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ORIGINAL CONTRIBUTION

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Vitamin E reduces lipid peroxidation in experimental hepatotoxicity in rats

Abstract *Background and aims* Lipid peroxidation is believed to be involved in the pathophysiology of a number of diseases and in the process of aging. This study investigates the effects of dietary supplementation with vitamin E (20 g/kg diet of all-rac-α-tocopheryl succinate for 3 weeks) on both non-enzymatic and enzymatic lipid peroxidation in experimental rats with carbon tetrachloride (CCl_4)-induced hepatotoxicity (2.5 mL/kg body). *Methods* Plasma, urine and liver samples from control rats (n = 6), CCl_4-treated rats (n = 6), and rats supplemented with vitamin E prior to CCl_4 treatment (n = 8) were collected. Non-enzymatic lipid peroxidation induced by free radicals was investigated by measurement of a major F_2-isoprostane, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$). Cyclooxygenase-catalyzed enzymatic lipid peroxidation was measured with a major $PGF_{2\alpha}$ metabolite, 15-keto–13,14-dihydro-prostaglandin $F_{2\alpha}$ (15-K-DH-$PGF_{2\alpha}$). Malondialdehyde and antioxidants in plasma were also quantified. *Results* CCl_4 treatment alone resulted in significantly higher levels of plasma, urinary and liver 8-iso-$PGF_{2\alpha}$, and of plasma and urinary 15-K-DH-$PGF_{2\alpha}$ compared to controls. Rats supplemented with vitamin E prior to CCl_4 treatment had significantly lower levels of urinary and liver 8-iso-$PGF_{2\alpha}$, urinary 15-K-DH-$PGF_{2\alpha}$, and plasma malondialdehyde than rats treated with CCl_4 alone. However, plasma 8-iso-$PGF_{2\alpha}$ and plasma 15-K-DH-$PGF_{2\alpha}$ were not affected by vitamin E supplementation. *Conclusion* Thus, both non-enzymatic and enzymatic lipid peroxidation during experimental hepatic oxidative injury were suppressed by dietary vitamin E supplementation in rats.

Key words Vitamin E – carbon tetrachloride – lipid peroxidation – rats

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Introduction

Free radical-mediated peroxidation of biological molecules, especially lipids, are implicated in a variety of pathological events, such as cardiovascular diseases, rheumatoid arthritis, inflammatory disorders, cancer, and also in the process of aging [1]. Several methods have been developed to assess free radical-induced non-enzymatic lipid peroxidation, but most of these methods have different shortcomings and limitations [2, 3].

In the early 1990s, a group of prostaglandin (PG) F_2-like compounds, F_2-isoprostanes, were reported to be formed by free radical-induced peroxidation of arachidonic acid independent of the cyclooxygenase pathway [4]. F_2-isoprostanes are initially formed in situ from esterified arachidonic acids in phospholipids and are then released in the free form into the circulation and excreted in the urine [5, 6]. 8-Iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), one of the major F_2-isoprostanes formed in vivo [7], excerts potent biological activity [4, 8] and is a potential biomarker of non-enzymatic lipid peroxidation and oxidative injury [9, 10].

Enzymatic oxidation of arachidonic acid via the cyclooxygenase pathway leads to the formation of prostaglandins [11]. Several pro-inflammatory stimuli

induces cyclooxygenase–2, an isoform of cyclooxygenase, in macrophages, epithelial cells, and fibroblasts leading to release of prostaglandins [12–15]. A major metabolite of the primary prostaglandin $F_{2\alpha}$, 15-keto–13,14-dihydro-prostaglandin $F_{2\alpha}$ (15-K-DH-$PGF_{2\alpha}$), can be used as an index of inflammation and enzymatic lipid peroxidation via cyclooxygenase-catalyzed oxidation of arachidonic acid [16].

Highly specific and sensitive radioimmunoassays for the measurement of both 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ were recently developed and validated [17, 16]. The use of these radioimmunoassays is an excellent approach for simultaneous measurement of non-enzymatic and enzymatic lipid peroxidation in vivo. Levels of both 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ in plasma and urine have been shown to be increased in an animal model of carbon tetrachloride (CCl_4)-induced hepatotoxicity [18] and in experimental septic shock [19, 20].

Lipid peroxidation induced by CCl_4 is a widely used experimental animal model of oxidative injury. The liver is the major target organ of CCl_4 toxicity owing to its high content of cytochrome P–450, which metabolizes CCl_4 to trichloromethyl radicals leading to peroxidation of unsaturated lipids [21]. Antioxidants are recognized to scavenge free radicals and may, therefore, prevent propagation of the CCl_4-induced lipid peroxidation process. Vitamin E is a well-characterized chain-breaking antioxidant with the particular function of preventing lipid peroxidation in membrane systems [22].

In a first part of a larger project we have reported that basal levels of F_2-isoprostanes and prostaglandin $F_{2\alpha}$ were suppressed by vitamin E supplementation in rats [23]. The aim of this study was to investigate if dietary supplementation with vitamin E could suppress the formation of both non-enzymatic and enzymatic lipid peroxidation products as induced in experimental hepatotoxicity in rats by CCl_4. The untreated control animals that are reported in this study are the same as in our previous study [23]. We measured 8-iso-$PGF_{2\alpha}$ as an index of non-enzymatic lipid peroxidation and 15-K-DH-$PGF_{2\alpha}$ as a biomarker of enzymatic lipid peroxidation in plasma, urine, and liver tissue. Malondialdehyde and antioxidants in plasma were also quantified.

Materials and methods

Animals and diets

Male Sprague-Dawley rats (6 weeks old, ~200 g) were purchased from B & K Universal (Sollentuna, Sweden). The rats had free access to tap water and food and were subjected to a 12 h light/12 h dark schedule. Powdered food was prepared for all rats from commercial food pellets (R36; Lactamin AB, Stockholm, Sweden) containing total lipids 4%, protein 18.5%, carbohydrates 55.7%, fibers 3.5%, and vitamin E at 63 mg/kg. For vitamin E treatment, all-rac-α-tocopheryl succinate (Merck, Darmstadt, Germany) was blended into the powdered food at a concentration of 20 g/kg diet resulting in a daily intake of ~2 g/kg body. All rats received powdered food for a period of 3 weeks.

Experiment and sample collection

For CCl_4 treatment, rats were gavaged with CCl_4 (2.5 mL/kg body) and samples were collected at 4 h after CCl_4 administration. Samples were collected from control rats (n = 6), CCl_4-treated rats (n = 6), and rats supplemented with vitamin E prior to CCl_4 treatment (n = 8). Urine samples were collected in petri dishes. The rats were weighed and surgical anesthesia was induced with ether. During laparotomy, livers were excised and blood samples were drawn from the abdominal aorta. Rats were killed by heart puncture. Blood samples were collected in heparinized glass vials and plasma was prepared by centrifugation at 1930 × g for 8 min. All samples were immediately stored at –20°C during the experiment and thereafter at –70°C until analysis. The animal experimental procedure was approved by the Animal Ethics Committee of the Medical Faculty of Uppsala University.

Preparation of liver tissues

Liver samples were weighed, diluted with 3 volumes of phosphate buffer, and homogenized under cold conditions. The homogenate was centrifuged at 1680 × g and 4°C for 10 min and the supernatant was stored at –70°C until further preparation within 1 week. For measurement of the total amount of 8-iso-$PGF_{2\alpha}$ (sum of free and esterified 8-iso-$PGF_{2\alpha}$), the homogenate was first subjected to base hydrolysis by incubation with 3 volumes of 3 mol/L KOH at 37°C for 60 min, before acidification to pH 3–4 with HCl and extraction with 3 volumes of ethyl acetate. Extracted fractions were centrifuged at 1680 × g and 4°C for 10 min and the supernatant was evaporated under nitrogen. Samples were finally rediluted in < 5% ethanol (total volume concentration) and phosphate buffer and stored at –70°C until analysis within 2–8 weeks.

Radioimmunoassay of 8-iso-$PGF_{2\alpha}$

Plasma, urine, and liver samples from this study were analyzed for 8-iso-$PGF_{2\alpha}$ using a newly developed radioimmunoassay [17]. In brief, an antibody was raised in rabbits by immunization with 8-iso-$PGF_{2\alpha}$ coupled to bovine serum albumin at the carboxylic acid by the 1,1'-

carbonyldiimmidazole method. The cross-reactivity of the antibody with 8-iso–15-keto–13,14-dihydro-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto–13,14-dihydro-$PGF_{2\alpha}$, TXB_2, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, and 8-iso-$PGF_{3\alpha}$ was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8, and 0.6 %, respectively. The detection limit of the assay was about 23 pmol/L. Unextracted plasma and urine samples of various volumes and dilutions were used in the assay for measurement of free 8-iso-$PGF_{2\alpha}$ concentrations. Levels of 8-iso-$PGF_{2\alpha}$ in urine were adjusted for creatinine concentration measured by a colorimetric method using IL test creatinine 181672–00 in a Monarch 2000 centrifugal analyzer (Instrumentation Laboratories, Lexington, MA). Various volumes and dilutions of the liver sample preparations were used in the assay for the quantification of total 8-iso-$PGF_{2\alpha}$.

Radioimmunoassay of 15-keto–13,14-dihydro-$PGF_{2\alpha}$

Plasma and urine samples from this study were analyzed for 15-K-DH-$PGF_{2\alpha}$ using a newly developed radioimmunoassay [16]. Briefly, an antibody was raised in rabbits by immunization with 15-K-DH-$PGF_{2\alpha}$ coupled to bovine serum albumin at the carboxylic acid by the 1,1'-carbonyldiimmidazole method. The cross-reactivity of the antibody with $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, PGE_2, 15-keto–13,14–dihydro-PGE_2, 8-iso–15-keto–13,14-dihydro-$PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, TXB_2, and 8-iso-$PGF_{3\alpha}$ was 0.02, 0.43, < 0.001, 0.5, 1.7, < 0.001, < 0.001, < 0.001, and 0.01 %, respectively. The detection limit of the assay was about 45 pmol/L. Unextracted plasma and urine samples of various volumes and dilutions were used in the assay. Levels of 15-K-DH-$PGF_{2\alpha}$ in urine were adjusted for creatinine concentration.

Measurement of malondialdehyde

Malondialdehyde levels in plasma samples were measured using HPLC with fluorescence detection as described previously by Öhrvall et al. [24]. In brief, a thiobarbituric acid reaction was initiated by mixing 200 µL of plasma sample with 750 µL of 0.15 mol/L phosphoric acid, 300 µL of water and 250 µL of 42 mmol/L thiobarbituric acid. The reaction mixture was incubated in a boiling water bath for 60 min and then cooled on ice. The malondialdehyde-thiobarbituric acid complex was extracted with methanol and 20 µL of the sample was injected into a HPLC column (Lichrospher 100 RP–18, 250 x 4 mm). The mobile phase contained methanol/50 mmol/L phosphate buffer (2/3). Fluorescence was measured with an excitation wavelength of 532 nm and an emission wavelength of 553 nm.

Measurements of antioxidants

Plasma α-tocopherol was determined using HPLC with fluorescence detection [25]. Briefly, 500 µL of plasma was extracted with 500 µL of ethanol containing 0.05 g/L butylated hydroxytoluene and 2 mL of hexane. A 20-µL volume of supernatant was injected into a HPLC column (LiChrospher 100 NH_2, 250 x 4 mm). Fluorescence was measured with an excitation wavelength of 295 nm and an emission wavelength of 327 nm.

Plasma antioxidative capacity was measured as trolox equivalents by a modified chemiluminescence assay described by Öhrvall et al. [26]. The assay is based on measurement of light emission when a chemiluminescent substrate, luminol, is oxidized by hydrogen peroxide in a reaction catalyzed by horseradish peroxidase. Suppression of the light output by antioxidants is related to the antioxidative capacity of the sample. Uricase was used to eliminate the urate content in the sample resulting in an antioxidative capacity value without urate.

Statistics

Data are presented as means ± SD. All variables were continuous and on an interval scale. Variables that were normally distributed or log-normally distributed were tested with parametric methods and other variables were tested with non-parametric tests. Differences between the three groups were first tested in an overall test using either analysis of variance or the non-parametric Kruskal Wallis test. In case of a significant overall test, pair-wise comparisons were made using unpaired Student's t-test or Mann Whitney's non-parametric test. All tests were two-tailed and the significant level was 0.05. The statistical analyses were performed using the statistical software package JMP (SAS Institute, Cary, NC).

Results

Effects of CCl_4 treatment

Oxidative injury was induced by oral administration of CCl_4. Both plasma and urinary 8-iso-$PGF_{2\alpha}$ levels were significantly higher in rats treated with CCl_4 compared to control rats ($P < 0.05$ and $P < 0.01$ respectively, Fig. 1). The levels of 8-iso-$PGF_{2\alpha}$ in the liver were also increased after CCl_4 treatment compared to the controls ($P < 0.01$, Fig. 2).

The effects of CCl_4 administration on the levels of 15-K-DH-$PGF_{2\alpha}$ were similar to those on 8-iso-$PGF_{2\alpha}$. Plasma and urinary levels of 15-K-DH-$PGF_{2\alpha}$ were significantly higher after CCl_4 treatment compared to the levels of control rats ($P < 0.001$, Fig. 3).

Plasma concentrations of malondialdehyde and α-

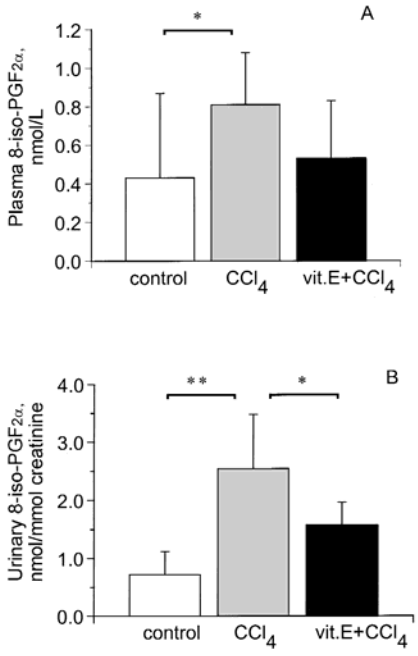

Fig. 1 Effect of CCl_4 treatment (2.5 mL/kg body) with or without prior supplementation with vitamin E daily for 3 weeks (2 g/kg body) on levels of 8-iso-$PGF_{2\alpha}$ in plasma (A) and urine (B) in rats. Values are means ± SD of controls (n = 6), CCl_4-treated rats (n = 6), and vitamin E-supplemented CCl_4-treated rats (n = 8). * Indicates differences between groups (* $P < 0.05$ and ** $P < 0.01$).

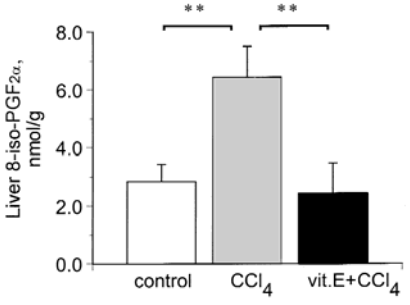

Fig. 2 Effect of CCl_4 treatment (2.5 mL/kg body) with or without prior supplementation with vitamin E for 3 weeks (2 g/kg body) on levels of total 8-iso-$PGF_{2\alpha}$ in liver samples in rats. Values are means ± SD of controls (n = 6), CCl_4-treated rats (n = 6), and vitamin E-supplemented CCl_4-treated rats (n = 8). *Indicates differences between groups (** $P < 0.01$).

tocopherol did not differ between the two groups, whereas the antioxidative capacity was slightly greater in CCl_4-treated rats compared to control rats ($P < 0.05$, Table 1). Both control rats and CCl_4-treated rats gained weight during the 3-week-intervention period and the final body weights did not differ between the groups (Table 1).

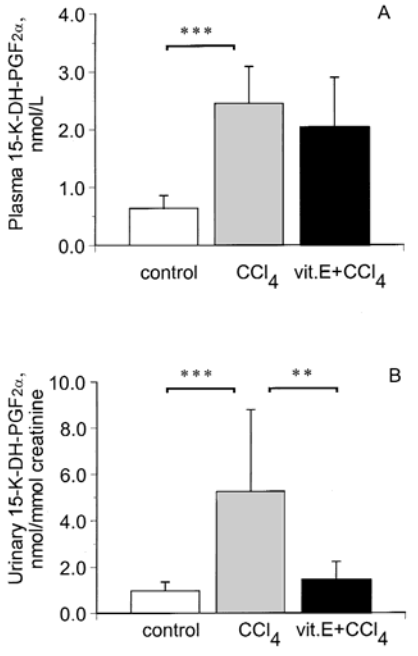

Fig. 3 Effect of CCl_4 treatment (2.5 mL/kg body) with or without prior supplementation with vitamin E for 3 weeks (2 g/kg body) on levels of 15-K-DH-$PGF_{2\alpha}$ in plasma (A) and urine (B) in rats. Values are means ± SD of controls (n = 6), CCl_4-treated rats (n = 6), and vitamin E-supplemented CCl_4-treated rats (n = 8). * Indicates differences between groups (** $P < 0.01$ and *** $P < 0.001$).

Tab. 1 Effects of CCl_4 treatment and vitamin E supplementation in rats on the levels of malondialdehyde, α-tocopherol and antioxidative capacity in plasma and on the body weight

	Control	CCl_4	Vitamin E + CCl_4
Malondialdehyde (μmol/L)	2.54 ± 1.19	2.44 ± 0.65	1.56 ± 0.47*
α-Tocopherol (μmol/L)	17.9 ± 1.7	15.5 ± 2.6	29.2 ± 5.6***
Antioxidative capacity (μmol trolox equivalents/L)	181 ± 6	212 ± 18§	292 ± 29***
Final body weight (g)	364 ± 9	369 ± 8	373 ± 7

Control rats are compared to rats administered CCl_4 (2.5 mL/kg body) with or without prior dietary supplementation with vitamin E daily for 3 weeks (2 g/kg body). Values are means ± SD of controls (n = 6), CCl_4-treated rats (n = 6) and vitamin E-supplemented CCl_4-treated rats (n = 8). § Indicates difference from control mean (§ $P < 0.05$) and * indicates difference from the CCl_4-treated group (* $P < 0.05$; *** $P < 0.001$).

Effects of vitamin E supplementation prior to CCl_4 treatment

Rats supplemented with vitamin E in the diet prior to CCl_4-induced oxidative injury had lower levels of urinary 8-iso-$PGF_{2\alpha}$ compared with the rats treated with CCl_4 alone ($P < 0.05$, Fig. 1B). In the liver, levels of 8-iso-$PGF_{2\alpha}$ were also lower in vitamin E-supplemented CCl_4-treated rats compared with rats treated with CCl_4 only ($P < 0.01$, Fig. 2). However, plasma 8-iso-$PGF_{2\alpha}$ levels did not differ between CCl_4-treated rats with and without vitamin E supplementation (Fig. 1A).

Vitamin E supplementation prior to CCl_4 treatment resulted in lower urinary levels of 15-K-DH-$PGF_{2\alpha}$ compared to CCl_4-treated rats ($P < 0.01$, Fig. 3B), whereas there was no difference in plasma 15-K-DH-$PGF_{2\alpha}$ levels between vitamin E-supplemented CCl_4-treated rats and rats treated with CCl_4 only (Fig. 3A).

Rats supplemented with vitamin E prior to CCl_4 treatment had lower levels of plasma malondialdehyde than CCl_4-treated rats ($P < 0.05$; Table 1). Regarding antioxidants in the circulation, both α-tocopherol levels and the antioxidative capacity in plasma were greater in vitamin E-supplemented rats even after CCl_4 treatment when compared to CCl_4 treatment alone ($P < 0.001$ and $P < 0.001$ respectively, Table 1). The final body weights did not differ between the CCl_4-treated rats and the rats supplemented with vitamin E prior to CCl_4 treatment (Table 1).

Discussion

In this study we investigated the effect of dietary supplementation with vitamin E on non-enzymatic free radical-induced and enzymatic cyclooxygenase-mediated lipid peroxidation in CCl_4-induced hepatotoxicity in rats. CCl_4 is a well-known model compound for induction of free radical damage in vivo [21]. The antioxidant vitamin E is a chain-breaking antioxidant preventing lipid peroxidation in membranes by scavenging of free radicals [22]. Vitamin E may, therefore, prevent propagation of the CCl_4-induced lipid peroxidation process through this mechanism. However, information on whether vitamin E also affects the inflammatory response through cyclooxygenase-mediated lipid peroxidation during hepatotoxicity is lacking. Our results indicate that both non-enzymatic and enzymatic lipid peroxidation during experimental hepatic oxidative injury can be suppressed by dietary vitamin E supplementation in rats.

The levels of 8-iso-$PGF_{2\alpha}$ (a major F_2-isoprostane) and 15-K-DH-$PGF_{2\alpha}$ (a major $PGF_{2\alpha}$ metabolite) in plasma and urine have been shown to be increased in experimental CCl_4-induced hepatotoxicity [17, 18] and in septic shock [19, 20], as measured with unique radioimmunoassays [17, 16]. Increased formation of 8-iso-$PGF_{2\alpha}$ after CCl_4 administration have also been reported in a series of previous experimental animal studies using GC/MS based methodologies [5, 6, 27]. In our study, significantly increased levels of plasma, urinary and total liver 8-iso-$PGF_{2\alpha}$, and of plasma and urinary 15-K-DH-$PGF_{2\alpha}$ were detected in CCl_4-treated rats compared to controls. However, these increases after CCl_4 treatment were less pronounced than increases reported earlier irrespective of the method used [5, 6, 17, 18]. The smaller increases in the formation of 8-iso-$PGF_{2\alpha}$ after CCl_4 treatment in our study could possibly be caused by less gastrointestinal absorption due to difficulties during CCl_4 administration to the rats.

To our knowledge, little is known about the effect of vitamin E on the formation of F_2-isoprostanes and $PGF_{2\alpha}$ metabolites after CCl_4-induced hepatotoxicity in vivo. A few studies have investigated the effect of vitamin E on 8-iso-$PGF_{2\alpha}$ in various animal models. We previously reported that vitamin E supplementation decreased the basal levels of both 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ in rats [23]. A reduced F_2-isoprostane generation was observed in apolipoprotein E-deficient mice [28] and in diabetic rats [29] after vitamin E supplementation, whereas vitamin E deprivation was related to increased levels of 8-iso-$PGF_{2\alpha}$ [30, 31]. In this study we used a high dose of vitamin E (2 g/kg body) to counteract the severe liver damage induced by CCl_4 and the subsequent lipid peroxidation. The dose of vitamin E used in our study is in the upper range used in various animal studies. In a review of safety studies of vitamin E intake by Kappus and Diplock [32], adverse effects were rarely observed with dosages up to 2 g/kg body in rats. All animals in this study gained weight and the final body weight did not differ between the groups. The supplementation with vitamin E was reflected by increased levels of α-tocopherol and antioxidative capacity even after CCl_4 administration.

We report here that supplementation with vitamin E prior to CCl_4 treatment significantly reduced the levels of urinary and liver 8-iso-$PGF_{2\alpha}$ and the levels of urinary 15-K-DH-$PGF_{2\alpha}$, as compared to CCl_4 treatment alone. However, plasma 8-iso-$PGF_{2\alpha}$ and plasma 15-K-DH-$PGF_{2\alpha}$ were not shown to be affected by vitamin E supplementation. The main reason why 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ in plasma was not suppressed by vitamin E supplementation, as it was in the urine or liver, is probably because the kinetics of formation and availability of 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ are different in the plasma, urine, and liver tissue. 8-Iso-$PGF_{2\alpha}$ is rapidly metabolized and efficiently excreted into the urine [33]. Urinary levels of 8-iso-$PGF_{2\alpha}$, therefore, reflect an earlier event of the biosynthesis and availability of 8-iso-$PGF_{2\alpha}$ in the body compared to plasma levels measured at the same time [33].

The reduced levels of 8-iso-$PGF_{2\alpha}$ indicate that the increased formation of free radicals after CCl_4 administration may have been scavenged by vitamin E to some degree and that the peroxidation of arachidonic acid thereby could partially have been prevented. Whether vitamin E has other functions, apart from scavenging of free radicals and reacting with active forms of oxygen, has not yet been established. However, interference of vitamin E with the cytochrome P–450 system and, thus, the lower production of 8-iso-$PGF_{2\alpha}$ cannot be ruled out. Furthermore, our study showed that the levels of a $PGF_{2\alpha}$ metabolite, 15-K-DH-$PGF_{2\alpha}$, in urine samples were decreased after CCl_4 treatment in the vitamin E-

supplemented rats compared to rats treated with CCl_4 alone. In an earlier experimental study of hepatotoxicity [18], it was shown that both the inflammatory response, as measured by 15-K-DH-$PGF_{2\alpha}$, and the oxidative injury, as measured by 8-iso-$PGF_{2\alpha}$, were increased. The oxidative injury increased before an increase in the inflammatory response could be seen, suggesting that the cyclooxygenase-dependent inflammatory response could possibly be a secondary effect of oxidative injury and a conceivable link between inflammation and oxidative stress [18].

Plasma malondialdehyde levels in this study were not affected by CCl_4 treatment and the concentration of malondialdehyde in plasma did not correlate with the increased levels of plasma 8-iso-$PGF_{2\alpha}$ after CCl_4 treatment, which is in agreement with previously described results [18]. The malondialdehyde methodology has been criticized for being non-specific, and artifacts may be produced during incubation with thiobarbituric acid in a boiling water-bath. The different responses after CCl_4 treatment between malondialdehyde and 8-iso-$PGF_{2\alpha}$ may also reflect different stages in the lipid peroxidation process.

In summary, this study demonstrates that dietary supplementation with vitamin E prior to CCl_4 treatment results in reduced levels of urinary and liver 8-iso-$PGF_{2\alpha}$, indices of non-enzymatic free radical-induced lipid peroxidation, as compared to levels after CCl_4 treatment alone. Vitamin E supplementation also reduced the level of urinary 15-K-DH-$PGF_{2\alpha}$, a biomarker of enzymatic lipid peroxidation. Thus, vitamin E supplementation may affect both free radical-induced oxidative injury and cyclooxygenase-catalyzed prostaglandin formation. The simultaneous non-invasive measurement of 8-iso-$PGF_{2\alpha}$ and 15-K-DH-$PGF_{2\alpha}$ is a promising approach for studies investigating the possible roles of lipid peroxidation under normal conditions and in the pathology of human diseases.

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