PROTECTIVE EFFECT OF LYCOPENE ON OXIDATIVE STRESS INDUCED BY DIFFERENT DOSES OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN IN BRAIN, LIVER, KIDNEY, AND HEART TISSUE OF RATS

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Abstract

The aim of this study was to investigate the possible protective role of lycopene (LYC) on liver, kidney, heart, and brain in male rats exposed to different doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Forty-eight rats were divided into six groups. The first group received 0.5 mL corn oil as control; the second group was treated with 10 mg/kg bw/day LYC. Groups 3 and 4 were treated to 50 and 500 ng/kg bw/day of TCDD, respectively. Groups 5 and 6 were subjected to 50 and 500 ng/kg bw/day of TCDD along with 10 mg/kg bw/day of LYC, simultaneously. The duration of the experiment was 13 weeks. While the exposure to TCDD increased the levels of malondialdehyde (MDA), it decreased glutathione (GSH) levels, and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities. However, LYC-treatment decreased the high MDA levels and increased GSH, SOD, GSH-Px, and CAT activities. In conclusion, LYC treatment decreased TCDD-induced lipid peroxidation and supported the antioxidant activity. It is suggested that LYC has a protective effect against oxidative stress induced by TCDD.

Rezumat

Scopul acestui studiu a fost investigarea posibilului rol protector al licopenului (LYC) asupra ficatului, rinichiului, inimii şi creierului şobolanilor masculi expuşi la doze diferite de 2,3,7,8-tetraclorodibenzo-*p*-dioxină (TCDD). 48 şobolani au fost împărţiţi în 6 grupuri. Primul grup a primit 0,5 mL ulei de porumb ca martor; al doile grup a fost tratat cu 10 mg/kg/zi LYC. Grupurile 3 şi 4 au fost tratate cu 50 şi respectiv 500 ng/kg/zi de TCDD. Grupele 5 şi 6 au fost supuse la 50 şi 500 ng/kg corp/zi de TCDD împreună cu 10 mg/kg/zi de LYC, simultan. Perioada de experimentare a fost de 13 săptămâni. Expunerea la TCDD a mărit nivelul de malonil dialdehida (MDA), a scăzut nivelul de glutation (GSH) şi activităţile superoxid dizmutazei (SOD), glutation peroxidazei (GSH-Px) şi catalazei

(CAT). Totuşi, tratamentul cu LYC a scăzut concentratia de MDA şi a crescut concentratia de GSH. De asemenea, activităţile enzimatice ale GSH, SOD, GSH-Px şi CAT au fost intensificate. În concluzie, tratamentul cu LYC a diminuat peroxidarea lipidică indusă de TCDD şi a susţinut activitatea antioxidantă. Se sugerează că LYC are un efect protector asupra stresului oxidativ determinat de TCDD.

Keywords: dioxin; toxicity; oxidative stress; lycopene

Introduction

Dioxins are known to be a class of highly toxic, widespread, and persistent environmental contaminants. Dioxins include 75 polychlorinated dibenzo-*p*-dioxins (PCDDs), 135 polychlorinated dibenzofurans (PCDFs), and 12 nonortho- and monoortho- polychlorinated biphenyls (coplanar PCBs), which are structurally similar to PCDDs and PCDFs. Among them, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been the most intensively studied for toxicity, and it has been classified as a human carcinogenic substance by the International Agency for Research on Cancer [1]. Although the mechanism of carcinogenesis by TCDD is unclear, it is now considered to act as a cancer promoter [2]. It is a known fact that cases of death have increased due to many developing diseases (cancer, cardio-vascular diseases, etc.) caused by dioxins and other congeners [3].

Prolonged exposure to TCDD may result in a wide variety of adverse effects in experimental animals and in humans, including immunotoxicity, neurotoxicity, hepatotoxicity, teratogenesis, and carcinogenesis [4-6]. Oxidative stress is considered an important mechanism in the toxicity of TCDD [7-10]. Oxidative stress induction is now recognized as an important etiological factor in the induction of several chronic diseases including cancer, cardiovascular diseases, osteoporosis, and diabetes [11]. However, the mechanism involved in the production of oxidative stress following TCDD exposure has not been fully elucidated.

Lycopene (LYC), as a dietary source of a carotenoid antioxidant, has attracted considerable interest in recent years as an important phytochemical with a beneficial role in human health. [12]. LYC has received particular attention as a result of studies that have reported that it is a highly efficient antioxidant with a singlet oxygen ($^1O_2$) and free radical- scavenging capacity [13-15].

The aim of the present study was to investigate toxicity of subchronic differential doses of TCDD and to determine the possible protective role of LYC using biochemical approaches in heart, kidney, liver, and brain tissues of male rats.

Materials and methods

Chemicals

TCDD was purchased from AccuStandard (New Haven, CT, USA). LYC 10% FS (Redivivo™, Code 7803) was obtained from DSM Nutritional Products (İstanbul, Turkey). Reduced glutathione (GSH), glutathione reductase, thiobarbituric acid (TBA), fosfotungustic acid, hydrogen peroxide, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide phosphate (NADPH), and other reagents were supplied from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

Animals and experimental design

The protocol for animal use was approved by the Institutional Review Board of the National Institute of Health and the Local Committee on Animal Research. The animals were obtained from Fırat University, Experimental Research Centre (Elazığ, Turkey) and were housed under standard laboratory conditions (temperature 24 ± 3°C, humidity 40–60%, a 12-h light: 12-h dark cycle). A commercial pellet diet (Elazığ Food Company, Elazığ, Turkey) and fresh drinking water were given *ad libitum*. Forty-eight healthy adult male Sprague-Dawley rats (6–8 weeks old, weighing 190–250g) were used. The rats were divided into six groups containing eight rats each. All administrations were applied by gavage as emulsion within 0.5 mL corn oil. The experiment lasted 13 weeks.
Experimental groups were as follows:
Group 1 (Control): The group received 0.5 mL corn oil only.
Group 2: The group was given a dose of 10 mg/kg bw/day LYC.
Group 3: The group was given a dose of 50 ng/kg bw/day TCDD.
Group 4: The group was given a dose of 500 ng/kg bw/day TCDD.
Group 5: The group was given doses of 50 ng/kg bw/day TCDD plus 10 mg/kg bw/day LYC.
Group 6: The group was given doses of 500 ng/kg bw/day TCDD plus 10 mg/kg bw/day LYC.

Sample collection and biochemical assays

The rats were decapitated under slight ether anesthesia at the end of the experiment. Heart, liver, kidney, and brains were removed immediately and then stored at -20°C until biochemically analysed.

The homogenization of tissues was carried out in a Teflon-glass homogenizer with a buffer containing 1.15% KCl to obtain 1:10 (w/v) whole homogenate. Homogenates were centrifuged at 16.000×g (+4 °C) for 3 min to determine malonildialdehide (MDA) and total protein levels.

Homogenates were centrifuged at 25.000×g for 45 min to determine glutathione (GSH) concentrations and glutathione peroxidase (GSH-Px) and catallase (CAT) activities. The obtained supernatants were centrifuged again at 25.000×g for 45 min to determine superoxid dismutase (SOD) activities.

The concentrations of MDA were determined according to a modified method of Ohkawa et al. [16] based on the reaction with TBA and were expressed as nmol/g protein. Tissue GSH concentration was measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by Ellman [17] and was expressed as nmol/mg protein. GSH-Px activity was determined by the procedure described by Beutler [18]. The procedure of analysis performed was based on the oxidation of reduced GSH by GSH-Px coupled to the disappearance of NADPH by GSH-reductase measured at 37°C and 340 nm and were expressed as U/g protein. CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi [19] and was expressed as k (U/g protein), where k is the first-order rate constant. SOD activity was determined according to the method of Sun et al. [20]. The principle of the method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. SOD activity was also expressed as units per gram protein (U/g protein). Protein concentrations were measured according to Lowry et al. [21].

Statistical analysis

All values are presented as mean ± S.E.M. Differences were considered to be significant at $P<0.05$. Nonparametric Kruskall-Wallis H and Mann-Whitney U tests were used for testing intergroup statistical differences. The SPSS/PC program (Version 10.0; SPSS, Chicago, IL) was used for the statistical analysis.

Results and discussion

MDA assay results are summarized in Table I. MDA levels of whole tissues increased in Groups 3 and 4 compared to the control group ($P<0.001$). In addition, MDA levels in the liver and heart tissues increased in Group 4 ($P<0.001$), compared with Group 3. These findings are in agreement with results reported in previous studies [9, 22, 23]. On the other hand, simultaneous TCDD and LYC treatment provided a marked reduction ($P<0.001$) in the increased MDA levels, when compared to only TCDD-administrated groups. It was determined in the previous studies that MDA levels increasing in the case of oxidative stress are reduced by LYC treatment [24-26]. In the current study, increased TCDD-induced MDA levels were considerably reduced by LYC treatment (P<0.001). The

decrease in the MDA level can be related to the fact that LYC is an efficient scavenger of singlet oxygen ($^{1}O_2$).

Table I

Mean ± S.E.M. values of MDA and GSH levels and SOD, CAT, and GSH-Px activities in tissues belonging to each group.

Parameters	Groups	Tissues			
		Liver	**Kidney**	**Heart**	**Brain**
MDA (nmol/g protein).	**Group 1**	34.19 ± 0.72^{b}	89.55 ± 1.39^{bc}	34.42 ± 1.62^{c}	63.86 ± 1.40^{b}
	Group 2	28.12 ± 1.21^{c}	79.17 ± 3.37^{cd}	22.23 ± 1.59^{d}	58.54 ± 3.26^{bc}
	Group 3	38.45 ± 1.35^{b}	135.85 ± 7.24^{a}	53.89 ± 2.87^{b}	78.75 ± 1.92^{a}
	Group 4	48.47 ± 0.88^{a}	126.58 ± 7.43^{a}	69.45 ± 2.10^{a}	81.51 ± 1.73^{a}
	Group 5	21.65 ± 0.52^{d}	100.44 ± 5.25^{b}	20.55 ± 0.88^{d}	63.25 ± 2.02^{b}
	Group 6	27.64 ± 0.82^{c}	62.50 ± 7.27^{d}	23.94 ± 3.72^{d}	48.06 ± 1.98^{c}
	Significance	***	***	***	***
GSH (nmol/mg protein).	**Group 1**	3.74 ± 0.60^{abc}	7.58±0.76	1.95 ± 0.06^{a}	9.19±0.40
	Group 2	4.04 ± 0.31^{a}	6.09±0.33	2.00 ± 0.06^{a}	12.91±2.00
	Group 3	2.35 ± 0.31^{bc}	4.92±0.57	1.48 ± 0.11^{b}	8.90±0.54
	Group 4	2.56 ± 0.15^{bc}	5.24±1.02	1.96 ± 0.07^{a}	8.17±1.20
	Group 5	3.31 ± 0.25^{ab}	6.67±1.00	2.59 ± 0.28^{a}	11.79±1.00
	Group 6	2.35 ± 0.09^{c}	4.89±0.71	1.92 ± 0.14^{ab}	9.89±1.78
	Significance	**	NS	**	NS
SOD (U/g protein).	**Group 1**	46.47 ± 2.35^{ab}	33.52 ± 3.23^{bc}	80.11±3.20	64.05 ± 5.67^{ab}
	Group 2	57.02 ± 2.42^{a}	47.23 ± 2.12^{a}	76.36±6.32	74.33 ± 1.96^{a}
	Group 3	40.47 ± 2.10^{b}	32.33 ± 1.99^{bc}	74.24±2.98	58.33 ± 3.96^{b}
	Group 4	37.41 ± 3.49^{b}	27.36 ± 1.89^{c}	76.74±2.78	55.24 ± 6.90^{b}
	Group 5	48.32 ± 2.74^{ab}	37.45 ± 1.83^{b}	77.50±6.52	72.17 ± 4.73^{ab}
	Group 6	43.24 ± 3.72^{b}	29.05 ± 2.22^{bc}	72.39±4.95	57.28 ± 3.23^{b}
	Significance	**	***	NS	*
CAT (U/g protein).	**Group 1**	67.17 ± 4.18^{b}	22.15 ± 1.50^{a}	4.48±0.35	1.29 ± 0.18^{a}
	Group 2	85.15 ± 3.39^{a}	18.73 ± 1.89^{ab}	4.71±0.36	1.09 ± 0.12^{a}
	Group 3	55.75 ± 3.79^{bc}	14.95 ± 1.46^{b}	3.34±0.41	0.82 ± 0.03^{ab}
	Group 4	18.47 ± 5.36^{d}	14.51 ± 3.37^{b}	4.71±0.72	0.71 ± 0.04^{b}
	Group 5	70.64 ± 4.00^{ab}	13.87 ± 0.83^{b}	4.31±0.46	1.01 ± 0.10^{ab}
	Group 6	41.94 ± 6.54^{cd}	15.00 ± 1.92^{b}	3.56±0.57	0.83 ± 0.18^{ab}
	Significance	***	*	NS	*
GSH-Px (U/g protein).	**Group 1**	84.20 ± 3.14^{ab}	45.11 ± 3.60^{ab}	35.78 ± 1.33^{b}	32.63 ± 1.52^{ab}
	Group 2	89.04 ± 2.66^{a}	49.51 ± 2.22^{a}	50.55 ± 3.17^{a}	33.77 ± 0.84^{a}
	Group 3	57.13 ± 2.73^{cd}	36.64 ± 1.29^{b}	37.05 ± 2.17^{bc}	27.39 ± 1.14^{bc}
	Group 4	51.95 ± 3.54^{d}	35.57 ± 1.26^{b}	29.98 ± 2.34^{c}	25.03 ± 1.06^{c}
	Group 5	69.89 ± 4.51^{bc}	41.36 ± 2.18^{ab}	40.11 ± 1.28^{ab}	29.84 ± 1.24^{abc}
	Group 6	58.01 ± 4.69^{cd}	38.04 ± 1.08^{b}	39.09 ± 2.01^{b}	28.59 ± 2.80^{abc}
	Significance	***	**	***	**

NS: Non significant, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, $^{a, b, c, d}$: Different letters within same column showed significant differences.

Previous studies, where different doses of TCDD were adminstered to rats, showed that SOD [27], GSH-Px [23, 27], and CAT [28] activities in tissues decreased, while another study [28] indicated that CAT activity decreased while SOD and GSH-Px activities did not show any changes. In the present study it was determined that SOD activities in liver, kidney, heart, and brain tended to decrease in TCDD-administered groups, related to doses compared to the control group. Compared to the control group, it was detected that CAT activities in liver, kidney, and brain of TCDD-administrated groups considerably decreased related to the TCDD doses ($P<0.05$), while CAT activities in the heart were not affected by TCDD administration. GSH-Px activities in TCDD-administrated groups were generally considerably decreased compared to those in the control group ($P<0.05$), depending on TCDD doses. In the present study, the decrease in enzymes activities could be linked to an increase in free-radical production and inhibition of enzymatic-antioxidant depends on depletion.

Among the carotenoids, LYC as an antioxidant is more effective against biological reactive oxygen species (ROS) and may contribute to the protection or amelioration of cells and tissues *in vivo* or *in vitro* [29, 30]. In previous studies [30, 31] where LYC was used to diminish the level of oxidative stress, it was reported that SOD, CAT, and GSH-Px activities increased in tissues of LYC-treated rats. Atessahin et al. [32] indicated that GSH-Px and CAT activities considerably decreased in cyclosporine-induced nephrotoxicity; however, LYC-treatment increased these enzyme activities close to the control level. In the current study, it was determined that TCDD-administration inhibited SOD activity; LYC treatments generally accentuated this lowering in SOD activity close to normal levels when compared to TCDD-administrated groups. LYC had a partially positive effect on liver and brain CAT activities in comparison with the group to which TCDD alone was administrated. It was observed that GSH-Px activities of the heart best responded to LYC treatment; LYC treatment increased these activities close to normal levels and GSH-Px activities in liver, kidney, and brain also tended to increase. It can be concluded that LYC reduced the oxidative stress induced by TCDD and decreased lipid peroxidation, as well as considerably preventing decreases in antioxidant enzyme activities.

In the current study, it was determined that TCDD administration decreased GSH levels compared to those in the control group. This finding was in accordance with Slezak et al. [9], while it was not in agreement with Shon et al. [23] who reported that the total GSH level increased in the liver of rats exposed to TCDD in a 25 μg/kg bw dose. Differences between the

available findings and the literature could be linked to the applied TCDD doses. As reported in previous studies [9, 23], this increase in GSH levels by administrating TCDD is only observed in low doses, while it does not appear in high doses. When compared to TCDD-administered groups, it was observed that LYC-treatment increased the GSH level close to normal levels in the heart ($P<0.01$) and brain. In addition, it was determined that LYC had more marked effect on GSH levels in a 50 ng/kg bw/day dose of the TCDD-administered group than in a 500 ng/kg bw/day of TCDD-administered group. These findings were in accordance with the results of both Atessahin *et al*. [24] and Gupta *et al*. [29].

Conclusions

Low or high doses of TCDD-induced lipid peroxidation led to antioxidant defense system weakness in liver, kidney, heart, and brain tissues of rats. However, lycopene administration at the dose of10 mg/kg bw/day strengthened the antioxidant defense system and eliminated the negative effects caused by TCDD-induced lipid peroxidation. Considering all of the positive effects as a whole, today this strong antioxidant has the potential to be used in the treatment of several chronic diseases, such as cancer, against oxidative stress regarded as a prominent cause of these diseases.

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