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The Protective Effects of Antioxidants and Propranolol on Hepatotoxicity of TCDD During Isolated Rat Liver Perfusion

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Abstract: The purpose of this study was to evaluate protective effects of vitamin E, selenium and propranolol on TCDD induced changes of biochemical parameters using the rat liver perfusion system. Various concentrations of TCDD (0.3, 3, 20, 30 μg L^{-1}) were added to the perfusion fluid and biochemical changes in perfusion fluid of isolated rat liver were examined within 2 h. The results showed that TCDD significantly increases the aminotransferase activities in a dose-dependent manner. It was determined that TCDD at 20 μg L^{-1} caused a maximum increase in biochemical parameters within 2 h ($p<0.0001$). The result also showed that propranolol (20 mg L^{-1}), sodium selenite (345 mg L^{-1}) and vitamin E (α-tocopherol, 700 mg L^{-1}) significantly decreases TCDD hepatotoxicity. Aminotransferase enzyme activity as well as glutathione and protein content significantly changed in treatment groups as compared to the TCDD group ($p<0.001$). Reduction in hepatotoxicity may be attributed to prevention of lipid peroxidation, although other mechanisms may also be involved.

Key words: TCDD, liver perfusion, antioxidants, glutathione, aminotransferase

INTRODUCTION

TCDD was first recognized in 1957 as a by-product in production of herbicide in Germany[1]. It is also formed during the chlorine bleaching processes by pulp of paper mills[2]. The environmental pollutant, TCDD, can occur in processes with organic chlorine material either by heat or catalysis in manufacture of polyvinyl chloride (PVC) and is a products of combustion of many chemical processes that involve heat e.g. forest fire and garbage[3,4]. Humans are exposed to TCDD during many industrial accidents or as a contaminant in food products. It bioaccumulates in the food chain due to its high lipophilicity nature[5]. The liver was found to be the primary and most important target tissue for TCDD in rats[6]. Various studies have shown that TCDD induces hepatic lipid peroxidation[7,8], alters the distribution of divalent cations[9] and produces DNA single strand break[9,10]. TCDD was reported to decrease hepatic glutathione peroxidase activity by almost 45 to 50% and alters cellular GSH and reactive oxygen intermediate production[11,12].

The isolated rat liver was chosen because it permits studies of liver function in a system that resembles normal physiology[13]. Elevation of aminotransferase activities were shown to be a good indicator of hepatocellular damage[14,15]. In addition, glutathione level was also shown to be an indicator of liver damage through lipid peroxidation[16,17]. In the present study, the inhibitory effects of some well-known antioxidant materials on hepatotoxicity of TCDD have been assessed in an isolated perfused rat liver.

MATERIALS AND METHODS

Chemicals: 2,3,7,8-tetrachlorodibenzo-p-dioxin was obtained from Cambridge Isotope Laboratories, Inc. The substance was dissolved in distilled water/DMSO (10:1) and kept in dark. Perfusion fluid was made of Krebs-Henseleit buffer. The perfusion medium consisted of 118.9 mM NaCl, 4.7 mM KCl and 1.19 mM KH_2PO_4, 2.55 mM $CaCl_2$ and 24.8 mM $NaHCO_3$ at 37°C. D-glucose (0.1% w/v) was added to provide energy source. The perfusion medium was gassed continuously

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with carbogen, 95% O_2, 5% CO_2[18]. Phosphate buffer, pH 7.2, 0.2 M was made of potassium phosphate monobasic and dibasic. Propranolol (dl- propranolol) was purchased from ICI (England), vitamin E (α-dl-tocopherol) from Sigma Chemical Co (St. Louise, MO, USA) and sodium selenite from E. Merck (Darmstadt, Germany). All other chemicals were obtained from Sigma Chemical Co (St. Louise, MO, USA) with the highest available quality.

Perfusion apparatus: The perfusion apparatus consisted of a perfusate reservoir thermostated to 37°C, an Inline filter (Millipore, 5 μm-pore size and 5 cm diameter) and a membrane oxygenator. Perfusion was carried out by a peristaltic pump (Harvard apparatus) at a constant flow rate of 12 mL min^{-1} [18].

Animals: Male albino Wistar rats (6-8weeks) weighing 200-300 g were used in these experiments. They were housed individually in standard cages in a room on a 12/12 h light-dark cycle at 22°C and 50±5% relative humidity and allowed to become acclimatized to standard laboratory condition for at least 7 days. Food and water were freely available except before the experiment.

Experimental design: Animals were divided into 11 groups. ach roup ontained our male rats. In groups 1-2, alone (control) or TCDD plus Krebs buffer. In group's 3-11 the livers were perfused by Krebs buffers containing vitamin E or sodium selenite or propranolol with TCDD which was applied as pre-treatment, simultaneously (combination), or as post-treatment. Before performing the experiment, the perfusate was circulated for 30 min to permit stabilization. Then, the perfusion was circulated with krebs buffer containing 20 μg L^{-1} of TCDD, 700 mg L^{-1} of vitamin E, 345 mg L^{-1} of sodium selenite or 20 mg L^{-1} of propranolol for 120 min, respectively. Pre-treatment groups received the drug 30 min before the TCDD was added to the perfusion solution. Combination treatment groups received the drug and TCDD added together to the perfusion solution. Post-treatment groups received the drug 30 min after the TCDD was added to the perfusion solution.

Operation and perfusion: The rats were anaesthetized with ether. Heparin (500 units; i.p.) was administrated to the rats to prevent blood clotting prior to the anesthesia[13,19]. An incision was made along the length of the abdomen to expose the liver. The bile was collected; sutures were placed loosely around the common bile duct, which then placed loosely around the inferior vena cava, above and below the renal veins. The distal suture around the vena cava was tightened and then an 18 g polyethylene catheter was inserted, placed above the renal vein and secured with the proximal suture. The portal vein was immediately cannulated with an 18 g catheter that was secured. The diaphragm was incised and the inferior vena cava was legated suprahepatically. Following attachment of the perfusion tubing to the cannulate, the liver was perfused in situ through the portal vein[13]. Temperature, perfusion pressure, flow rate and perfusion fluid pH were closely monitored during the perfusion, particularly during the first 30 min of equilibration[13]. These parameters were initially checked every 10 to 15 min and the experiment were not begun until they had reached a constant and acceptable value. The temperature in the perfusion system was also set and maintained at 37°C. Perfusion pressure was not raised above 10-15 cm water with a flow rate of approximately 2 mL min^{-1} g^{-1} liver weight to provide adequate oxygenation. The perfusion fluid pH was always set between 7.2 and 7.4 by adjusting the CO_2 gases. As soon as perfusion was begun, the liver had an even, light brown colour, was soft and was kept moist. Blotches or discoloration means that the liver is not well perfused. Serum aminotransferase activities (SGPT and SGOT) serve as indicators of liver viability during perfusion, which were determined in samples of perfusion medium.

Biochemical determinations: The activities of aspartate aminotransferase (AST) activity and Alanine aminotransferase (ALT) activity in the perfusion fluid and homogenate sample were assayed using a commercial Kit of Zist Chimie (Tehran, Iran). Reduced glutathione (GSH) was estimated by Ellman's method[20]. Total protein was determined according to the Bradford method in the perfusion fluid and homogenate samples of hepatic tissue (which were prepared immediately after homogenization) using coomassi brilliant blue G-250 reagent[21].

Light microscopy: The liver was completely excised and freed of any extraneous tissue. Multiple samples were then taken from each liver (mean 3 mm) and placed in 10% neutral buffered formalin. Liver was cut into small pieces; sections were prepared, stained by eosin- hematoxylin and examined for histopathological changes[19].

Statistical analysis: All values were expressed as mean±SE of four rats. Multiple analysis of variance (MANOVA) followed by student newmans-keuls test was used to evaluate the significance of the results obtained. All computations were analysis by computer using SPSS software.

RESULTS

Effect of TCDD on biochemical changes in rat liver perfusion in comparison with control: Perfusion of rat liver with Krebs-Henseleit buffer containing 20 μg L^{-1} of TCDD showed a significant time-dependent increase in

Table 1: Effect of vitamin E, selenium and propanolol on TCDD-induced hepatotoxicity and protein contents in isolated rat liver perfusion

	ALT (IU/L)				
	Time (min)				
Groups	0	30	60	90	120
Control	15.8±2.2	20.4±2.2	26.5±3.6	29.0±3.9	34.9±3.7
TCDD	16.8±2.1	80.5±0.9†††	117.8±3.2†††	184.3±5.2†††	220.4±1.7†††
Vit-E Pre.	18.0±1.1	72.5±1.0**	106.0±2.1***	113.0±4.2***	154.0±3.2***
Comb.	16.0±3.4	75.4±3.1	100.0±4.2***	124.5±5.2***	147.5±4.2***
Post.	17.0±2.5	27.5±2.1***	56.5±3.5***	75.5±1.0***	112.3±5.2***
Sel Pre.	20.4±3.2	54.7±1.9***	78.3±2.7***	129.0±2.5***	182.8±9.9***
Comb.	12.5±2.1	61.3±3.7***	65.5±2.8***	163.3±6.7***	174.0±4.3***
Post.	15.3±1.0	67.0±2.4***	84.4±4.7***	156.0±2.1***	179.3±2.1***
Prop Pre.	14.4±1.3	31.5±4.8***	43.0±2.8***	51.5±4.6***	82.3±6.9***
Comb.	15.6±1.1	37.5±2.5***	64.6±5.3***	120.1±5.2***	133.1±3.8***
Post.	14.4±1.3	45.2±3.2***	58.4±2.6***	112.5±4.0***	128.8±4.6***
	AST (IU/L)				
	Time (min)				
Groups	0	30	60	90	120
Control	15.1±1.5	24.8±3.1	20.4±2.7	37.8±4.3	41.8±4.8
TCDD	15.5±2.9	86.0±2.9†††	104.0±4.5†††	146.2±4.1†††	174.2±5.2†††
Vit-E Pre.	17.0±2.8	50.0±2.2***	70.0±4.5***	85.0±4.9***	136.0±6.5***
Comb.	17.0±1.0***	42.7±2.5***	65.9±4.7***	78.2±6.3***	147.5±3.2***
Post.	16.5±1.1	50.3±2.6***	72.0±4.5***	106.0±2.9***	130.0±5.5***
Sel Pre.	15.6±1.2***	64.7±3.9***	86.0±2.3***	106.7±3.9***	131.8±1.5***
Comb.	14.4±1.9	60.0±3.2**	78.5±4.2***	116.8±2.1***	135.0±3.5***
Post.	15.3±1.0	67.8±2.2***	87.0±3.6***	132.0±1.7***	146.3±8.2***
Prop Pre.	12.5±1.1	41.5±4.9***	53.8±6.8***	122.5±3.2***	246.8±7.5***
Comb.	12.5±3.8	59.9±2.8***	85.0±3.5***	105.3±3.2***	126.3±4.8***
Post.	12.5±1.1	32.5±1.2***	61.3±5.7***	82.4±5.4***	138.2±4.0***
	Protein (μg mL^{-1})				
	Time (min)				
Groups	0	30	60	90	120
Control	15.0±1.3	5.7±1.7	69.3±0.9	75.9±2.1	117.5±3.2
TCDD	22.5±4.1†	147.5±4.3†††	470.5±8.5†††	533.0±10.8†††	637.5±12.3†††
Vit-E Pre.	18.0±2.9	128.0±4.3***	226.2±2.2***	248.0±4.3***	250.0±1.7***
Comb.	16.5±1.0	71.0±4.2***	220.0±6.3***	240.0±2.9***	279.3±6.2***
Post.	17.3±3.8	114.8±3.5***	232.0±1.9***	248.0±3.2***	323.0±5.5***
Sel Pre.	15.8±4.4	104.9±4.9***	237.5±2.4***	243.1±4.5***	257.7±5.2***
Comb.	12.8±2.7	68.0±5.3***	235.0±3.6***	248.0±1.3***	280.0±1.3***
Post.	15.5±1.0	112.9±2.4***	187.5±5.7***	238.0±7.3***	325.5±8.5***
Prop Pre.	12.5±1.9	63.6±4.0***	136.4±5.1***	184.9±8.7***	239.4±5.9***
Comb.	15.2±1.1	44.6±4.0***	153.9±4.5***	257.5±5.5***	289.8±6.7***
Post.	15.0±2.2	133.9±6.6*	325.9±6.8***	348.9±6.9***	377.2±5.8***

Data are means±SEM of four measurements. Vitamin E (700 mg L^{-1}), selenium (345 mg L^{-1}) and propranolol (20 mg L^{-1}) were used as Pre = before treatment, Comb=combination, Post= after treatment. **($p<0.01$) and ***($p<0.001$) significantly different when compared with TCDD 20 μg L^{-1} alone. † ($p<0.05$) and ††† ($p<0.001$) significantly different when compared with control (Krebs-Henseleit buffer)

aminotransferase activity and total protein in isolated rat liver perfusion ($p<0.001$) (Table 1). Antioxidants and propranolol have been shown to inhibit these hepatotoxic effect of TCDD ($p<0.01$).

Effect of TCDD on glutathione level in bile duct and total protein in homogenate as compared to control: Table 2 showed a significant decrease in glutathione level in bile duct and increase in protein level in liver homogenates ($p<0.001$). Antioxidants and propranolol have shown to inhibit these heptotoxic effect of TCDD ($p<0.01$).

Light microscopic observations: Histological studies using light microscope showed hepatocellular damages including necrosis and infiltration due to TCDD (20 μg L^{-1}) (Fig. 1c). In addition, other histopathological parameters including number of kupffer cell and mononuclear, edematous, necrosis and cell degeneration

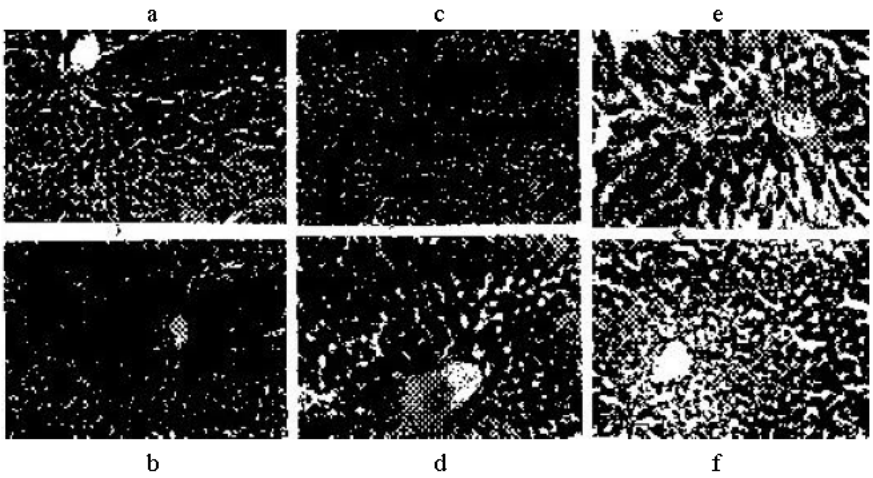

Fig. 1: Photomicrograph of rat liver lobule in control (a), Krebs-Henseleit buffer (b), TCDD (20 μg L^{-1}) perfused (c), TCDD with propranolol (20 mg L^{-1}) perfused (d), TCDD with selenium (345 mg L^{-1}) perfused (e), TCDD with vitamin E (700 mg L^{-1}) perfused (f). Hematoxylin and eosin (X25)

Table 2: Effect of vitamin E, selenium and propanolol on TCDD-induced hepatotoxicity and protein and glutathione contents in isolated rat liver perfusion

Group		Protein (μg mL^{-1})	GSH (μg L^{-1})
Control		148.9±4.2	21.1±0.5
TCDD 20 μg L^{-1}		1373.0±16.2†††	16.31±0.7†
Vit-E	Pre-	561.0±4.1***	28.5±1.5***
	Comb-	622.7±2.8	25.1±0.7***
	Post-	530.0±7.4	27.3±2.2***
Selnium	Pre-	744.3±7.4***	24.5±0.7***
	Comb-	570.6±6.2***	23.0±1.6***
	Post-	680.5±9.7***	25.0±1.2***
Propranolol	Pre-	903.8±4.6***	22.5±0.5
	Comb-	1037.7±7.8***	28.5±1.7**
	Post-	731.0±8.8***	28.1±4.4**

Data are means±SEM of four measurements. Vitamin E (700 mg L^{-1}), selenium (345 mg L^{-1}) and propranolol (20 mg L^{-1}) were used as Pre = before treatment, Comb=combination, Post= after treatment. **($p<0.01$) and ***($p<0.001$) significantly different when compared with TCDD 20 μgL^{-1} alone. † ($p<0.05$) and ††† ($p<0.001$) significantly different when compared with control (Krebs-Henseleit buffer)

were changed due to TCDD (Fig. not shown). These changes were alleviated using antioxidant or propanolol (Fig. 1).

DISCUSSION

In order of elucidate the TCDD-induced hepatotoxicity, the effects of TCDD on transaminase enzyme effect and total glutathione and protein content of isolated perfused rat liver were determined. Moreover the effect of antioxidant, vitamin E (α-dl tocopherol) selenium (sodium selenite) and a membrane-stabilizing agent also β-blocker (propranolol) on TCDD-induced hepatotoxicity was determined. We found previously that TCDD dose dependently caused histopathological changes especially at 20 μg L^{-1}. TCDD heptotoxicity was more evident with increase amount of necrosis. In addition, other histopathological parameters including number of Kupffer cell and mononuclearcells, edematous and cell degeneration were changed considerably at the concentration of 20 μg L^{-1} of TCDD[22,23]. In the present study, we sought to determine how we can prevent or decrease the hepatotoxic effect of TCDD. The activity of aminotransferase enzyme was increased significantly which was well correlated with total protein and decrease of glutathione[24]. On the other hand when antioxidants were used, this hepatotoxicity effect on enzyme was reduced almost to 40-50% of control. Glutathione depletion has been shown to correlate with lipid peroxidation in liver[16]. Moreover, TCDD also was shown to be an inhibitor of glutathione peroxidase, which catalyzes the destruction of H_2O_2 of lipid hydroperoxide by reduced glutathione. Therefore, with inhibition of glutathione peroxidase, there is a reduction in GSH which resulted in accelerated lipid peroxidation[25].

Antioxidants such as vitamin E and selenium have been proposed to prevent membrane damage of lipid peroxidation not only through glutathione peroxidase but also by allowing hydrogen to be abstracted from their own structure rather than from the allylic hydrogen of on unsaturated lipid, thus interrupting the free radical chain reaction[26]. Treatments with vitamin E and selenium have been shown to significantly decrease the toxicity of TCDD (Table 1 and 2). This may be through the mechanism mentioned above. Propranolol is also shown to decrease the toxicity of TCDD, this may be due to it membrane stabilizing function. Through

stabilization of the membrane the propranolol may reduce the lipid peroxidation effect of TCDD[27]. Increase in aminotransferase activity may be related to the depletion of glutathione. Other chemicals such as paraquat and nitrofurantoin have shown similar effects[28]. Moreover, our study shows that concurrent perfusion of TCDD with antioxidant and propanolol decreased the hepatotoxicity of TCDD. These findings are well correlated with biochemical parameter measured (Table 1). More studies are needed to further elucidate the mechanism of toxicity of TCDD.

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