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Dose and duration dependent toxicity of Dioxin (2,3,7,8 TCDD) to few lysosomal enzymes in mice kidney

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Abstract: *TCDD, a highly toxic lipophilic Dioxin, causes many health problems in animals including human when exposed through dietary intake of fat. Present communication reports in vivo dose and exposure duration dependent cellular toxicity of environmentally available concentration of TCDD to few lysosomal enzymes in mice kidney. The study tests two hypotheses (a) environmentally available low concentration of TCDD provokes dose and exposure duration dependent toxic effects to lysosomal enzymes and, (b) Low dose TCDD exposure may trigger cellular apoptosis by altering different lysosomal enzymes. Groups of female Swiss albino mice were subjected to different concentration of TCDD (0.004 mg/kg bw/d, 0.04 mg/kg bw/d) by oral gavage for 2,4 and 6 days of exposure duration. The observed results suggested that significantly dose and exposure duration dependent effects were found in kidney cells of mice. The results indicate TCDD possibly causing oxidative stress and disturb cell homeostasis by increasing intracellular ions. ROS and increased ions may be responsible for the alteration of different physiological activity of cell. Though the exact process is not clear at present, but it is also possible that altered functions of key lysosomal enzymes might have evoked the process of cell destruction and cell apoptosis.*

***Keywords*:** *Dioxin, TCDD, dose and duration dependent effects, lysosomal enzymes, kidney, mice*

I. Introduction

TCDD is one of the most toxic congener of dioxin family that has low degradable capacity and high bioavailability. TCDD induced cell death was reported as a consequences of loss of body weight (Wasting syndrome) and inhibition of gluconeogenesis with appetite suppression [1]. TCDD exposure caused hemorrhagic cystitis and focal pyelonephritis in an exposed child [2]. TCDD was reported to affect the variety of enzymes like Uridine diphosphate, Glucuronosyl transferase and multi-functional enzyme systems involved in detoxification and metabolism of a wide variety of endogenous and exogenous compounds. It was also reported that an apparent structure activity relationship were found between the located hydrogen atom on TCDD molecules and the activity inducing factor of other enzymes in *in vivo* and *in vitro* conditions [3,4]. Kidney being an excretory organ of living organism, accumulation of waste toxic products occurs into the renal cells and proximal convoluted tubules. TCDD intake by human beings occurs primarily via food products such as milk, dairy products. The metabolites of organochlorine may be responsible for renal cellular damage [5]. It was reported earlier that TCDD induces oxidative and energy stress to the developed chicken kidney [6]. Dysregulation of autophagy process in cell had been found to be linked with pathogenesis of renal disease. Recent studies showed clear evidences of TCDD induced significant cell proliferation and signs of apoptosis but indicated no activation of relevant caspases like caspase 8, 9 or 3 [7]. It has been reported previously that TCDD causes major urinary problems, however, histological studies showed no renal lesions [8]. Going through the literature it was observed that studies on the toxic effects of TCDD to lysosome is rare. Therefore, the present study was undertaken to assess the dose and duration dependent effects of a dioxin TCDD to few key lysosomal enzymes in mice kidney. The study tests two hypotheses (a) environmentally available low concentration of TCDD provokes dose and exposure duration dependent toxic effects to lysosomal enzymes and, (b) Low dose TCDD exposure may trigger cellular apoptosis by altering different lysosomal enzymes.

II. Materials And Methods

Inbred healthy female Swiss albino mice, around 3 months of age and weighing 30 ± 5 g, were taken for the study. A total of 65 animals groups were divided into different groups and were provided with commercially available rodent diet and water *ad libitum* and kept under highly hygienic conditions in the animal house facilities. The mice were kept under controlled humidity, temperature (25 ± 2 ^{0}C) and diurnal cycle of 14: 10 h. All experiments were conducted according to ethical norms provided by CPCSEA India (permission No. CPCSEA/CH/RF/ACK-2003). 2,3,7,8 TCDD was obtained from Sigma-Aldrich Chemicals Pvt. Ltd. (CAS No. 1746-01-6). All other chemicals used for this study were of analytical grade. Different groups of mice were administered of TCDD (0.004 mg/kg bw/d, 0.04 mg/kg bw/d) dissolved in corn oil (vehicle) for three different exposure durations of 2, 4 and 6 days. The selection of the doses were based on the available reports of the doses causing effects on enzymatic activity in the vital tissues of mice, acute to sub-acute exposure and

evaluation of toxicity studies and application of factors (LOAEL) for extrapolating from animal model to human for TCDD administered through oral route [8]. The doses selected for the study were very low concentration of TCDD and comparable to that of the human exposure through different environmental sources.

Kidney tissue from at least three animals for each dose group was suspended in chilled Sucrose-EDTA-Imidazole (SEI) buffer (pH 7.1) to remove blood and other membranous substances. Known amount of tissue was sampled from the pooled kidney tissues and homogenized in chilled phosphate buffer (pH 7.0) to obtain a 10 % (w/v) homogenate. Enzyme extract preparation for purified lysosomal enzymes was carried out by the method of Beaufay [9]. The homogenate was then centrifuged at 2000 rpm for 8 min at 4℃. The obtained supernatant was re-suspended in phosphate buffer and centrifuged at 11,000 rpm for 40 minutes. The resultant sediment was re-suspended in phosphate buffer with 0.1% Triton X 100 to obtain a supernatant of lysosomal fraction. The specific activity of Acid Phosphatase, α-Galactosidase, β-Glactosidase and β-Glucuronidase were estimated using this lysosomal fraction. The enzyme assays were done as per the method of Tettamanti and Masserini [10]. Protein concentration of the tissue homogenate was determined by the method of Lowry et al [11], using bovine serum albumin as the standard. The obtained data were subjected to different statistical analyses like one-way and two-way nested ANOVA and 't' test for their cumulative acceptability and hypotheses testing. All statistical analyses were done as per Sokal and Rohlf [12].

III. Results And Discussion

Results of the present study showed drastic changes in the specific activity of lysosomal enzymes exposed to the doses of TCDD for all exposure duration in kidney cells of mice. The specific activity of acid phosphatase showed inhibitory trend in highest dose and exposure duration followed by slight stimulation after 4 days of exposure duration in lower dose (Fig. 1). Similarly, the specific activity of α- galactosidase showed inhibitory trend in higher dose of TCDD after 6 days of exposure. However, slight stimulatory effect were observed in lower dose of TCDD exposed for 2 days (Fig. 1). The specific activity of β- galactosidase showed stimulation in higher dose of TCDD and exposed for 2, 4 and 6 days whilst, lower dose of TCDD showed inhibition in all exposure durations (Fig. 1). The specific activity of β-glucuronidase showed inhibitory trend in all exposure duration after the exposure in both the doses of TCDD (Fig. 1).

As the studies of the dose and duration dependent effects of TCDD on mammalian lysosomal enzymes are rare, the purpose of this study was to examine the very early effect of very low doses of TCDD to lysosomal enzymes more closely. The result suggests that the TCDD caused rapid changes in the specific activity of key lysosomal enzymes in kidney cells of mice. Dioxin like compounds are highly toxic environmental stressors that accumulated in lipid rich tissue of human and wildlife and relevant symptoms of renal disease were seen especially during starvation [13,14]. The intracellular accumulation of TCDD and its byproducts or free molecules of TCDD was reported to be depended mainly on the exposure time and secondarily on the dose of toxicant pumped into the animal [15]. Lysosome is recognized as intracellular catabolic center for different chemical reactions. Most of the environmental endocrine disruptors are accumulated within lysosome of kidney cells especially in the proximal convoluted tubule cells, where it may trigger cell proliferation and cell growth [16]. TCDD and few other dioxin like compounds induce formation of toxic proteins which might have created the intracellular toxic load. TCDD, PCBs and other PAH chemicals are reported to be interacting with AhR which can initiate the formation of apoptosome or trigger apoptosis [17]. Though it is not very clear at this moment but the results of the present study indicated the observed nephrotoxicity by TCDD was probably caused by some byproduct or intermediate compounds produced elsewhere. These compounds were transported to kidney and ultimately accumulated in lysosome or endocytosed, degraded by lysosome [18-19]. TCDD affects the signal pathway after binding with AhR receptor in cytosol [20] and induce enzymatic activity such as phenobarbital [21, 22]. The results of two way nested ANOVA showed a clear exposure duration dependent effects of TCDD in almost all the enzymes estimated, except β-glucuronidase. β-glucuronidase showed highly significant dose and duration dependent effects of TCDD (Table 1). The observed results indicated that even very low doses of TCDD can alter the lysosomal enzyme stability in kidney cells. On the other hand, the results of't' tests showed significant variations in the activities of key lysosomal enzymes over their respective controls. Highly significant variations in the specific activity of α-galactosidase were observed after the exposure of higher dose of TCDD (Table 2). Similar results were also observed in one way ANOVA between individual exposure duration within each dose group (Table 3). The present study indicated that the cytotoxic effects of lipophilic TCDD might have altered the morphological and functional aspects of lysosomal enzyme studied. It has been reported that the ROS, or oxidative stress produced by environmental stressors, activate the caspase 3 or 9. This caspases are activate the preliminary process of apoptosis through alteration of signaling pathway in kidney cells [23]. The observed results are in accordance with the report that the functional integrity of lysosomal is generally the common target for most environmental stressors [24]. Dysfunctions of lysosomal enzymes are related with many pathological conditions associated with toxic and degenerative renal disease [25]. The observed results are also in accordance with the fact reported earlier that TCDD induces expression of CYP1A1 protein [26], altered

intracellular volume of cell that affects the metabolic pathways where metabolism of different macromolecules might have arrested [27,28]. Thus, the intracellular ROS induces lipid peroxidation and conversion of fatty acid via lipooxygenase pathway, inhibiting receptor mediated mechanism in glomerular mesangium [29]. The predominant exposure duration dependent disturbances in the specific activities of the selected lysosomal enzymes in the present study possibly indicating a disturbances in cellular homeostasis which may affect various cellular functions [30,31]. These organochlorine pollutants are lipophilic and tend to accumulates in adipose tissue so symptoms relevant to toxicity manifest after starvation condition of living organism [32-34]. The alterations in the enzyme activities possibly showed cell defense toward incoming ROS which might have formed outside renal cells [35].

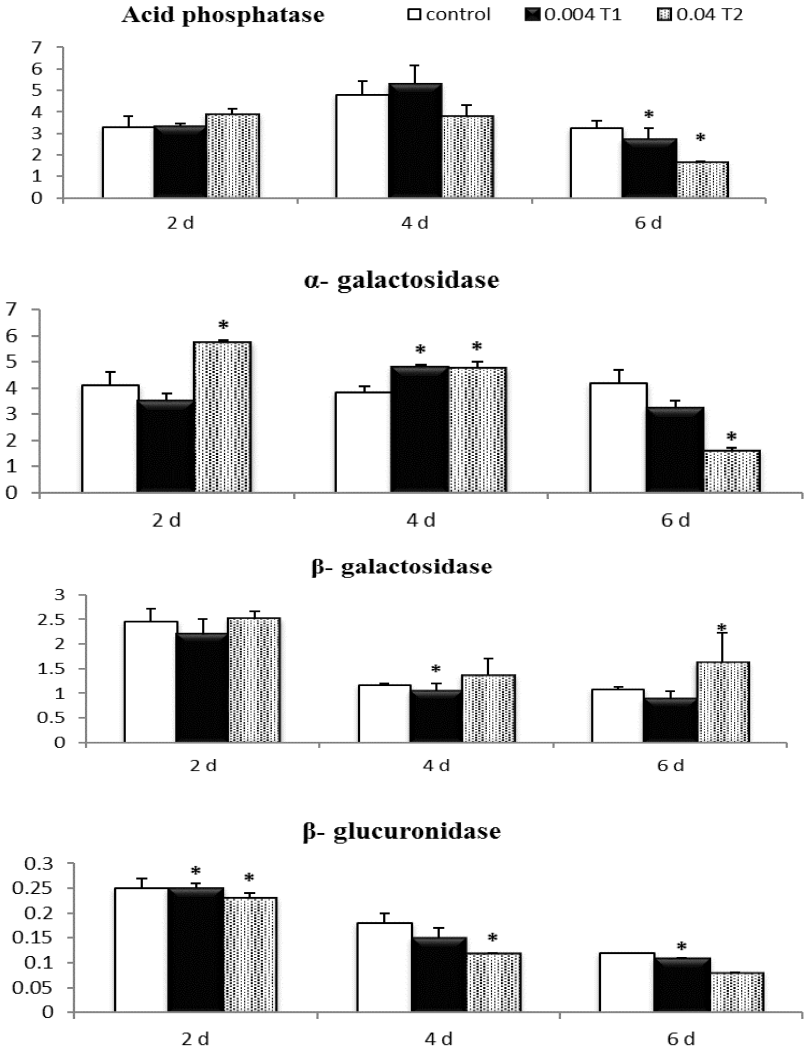

Fig.1. Histograms showing dose and duration dependent alterations in the specific activity of different lysosomal enzymes after *in vivo* TCDD intoxication. Error bars represent standard deviation and '*' sign represents the significant variations at p = 0.05 level. T_1 & T_2 represent toxicated groups.

TABLE 1. Results of two-way nested ANOVA between control and toxicated groups.

	Acid Phosphatase	α- Galactosidase	β- Galactosidase	β-Glucuronidase
Amongst doses	0.35	1.57	0.07	3.68*
Within durations	12.40**	12.49**	24.75**	52.10**

*Significance at P = 0.05 (F *crit* of dF = 3,8) = 3.63
**Significance at P = 0.05 (F *crit* of dF = 8,35) = 2.59

TABLE 2. Results of t-test between control and individual TCDD exposure durations within each dose group, in kidney tissue of mice.

	Acid Phosphatase		α- Galactosidase		β-Galactosidase		β-Glucuronidase	
	0.004 mg	**0.04 mg**	**0.004 mg**	**0.04 mg**	**0.004 mg**	**0.04 mg**	**0.004 mg**	**0.04mg**
2 days	0.11	1.81	1.78	3.18*	1.07	0.31	7.43*	5.58*
4days	0.79	2.04	7.02*	5.05*	2.84*	1.49	0.53	4.01*
6 days	5.73*	2.81*	1.14	7.86*	1.85	3.63*	3.88*	1.94

*Significance at P = 0.05 (F *crit* = 2.77)

TABLE-3. Results of one-way ANOVA between individual exposure duration within each dose group.

	Acid Phosphatase	α-Galactosidase	β-Galactosidase	β-Glucuronidase
Control	8.70	0.45	67.73	45.78
T1 (0.004)	28.82*	5.27*	25.58*	6.48*
T2 (0.04)	11.97*	70.19*	28.55*	2.21

*Significance at P = 0.05 (F *crit* = 5.14)

IV. Conclusion

The overall results indicated that even very low and environmentally available concentration of TCDD provoked dose and duration dependent effects on key lysosomal enzymes studied. TCDD altered the lysosomal enzyme stability and possibly produce intracellular reactive oxygen species. These ROS might be responsible for an increase in the intracellular ions. Though it is not very clear at this point, but all these effects cumulatively might have initiated the preliminary process for cell damage and possibly cell apoptosis.

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