHT), sodium dodecyl sulfate (SDS), and hydrogen peroxide were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Donor horse serum and donor bovine serum were purchased from Atlanta Biologicals (Lawrenceville, GA, USA).

2.2. Oxidation of cod liver oil by Fenton's reagent

Cod liver oil was oxidized using a previously reported method (Dennis & Shibamoto, 1989). An aqueous solution (5 ml) containing 0.25 mmol phosphate buffer (pH 7.4), 1 μmol ferrous chloride, 0.5 μmol hydrogen peroxide, 0.75 mmol potassium chloride, 0.2% surfactant sodium dodecyl sulfate and 10 μl cod liver oil fatty acid methyl esters was prepared. Samples were vortexed for 10 s and incubated in a water bath at 37 °C for 16 h. While incubating, the samples were covered in aluminum foil to prevent any influence of light on the peroxidation system. After incubation, oxidation was stopped by adding 50 μl 4% BHT and vortexing for 10 s according to a previously reported method (Ichinose, Miller, & Shibamoto, 1989).

2.3. Oxidation of cod liver oil in the presence of transition metals

Samples were prepared exactly as above, but cadmium chloride, chromium chloride, and lead chloride were substituted for ferrous chloride in the Fenton system. Different amounts of each metal (0.25, 0.50, 1.0, 2.0 or 4.0 μmol) were added and the samples were vortexed and incubated as above.

2.4. Analysis of malonaldehyde formed from cod liver oil oxidized with Fenton's systems

To each of the samples produced by the methods described above, 50 μl *N*-methylhydrazine was added and the samples were vortexed for 10 s. The samples were then placed back into the water bath for 45 min to complete the derivatization of malonaldehyde as 1-MP. Samples were then loaded onto a C18 SPE cartridge (Varian, Inc., Chicago, IL, USA), which had been previously conditioned with two column volumes of HPLC-grade methanol and two column volumes of deionized water. Samples were loaded drop-wise at a rate of 1 ml/min, washed with a 2 ml aliquot of deionized water and dried under vacuum for 30 min. 1-MP was eluted with 5 ml ethyl acetate and the column was rinsed with a 2 ml aliquot of ethyl acetate. The solution was filtered through sodium sulfate and 50 μl of a 2-methylpyrazine solution (1 mg/ml ethanol) was added as an internal standard. More ethyl acetate was added to make a final volume of 5 ml. Except for the samples containing cod liver oil, all samples were then concentrated to a volume of 1 ml by removing ethyl acetate under a purified nitrogen stream for analysis by a gas chromatograph/nitrogen phosphorous detector (GC/NPD).

An Agilent 6890 GC equipped with a DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 μm) (J&W Scientific, Folsom, CA) and a NPD was used for quantification of 1-MP. The initial oven temperature was 40 °C, held for 1 min, and programmed at 10 °C/min to 90 °C. The injector temperature was 200 °C and the detector temperature was 325 °C. The helium carrier gas flow rate was 1 ml/min.

3. Results and discussion

It is well known that iron serves as a catalyst for the formation of the highly reactive hydroxyl radical via Fenton reaction (Goldstein, Meyerstein, & Czapski, 1993; Yamazaki & Piette, 1991):

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + HO^{\cdot} + HO^{-}$$

In addition to ferrous ion, many metal ions including Cu(I), Cr(II), and Co(II) were found to have the oxidative features of the Fenton reagent. Therefore, the mixtures of these metal compounds with H_2O_2 were named "Fenton-like reagents" (Czapski, Samuni, & Meisel, 1971; Davies, Sutin, Kay, & Walkins, 1970; Moorhouse, Halliwell, Grootveld, & Gutteridge, 1985). In actual *in vivo* systems, once organic peroxides (ROOH) are formed by the action of ROS, heat, and/or photoirradiation, ROOH can be substituted for HO and ROOH reacts with metal ions to form alkoxyl radicals (Sheldon & Kochi, 1981). Therefore, the general form of the Fenton-like reaction is:

$$ROOH + M^{n+} + H^{+} \rightarrow M^{n+1} + H_2O + RO^{\cdot}$$

Subsequently, a chain reaction of lipid peroxidation occurs.

In order to investigate the role of relevant metals in *in vivo* systems, Fe (II), Cr(II), Pb(II), and Cd(II) were used

in Fenton-like reagent systems to induce lipid peroxidation of cod liver oil. Cod liver oil was used, because it contains high levels of ω-3 fatty acids including (10% of EPA and 30–33% of DHA) (Kinsella, 1987). ω-3 Fatty acids are known to posses various medicinal effects such as the reduction of coronary heart disease (Kinsella, Lokesh, & Stone, 1990), the effect on hypertension (Simpopoulos, 1991), and the effect on atherosclerosis (Robinson & Stone, 2006), and the effect on cardiovascular (Leaf & Weber, 1988; Von Schacky & Harris, 2007) and have been used to treat various diseases (Von Schacky, 2006). Therefore, these ω-3 fatty acids may contact metal ions and subsequently lipid peroxidation occurs *in vivo*.

Fig. 1 shows the amounts of MA formed from cod liver oil oxidized by the Fenton-like reagents. The values are mean ± SD ($n = 3$). Monitoring MA formation from lipids during oxidation is the most widely and commonly used method to investigate the lipid peroxidation associated with various diseases (Shibamoto, 2006). Generally, the lower the third ionization potential of the metal (Ionization energies of the elements, 2007) used the higher MA formation that was observed. In increasing order, the third ionization potential of the metals used is Fe(II)(2957 kJ/mol) < Cr(II)(2987 kJ/mol) < Pb(II)(3081.5 kJ/mol) < Cd(II)(3616 kJ/mol). The decreasing order of MA formed in the metal systems at the level of 1 μmol is Fe(II)(837.0 ± 19.1 μmol) > Cr(II)(274.1 ± 20.1 nmol) > Pb(II)(150.7 ± 13.0 nmol) > Cd(II) (95.4 ± 6.7 nmol). The MA production exhibited dose response and the greatest amount (837.0 ± 19.1 nmol) was obtained by the Fe(II) system at the level of 1 μmol. However, the highest level of Fe(II) (4 μmol) in the testing system showed less MA (223.9 ± 11.1 nmol) than the 0.5 μmol level (697.6 ± 19.8 nmol). The excess metal ion may inhibit MA formation. There may be many factors influencing MA formation from lipids by various metals. For example, the quantitative measurement of reaction of Fe(II) and H_2O_2 shown above had reportedly shown that a stoichiometric amount of ·OH is spin-trapped when ion concentration was less than 1 μM, suggesting that the strength of the Fenton system depended on the metal concentration (Yamazaki & Piette, 1991). Since Fenton reported that a mixture of hydrogen peroxide and ferrous salts was an effective oxidant of a large variety of organic substrates in 1894 (Fenton, 1984), this reagent (the so-called Fenton's reagent) has been used to investigate many subjects related to *in vitro* oxidation of organic substrates including lipids (Halliwell & Gutteridge, 1985).

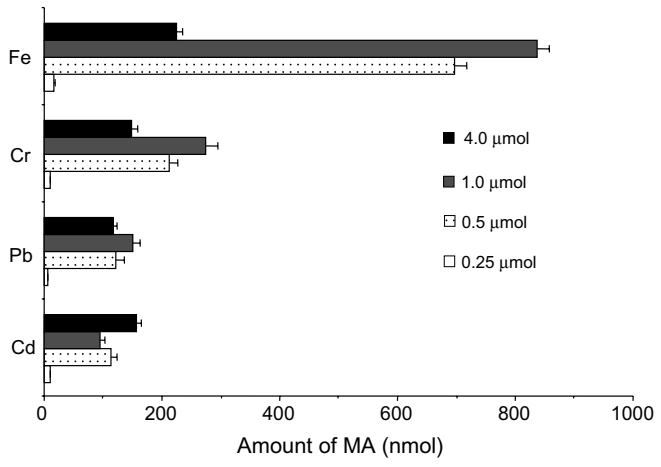

Fig. 1. Amounts of MA formed from cod liver oil oxidized by the Fenton-like reagents. Values are mean ± SD ($n = 3$).

The amounts of MA formed in the Cr(II), Pb(II), and Cd(II) systems were considerably lower than those in the Fe(II) system. The relative formations of MA in the Cr(II) and Pb(II) were similar to those in Fe(II) system. In the case of the Cr(II) system, the dose did not seem to be correlated to the MA formation at levels higher than 0.5 μmol. Chromium is a widely used industrial metal, with uses in steel and chrome production, as well as paints and wood treatment. Cr(III) occurs in nature and is an essential trace element used in the regulation of blood glucose levels. Cr(III) reacts with a superoxide ($O_2^{\cdot -}$) and produces Cr(II) as follows:

$$Cr^{3+} + O_2^{\cdot} \rightarrow Cr^{2+}O_2$$

Subsequently Cr(II) yields ·OH via Fenton-like reaction with H_2O_2 to initiate lipid peroxidation (Samuni, Meisel, & Czapski, 1972).

The Pb(II) system produced similar amounts of MA (116–150 nmol) at the three levels tested (0.5, 1 and 4 μmol). Lead is found in nature and is one of the most abundant metals in the environment. It has been used in paint, gasoline, and pesticides in large amounts. In particular, lead from automobile exhaust gases was the major metal contaminants in the ambient air prior to 1976 (NAPE, 1993). Adverse effects of lead have been well documented and children of all races and ethnic origins are reportedly at risk of lead poisoning throughout the United States (Kinder, 1997). There are several reports on the role of lead in lipid peroxidation associated with metal toxicities (Donaldson & Knowles, 1993). For example, the administration of lead compounds to rats resulted in an increase of lipid peroxidation in the brain (Rehman, 1994). These reports and the present study indicate that lead promotes *in vivo* lipid peroxidation.

MA formation in the Cd(II) system behaved somewhat differently from the other systems. Dose dependent responses were not observed within the testing range (0.25–4.0 μmol). The greatest amount of MA (157.6 ± 7.9 nmol) was obtained at the highest level of Cd(II) (4 μmol). Cadmium is a highly toxic metal that is becoming a growing environmental concern as result of its use in industry, such as electroplating, paint production, and battery manufacturing. There have been many reports on the adverse effects, including cancer, caused by cadmium toward various organs such as lung, prostate, pancreas, kidney, and brain as well as heart central nervous system (Nawrot et al., 2006; Waalkes, 2003). There is some evidence that cadmium induces *in vivo* lipid peroxidation.

For example, cadmium intoxication was shown to increase lipid peroxidation in rat liver, kidney and heart (Sarkar, Poonam, & Bhatnagar, 1997). However, the mechanisms of cadmium toxicity are not fully understood. Cadmium indirectly affects the generation of various radicals including superoxide and hydroxyl radical (Galan et al., 2001). The generation of hydrogen peroxide by cadmium ion may become a source of radicals in the Fenton system (Watanabe, Henmi, Ogawa, & Suzuki, 2003).

As mentioned above, we are exposed to many different metals, regardless of whether they are essential metals, such as iron and chromium, or not. Once they enter our physiological systems, these metals may play a role in oxidative adverse effects. Some transition metals including iron, chromium, lead, and cadmium generated lipid peroxidation in cod liver oil in the present study, suggesting that metals contribute oxidative effects to lipid peroxidation followed by various diseases.

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