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Antioxidative effects of curcumin, β-myrcene and 1,8-cineole against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin–induced oxidative stress in rats liver

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Abstract

The aim of this study was to investigate the effectiveness of curcumin, β-myrcene (myrcene) and 1,8-cineole (cineole) on antioxidant defense system in rats given a persistent environmental pollutant (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD). Rats ($n = 112$) were divided randomly into 8 equal groups. One group was kept as control and given corn oil as carrier. TCDD was orally administered at the dose of 2 μg/kg/week. Curcumin, myrcene and cineole were orally administered at the doses of 100 mg/kg/day, 200 mg/kg/day and 100 mg/kg/day, respectively, by gavages dissolved in corn oil with and without TCDD. The liver samples were taken from half of all rats on day 30 and from the remaining half on day 60 for the determination of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GSH-Px) and CuZn-SOD levels by spectrophotometric method. The results indicated that although TCDD significantly ($p \leq 0.01$) increased formation of TBARS, it caused a significant decline in the levels of GSH, CAT, GSH-Px and CuZn-SOD in rats. In contrast, curcumin, myrcene and cineole significantly increased GSH, CAT, GSH-Px and CuZn-SOD levels but decreased formation of TBARS. Additionally, the antioxidative effects of curcumin, myrcene and cineole were increased at day 60 compared to day 30. In the TCDD groups given curcumin, myrcene and cineole, oxidative stress decreased by time. In conclusion, curcumin, myrcene and cineole showed antioxidant activity and eliminated TCDD-induced oxidative stress in rats in a time-dependent manner.

Keywords

2, 3, 7, 8-TCDD, curcumin, myrcene, cineole, oxidative stress

Introduction

2,3,7,8-Tetracholorodibenzo-*p*-dioxin (TCDD) is a environmental contaminant that has been recently found to produce some adverse effects in experimental animals (Alsharif and Hassoun, 2004; Hassoun et al., 2004). TCDD is formed as an unwanted by-product in the manufacture of chlorinated hydrocarbons. It is also formed in incineration processes, paper and pulp bleaching and emissions from steel foundries and motor vehicles (Latchoumycandane et al., 2003). Much of the concern with exposure to TCDD is due to their environmental and biological persistence, which may result in the bioconcentration and bioaccumulation of the chemicals up to the food chain

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(Guo et al., 2001). Therefore, long-term toxicity of TCDD has been the major focus of several studies that demonstrated histopathological changes, wasting syndrome, immunological disrupts and carcinomas after subchronic and chronic exposures of mice and rats (Ciftci et al., 2010; Hassoun et al., 2004; Slezak et al., 2000). Several studies have demonstrated that oxidative stress is an important constituent in the mechanism of TCDD toxicity (Hassoun et al., 1998; Slezak et al., 2000).

The liver has been long considered as a major target organ for the toxic effects of TCDD. It has been shown to enhance in vitro and in vivo hepatic and extrahepatic lipid peroxidation, decrease glutathione (GSH) content, decrease hepatic membrane fluidity increase DNA damage and decrease nonprotein sulfhydryl and NADPH content (Alsharif et al., 1990; Stohs, 1990). Reactive oxygen species have been implicated in TCDD-induced lipid peroxidation (Hassoun et al., 1998) and DNA damage in the hepatic of rodents and other animals (Alsharif and Hassoun, 2004; Hassoun et al., 2000, 2004; Slezak et al., 2000).

Curcumin, which gives the yellow color to turmeric derived from the *Curcuma longa*, is commonly used as a spice in curries, food additive and also as a dietary pigment (Aggarval et al., 2007). Curcumin reportedly possesses several pharmacological properties including antioxidant, anti-inflammatory, antiviral, antimicrobial and antifungal activities (Araujo and Leon, 2001). The antioxidant effect of curcumin has been assessed in various in vitro systems and in experimental animal systems (Kuhad et al., 2007). The chemoprotective properties of curcumin have also been extensively investigated and are linked to its antioxidant activities (Piper et al., 1998). Myrcene is an acyclic monoterpene found in the essential oils of a large variety of useful plants such as lemongrass, hop, verbena, bay and others (De-Oliveira et al., 1997). Myrcene is mainly used in the manufacturing of cosmetics fragranced products in shampoos, soaps and detergents. It has antioxidant and antibacterial properties (Mitić-Culafić et al., 2009). Cineole, also known as eucalyptol or cajeputol, is a colorless substance bearing a strong odor. This substance is present in a large quantity of plants such as *Rosmarinus officinalis* and various species of the eucalyptus gender (Ferreira da-silva et al., 2009). This compound was reported to have various pharmacological effects, such as smooth-muscle relaxant, anti-inflammatory, antioxidant and hypotensive (Ferreira da-silva et al., 2009).

The protective effects of many substances obtained from plants on oxidative stress are well known. As curcumin, myrcene and cineole have antioxidant properties, they can successfully be used against side effects of TCDD. Therefore, the aim of the current study was to produce oxidative stress in rats by TCDD and to compare the effectiveness of curcumin, myrcene and cineole on reversing TCDD-induced oxidative damage in rat liver.

Materials and Method

Chemicals

2,3,7,8-TCDD (purity >99%) was obtained from Accustandart, Inc. (New Haven, Connecticut, USA). All other chemicals, including curcumin, myrcene and cineole, were purchased from Sigma Chemical Co. (St Louis, Missouri, USA) and were of analytical grade or of the highest grade available.

Animals and Treatment

A total of 112 healthy young adult female Spraque-Dawley rats (between 3 and 4 months old and 280-310 g in weight) were obtained from Experimental Animal Institute, Elazig, Turkey, for this experiment. Animals were housed in sterilized polypropylene rat cages, in 12-h light-dark cycle, at an ambient temperature of 21°C. Diet and drinking water were given ad libitum. Experiments were performed based on animal ethics guidelines of Institutional Animals Ethics Committee.

Rats were randomly divided into 8 equal groups ($n = 14$ in each group). Group 1 served as negative control and received only corn oil (Control). In group 2 (TCDD group or positive control), TCDD, stock solution dissolved in acetone was diluted with corn oil, and then the acetone was evaporated under nitrogen before administration. TCDD was orally administered at the dose of 2 μg/kg/week by gavages. Rats in the groups 3, 4, 5 were treated with curcumin (group 3), myrcene (group 4) and cineole (group 5), suspended in corn oil, at the doses of 100 mg/kg/day, 200 mg/kg/day and 100 mg/kg/day, respectively, by gavages. In groups 6, 7, 8, rats were treated with TCDD and curcumin (TCDD + curcumin group), myrcene (TCDD + myrcene group), cineole (TCDD + cineole group) at the same time. Tissue samples were taken firstly at 30th day from seven animals of each group and then at 60th days from the remaining seven animals. The animals were sacrificed under slight ether

anesthesia and the livers were immediately removed and dissected over ice-cold glass. Tissues were stored at −45°C in deepfreeze until analysis.

The homogenization of tissues was carried out in Teflon glass homogenizer with 150 mM KCl (pH 7.4) to obtain 1:10 (w/v) dilution of the whole homogenate. The homogenates were centrifuged at 18,000 x *g* (4°C) for 30 min to determine thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) levels and catalase (CAT) activities and at 25,000 x *g* for 50 min to determine glutathione peroxidase (GSH-Px) activities.

Biochemical assay

The levels of homogenized tissue TBARS, as an index of lipid peroxidation, were determined by thiobarbituric acid reaction using the method of Yagi (1998). The product was evaluated spectrophotometrically at 532 nm and results are expressed as nmol/g tissue.

The GSH content of the liver homogenate was measured at 412 nm using the method of Sedlak and Lindsay (1968). The GSH level was expressed as nmol/mg protein.

CuZn-SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O_2^- generated by the xanthine/xanthine oxidase system (Sun et al., 1988). One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The product was evaluated spectrophotometrically at 560 nm. Results are expressed as IU/mg protein.

CAT activity of tissues was determined according to the method of Aebi (1974). The enzymatic decomposition of H_2O_2 was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The enzyme activities are given in k/mg protein.

GSH-Px activity was measured by the method of Paglia and Valentina (1967). In the presence of glutathione reductase and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured. GSH-Px activity is expressed as IU/mg protein.

Determination of protein content. Tissue protein content was determined according to the method developed by Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

All values were presented as mean ± SEM. Differences were considered to be significant at $p \leq 0.01$. Statistical analyses were performed using one-way ANOVA and post hoc Tukey's significant difference test by SPSS/PC computer program (SPSS Inc., Chicago, Illinois, USA).

Results

The liver SOD, GSH-Px, CAT, GSH and TBARS levels are given in Table 1 for day 30. In rats treated with TCDD for 30 days, liver SOD, GSH-Px, CAT and GSH levels were significantly ($p \leq 0.01$) decreased whereas TBARS levels were significantly ($p \leq 0.01$) increased compared with negative control and other groups. In general, curcumin, myrcene and cineole increased SOD, GSH-Px, CAT, GSH levels while reducing TBARS levels. These substances when given together with TCDD brought SOD, GSH-Px, CAT, GSH and TBARS levels closer to the control level.

The levels of SOD, GSH-Px, CAT, GSH and TBARS are given for day 60 (Table 2). The liver SOD, GSH-Px, CAT and GSH levels were highly significantly ($p \leq 0.01$) decreased whereas TBARS levels were highly significantly ($p \leq 0.01$) increased in TCDD group compared with negative control and other groups. On day 60, curcumin, myrcene and cineole significantly ($p \leq 0.01$) increased SOD, GSH-Px, CAT, GSH levels while reducing TBARS levels. Curcumin, myrcene and cineole when given together with TCDD, increased ($p \leq 0.01$) SOD, GSH-Px, CAT, GSH but decreased TBARS levels in rat liver.

We showed that duration of drug administration significantly altered oxidative stress (Tables 1 and 2). The lipid peroxidation was significantly ($p \leq 0.01$) higher in rats treated with TCDD for 60 days compared with rats treated with TCDD for 30 days. For this reason, we determined that TCDD induced oxidative stress in a time-dependent manner. The antioxidative effects of curcumin, myrcene and cineole were increased at day 60 compared to day 30. In the TCDD groups given curcumin, myrcene and cineole, oxidative stress decreased by time.

Table 1. The levels of CuZn-SOD, GSH-Px, CAT, GSH and TBARS in rats drug administered during 30 days[a]

30th days	Control	TCDD	Curcumin	Myrcen	Cineole	TCDD + Curcumin	TCDD + Myrcen	TCDD + Cineol
SOD (U/mg protein)	365.0 ± 11.0^{ad}	250.0 ± 17.3^{b}	511.0 ± 15.2^{ce}	438.0 ± 30.1^{cd}	538.8 ± 16.9^{e}	292.8 ± 14.3^{ab}	320.6 ± 23.5^{ab}	267.8 ± 17.8^{b}
GSH-Px (U/mg protein)	291.2 ± 6.5^{a}	216.0 ± 8.5^{b}	476.0 ± 10.2^{c}	314.3 ± 8.2^{a}	416.0 ± 12.1^{d}	304.1 ± 9.6^{a}	276.0 ± 4.8^{a}	314.0 ± 9.5^{a}
CAT (k/mg protein)	7.69 ± 0.4^{a}	5.56 ± 0.3^{b}	11.3 ± 0.3^{c}	11.6 ± 0.2^{c}	8.31 ± 0.4^{d}	7.95 ± 0.1^{ad}	6.94 ± 0.4^{a}	9.82 ± 0.4^{cd}
GSH (nmol/mg protein)	2.15 ± 0.11^{ac}	1.57 ± 0.08^{b}	2.47 ± 0.16^{c}	2.14 ± 0.08^{ac}	2.28 ± 0.09^{ac}	1.78 ± 0.10^{ab}	1.75 ± 0.21^{ab}	1.80 ± 0.11^{ab}
TBARS (nmol/g tissue)	131.2 ± 4.2^{ad}	173.8 ± 3.3^{b}	91.3 ± 4.7^{c}	106.1 ± 5.9^{ac}	87.6 ± 6.5^{c}	132.9 ± 8.1^{d}	147.9 ± 6.7^{bd}	139.0 ± 4.3^{d}

Abbreviations: CuZn-SOD: copper-zinc superoxide dismutase, GSH-Px: glutathione peroxidase, CAT: catalase, GSH: glutathione, TBARS: thiobarbituric acid reactive substances, TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[a] Different letters a, b, c, d and e within the same row in the body of the table showed significant ($p \leq 0.01$) differences between groups.

Table 2. The levels of CuZn-SOD, GSH-Px, CAT, GSH and TBARS in rats drug administered during 60 days[a]

60th Day	Control	TCDD	Curcumin	Myrcen	Cineol	TCDD + Curcumin	TCDD + Myrcen	TCDD + Cineol
SOD (U/mg protein)	367.5 ± 7.5^{a}	202.0 ±23.8^{b}	510.1 ±26.2^{c}	409.8 ±20.7^{ac}	483.2 ±27.5^{c}	434.4 ±25.2^{ac}	373.4 ±29.1^{ac}	400.2 ±22.5^{ac}
GSH-Px (U/mg protein)	296.2 ± 9.6^{a}	199.8 ± 6.9^{b}	456.0 ± 24.8^{c}	340.4 ± 14.7^{a}	439.6 ± 20.8^{c}	334.0 ± 11.8^{a}	307.6 ± 25.9^{a}	347.6 ± 19.6^{a}
CAT (k/mg protein)	9.95 ± 0.31^{a}	3.84 ± 0.18^{b}	19.3 ± 0.47^{c}	15.5 ± 0.56^{d}	14.5 ± 0.52^{d}	11.8 ± 0.33^{ae}	9.7 ± 0.38^{a}	13.5 ± 0.80^{de}
GSH (nmol/mg protein)	2.22 ± 0.11^{ac}	1.38 ± 0.08^{b}	2.71 ± 0.16^{c}	2.15 ± 0.08^{ac}	2.32 ± 0.09^{ac}	1.85 ± 0.10^{ab}	1.88 ± 0.21^{ab}	1.82 ± 0.11^{ab}
TBARS (nmol/g tissue)	137.3 ± 4.0^{a}	268.7 ± 6.8^{b}	72.7 ± 4.3^{c}	85.1 ± 5.0^{c}	68.9 ± 4.9^{c}	114.6 ± 2.8^{d}	125.0 ± 3.1^{ad}	107.2 ± 2.8^{d}

Abbreviations: CuZn-SOD: copper-zinc superoxide dismutase, GSH-Px: glutathione peroxidase, CAT: catalase, GSH: glutathione, TBARS: thiobarbituric acid reactive substances, TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

[a] Different letters a, b, c, d and e within the same row in the body of the table showed significant ($p \leq 0.01$) differences between groups.

Discussion

In the current study, antioxidant enzymes decreased but lipid peroxidation increased in liver tissue samples reflecting oxidative stress induced by TCDD. A similar effect is equivocally observed by the other studies (Alsharif et al., 1990; Hassoun et al., 2000, 2004). Additionally, several workers (Alsharif and Hassoun, 2004; Hassoun et al., 2000; Sweeney et al., 1984) demonstrated that the liver is a major organ for toxic effects of TCDD revealed by histopathological changes and biochemical liver function tests. Although these variables were not assessed in the current study, our results clearly show that TCDD negatively affects liver function and this is reversed by the antioxidative substances (curcumin, myrcene, cineole) used.

TCDD and oxidative balance

Our study shows that TCDD produces time-dependent increases in the formation of TBARS levels in the liver. Many other studies reported that similar increases in TBARS formation in the liver, kidney, thymus and brain tissues from TCDD-treated animals (Alsharif and Hassoun, 2004; Dhanabalan and Mathur, 2009; Hassoun et al., 2003, Slezak et al., 2000). Besides, we determined that GSH levels in rats treated with TCDD significantly decreased time-dependency. Similarly, Hassoun et al. (2006) showed that subchronic treatment of rats with TCDD resulted in significant suppression of GSH levels. On the other hand, Hung et al. (2006) claimed that the total GSH level increased in mice treated with TCDD. We think that this difference could be due to the single and low dose of TCDD used in the study of Hung et al. (2006). Additionally, in current study, we showed that SOD, CAT and GSH-Px activity in the liver of rats treated with TCDD were significantly reduced time-dependently. These results agree with previous studies (Dhanabalan and Mathur, 2009; Hassoun et al., 2006; Lim et al., 2007). Therefore, our results clearly demonstrate that TCDD induces oxidative damage in the liver of the rats.

Curcumin and oxidative balance

Curcumin treatment at the doses of 100 mg/kg/day for 30 or 60 days significantly reduced lipid peroxidation in TCDD-treated rats. There are a few reports that describe the antioxidant effects of curcumin against lipid peroxidation. Suryanarayana et al. (2007) showed that curcumin significantly inhibited formation of TBARS in the liver tissues of diabetic rats. Similarly, curcumin significantly and dose-dependently attenuated lipid peroxidation in cisplatin-treated rats (Kuhad et al., 2007).

Curcumin treatment in the current study increased GSH levels time-dependently, as revealed also by Kaur et al. (2006), and it prevented a decrease in GSH levels by TCDD. Additionally, we showed that curcumin increased the activities of SOD, CAT and GSH-Px in liver. Similarly, previous study (Kalpana et al., 2007) has shown that curcumin is potent inducer of detoxifying enzymes and thereby prevent the toxicity induced by chemical carcinogens. Kaur et al. (2006) demonstrated that curcumin significantly attenuated hepatic dysfunction along with lipid peroxidation and restored the levels of GSH in rats. Therefore, it is obvious that curcumin level used in the current study positively affects oxidative balance and seems to be beneficial for reversing the negative effects of TCDD.

Myrcene, cineole and oxidative balance

Our study showed that myrcene and cineole significantly and time-dependently decreased TBARS levels in rats. Myrcene and cineole increased the level of GSH and the activities of SOD, CAT and GSH-Px in liver. To the best of our knowledge, there is no study on the effects of myrcene and cineole on lipid peroxidation, GSH level and antioxidant enzyme activities in rat. However, a few studies (Wang et al., 2005) indicate that monoterpenes have effective antioxidant properties. Similarly, previous studies (Lima et al., 2004; Mitić-Culafić et al., 2009) have shown that myrcene- and cineole-containing plants are potent inducers of detoxifying enzymes and thereby prevent the oxidative damage. Additionally, a few studies indicate that the myrcene, cineole and other some monoterpenes efficiently protect bacterial and human cells against oxidative damage (Wang et al., 2008; Mitić-Culafić et al., 2009). Together with the evidences obtained from the above studies, the current study indicates that myrcene and cineole protects rat liver from oxidative damage induced by TCDD.

Conclusion

The present study shows that (1) TCDD at 2 μg/kg/week induces oxidative damage in rats in a time-dependent manner; (2) curcumin (100 mg/kg/day), cineole (100 mg/kg/day) and myrcene (200 mg/kg/

day) had strong antioxidatıve potentials and (3) these substances appears to have protective effects against the oxidative damage induced by TCDD treatment. Furthermore, the highest protective effects were observed for curcumin followed by cineole and β-myrcene. Thus, curcumin, cineole and myrcene, which reduce oxidative stress, may be useful as a new category of anti-TCDD toxicity agent. Their protective effect lends more support to the role of oxidative stress in the overall toxicity of TCDD.

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