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TCDD (2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN) CAUSES REDUCTION IN THE LOW DENSITY LIPOPROTEIN (LDL) RECEPTOR ACTIVITIES IN THE HEPATIC PLASMA MEMBRANE OF THE GUINEA PIG AND RAT

David W. Bombick, Fumio Matsumura and B. V. Madhukar

Pesticide Research Center, Michigan State University, E. Lansing, MI 48824

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SUMMARY: Administration of 1 μg/kg (single intraperitoneal injection and studied after 10 days) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to young male guinea pigs was found to cause a significant reduction in binding of low-density lipoprotein (LDL) to its receptor on the hepatic plasma membrane. This reduction in LDL binding is not caused by the decrease of food intake by treated animals since pair-fed control animals had significantly higher LDL binding than treated animals. It was also found that primary hepatocytes from treated animals had a reduced ability to internalize LDL than controls. Such a change in the plasma membrane function may explain the resulting hyperlipidemia particularly hypercholesterolemia which occurs in this species as a result of TCDD administration.

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a highly toxic chemical. Its oral LD_{50} (single dose) to male guinea pigs is approximately 0.6 μg/kg (1). Its toxic manifestations are, however, known to vary from species to species (2). The most clear-cut example of species variation in response to TCDD administration is the difference of LD_{50}s between three rodent species: guinea pigs, rats (60 to 100 μg/kg) and hamsters (1000 to 5000 μg/kg) (1,3).

An obvious sign of TCDD's toxicity which occurs both in the rat and hamster are hepatic changes, particularly induction of mixed-function oxidases which occur at low doses (4). However, TCDD-treated guinea pigs appear to be non-responsive (5,6) with regard to these biochemical and morphological changes in the liver. Rather in this species a very noticeable sign of TCDD's toxic effect is the unusual accumulation of cholesteryl ester and triglyceride carrying lipoproteins in the serum, specifically low-density lipoproteins (LDL) and very low density lipoproteins (VLDL) (7). According to Swift et al. (7) the VLDL and LDL levels in TCDD-treated guinea pigs (2 μg/kg, single i.p.) are 18.3 and 3.3 times above pair-fed control values, and are the primary cause for the treated animals' hypertriglyceridemia and hypercholesterolemia. Curiously, treated animals exhibiting hyperlipidemia also lose body weight when compared against controls matched for food consumption (i.e., pair-fed control animals).

Recently, it has been reported from this laboratory that hepatic plasma membranes from rats treated with TCDD in vivo have lower binding capacities to a number of ligands and also show reduced activity in a number of membrane bound enzyme functions (8). To relate these observations to TCDD-induced hyperlipidemia

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we have made an attempt to study plasma membrane functions which may be critical to serum lipoprotein metabolism. We have found that LDL binding to hepatic plasma membrane and its subsequent internalization are significantly reduced in TCDD-treated pigs. The results reported can potentially explain several key toxic expressions of TCDD.

MATERIALS AND METHODS

Guinea pigs (approximately 200 grams) were obtained from Michigan Department of Health and maintained on Purina Guinea Pig Chow diet. Food and water were provided ad libitum unless specifically stated. TCDD (99% purity) was obtained as a gift from Dow Chemical Company (Midland, Michigan). The proper amount of TCDD dissolved in corn oil:acetone (9:1) was used for single intraperitoneal injection (i.p.) at the stated dose.

Guinea pig LDL was separated by ultracentrifugation (9). Its characteristics were confirmed by electron microscopy and TLC lipid analysis. Isolated LDL was iodinated by a slightly modified Bio-Rad enzymobead method (10) utilizing a lactoperoxidase method. The modification consists of increasing the sample volume to 1-2 ml and reducing the enzymobead reagent and $Na^{125}I$ by half. This procedure resulted in 90% labeling of the LDL protein with a specific activity of approximately 25 - 100 cpm/ng protein.

Hepatic plasma membrane for binding studies was isolated by the method described by Peterson et al. (11,12). Specific binding of LDL was determined in treated animals, pair-fed controls, and ad lib controls by the method described by Kovanen et al. (13).

Uptake of LDL was studied with liver slices obtained by using a McIllwain tissue chopper. Liver slices of approximately 100 mg from treated (1.0 µg/kg TCDD single i.p.) and control animals were incubated in 3 mls of Eagles medium (Gibco Laboratories) with either 35 µg of ^{125}I-LDL (from determined protein) or 35 µg ^{125}I-bovine serum albumin (specific activity of 708 cpm/µg). After the specified time periods slices were rinsed with Eagles medium, solubilized with NaOH, and counted on a scintillation counter. Values are reported as ng LDL uptake per mg liver slice.

Subcellular distribution of ^{125}I-LDL was determined as follows: approximately 3 grams of control and treated livers slices were obtained ten days after animals were dosed with 1.0 and 0.01 µg/kg TCDD single i.p. dose or ten days with daily i.p. doses of 200 mg/kg DDT. The slices were incubated at 37°C in 3 mls of Eagles medium containing approximately 35 µg of ^{125}I-LDL. After 20 minutes these slices were homogenized and cellular fractions obtained off the sucrose density gradient (same method utilized to obtain plasma membrane). A parallel set of control and treated liver slices also underwent 20 minutes of incubation in ^{125}I-LDL followed by additional 20 minutes of incubation with unlabeled LDL (approximately 350 µg LDL protein). These slices were also homogenized and fractionated. Aliquots of all fractions were solubilized and counted in a scintillation counter as above. The values reported are percentages of radioactivity in each fraction versus total radioactivity in the gradient.

Internalization of LDL was studied using isolated guinea pig hepatocytes (14). Hepatocytes were isolated ten days after a single i.p. dose of 1.0 µg/kg TCDD. Pair-fed animals dosed with just vehicle were used as controls. LDL internalization was determined using a modified method described by Soltys and Portman (15). Other details are: approximately 2 X 10^5 hepatocytes were incubated in 1 ml of Eagles medium containing 500 µg of unlabeled LDL for 60 min at 37°C. ^{125}I-LDL (35 µg) was added and after specified incubation periods the cells were gently spun down and rinsed twice with one ml of Eagles medium. They were then treated with 1 ml of trypsin in Eagles medium (500 units) for 10 min, centrifuged, washed once with one ml of fetal calf serum, solubilized and counted in a scintillation counter. Hepatocyte viability was assayed by trypan blue exclusion during the entire experimental period.

In addition, protein determinations were performed on the trypsin digested supernatants to assess the integrity of the hepatocyte membranes. Values reported are μg LDL internalized for 2 X 10^5 hepatocytes.

RESULTS

When slices of liver tissues from treated and control animals were incubated with the medium containing ^{125}I-labeled bovine serum albumin or ^{125}I-labeled LDL for up to 120 minutes, the slices from the treated guinea pigs show reduced total uptake of these labeled materials from the medium (Fig. 1). The difference was also observed in terms of the rate of uptake. In the control preparations the level of radiolabels show a steady increase from 30 to 120 minutes whereas with slices from the treated guinea pigs no significant increase could be observed in the same time period.

To examine a cause for a reduction in LDL uptake by the slices, the pattern of intra-tissue distribution of ^{125}I-LDL was studied as described in the Methods section.

The results (Table 1) indicate that the highest degree of displacement of the ^{125}I-LDL by the second addition of nonlabeled LDL occurred in the slices from the guinea pigs treated with 1.0 μg/kg TCDD. Those from the DDT-treated and the low dose TCDD-treated (0.01 μg/kg) animals showed less significant difference from the control counterpart. Within centrifugally separated fractions the most signficant difference was recorded among lighter density fractions (i.e., plasma membrane fraction) whereas radioactivity levels in mitochondrial and microsomal fractions were not significantly different among all preparations (data not shown).

In the next series of experiments the LDL binding was directly studied using isolated hepatic plasma membrane fractions. The results summarized in

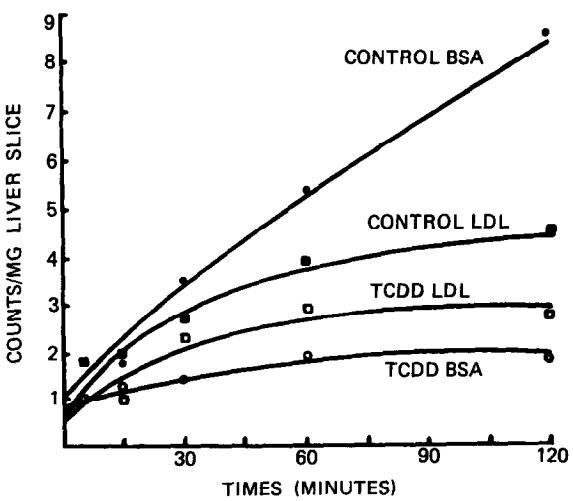

Fig. 1. Uptake of ^{125}I-LDL and ^{125}I-BSA in liver slices from treated (1.0 μg/kg single i.p. injection) guinea pigs and controls ten days after dosing. Specific activity of ^{125}I-BSA and ^{125}I-LDL was 708 cpm/μg protein and 80 cpm/ng protein, respectively. Other details are outlined in Materials and Methods section.

Table 1. Changes in distribution of ^{125}I-LDL among subcellular fractions after second 20 minute incubation of the liver tissue slices from control and treated guinea pigs with non-labeled LDL medium following the initial 20 minute incubation with the medium containing ^{125}I-LDL.

Subcellular fractions separated by centrifugation (sucrose conc.)	Treatments (μg/kg, i.p. single dosing)	% Recovery of originally found ^{125}I radioactivity		% Changes (-) reduction
		Incubation with ^{125}I-LDL	Incubation with LDL	
Plasma membrane (1.16-1.18 M)	Control	16.8 ± 1.7	11.5 ± 2.3	-31.5
	TCDD (0.01)	17.1 ± 1.9	5.4 ± 1.0	-68.4
	TCDD (1.0)	16.1 ± 2.7	4.2 ± 1.4	-73.9
	DDT (2×10^5)	22.5 ± 1.7	13.9 ± 2.4	-38.2

Table 2 clearly show that isolated plasma membranes from the TCDD-treated animals have a lower capacity to bind with the exogenously added ^{125}I-LDL. Since activity of the LDL receptor is known to be influenced by the nutritional status of the animals the same experiment was repeated with the matched pair-fed controls (i.e., control animals were fed with the same amount of food as the treated animals). The results were similar in that plasma membranes from treated animals always showed a lower LDL-binding capacity than that of paired control animals. Since it has been reported by several workers (16,17,18) that TCDD causes increases in serum cholesterol levels in rats, a similar experiment was also conducted

Table 2. Differences in the levels[a] of specific binding to ^{125}I-LDL of the hepatic plasma membrane of the hepatic and the isolated hepatocytes from the untreated (i.e., control) and TCDD treated guinea pigs and rats.

Source	Treatment (μg/kg, single i.p.)	^{125}I-LDL binding (pg/μg protein)	
		Control	TCDD-treated
Guinea pigs			
Plasma membrane	1.0	210.4 ± 21.1 (3)	78.7 ± 7.4 (3)*
Plasma membrane (control, pair-fed)	1.0	320.0 ± 9.7 (3)	84.9 ± 18.8 (4)*
Hepatocytes	1.0	1.630[b] ± 2.21 (1)	0.269[b] ± 0.14(1)
Rats			
Plasma membrane	25	1633 ± 386 (3)	873 ± 116 (3)

[a]The data expressed in mean ± standard error (number of independent tests with different animals for each test).
[b]μg of LDL bound per 10^6 cells. Each value represents three replicates of a single animal.
*Statistically significant at p = 0.05.

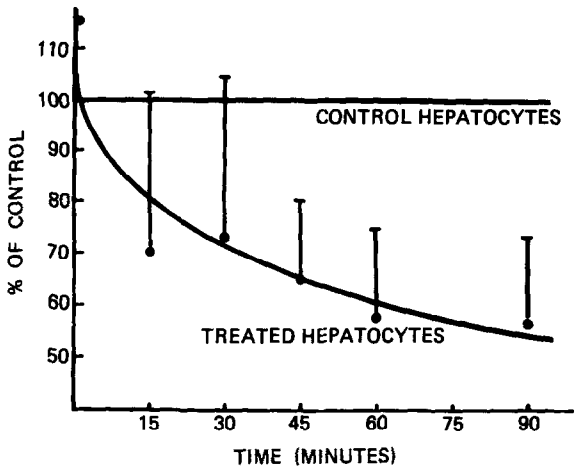

Fig. 2. Comparison (as a percentage) of ^{125}I-LDL internalization in primary hepatocytes from treated guinea pigs (1.0 µg/kg single i.p. injection) and control primary hepatocytes. Control values (µg ^{125}I-LDL/200,000 cells) for 0, 15, 30, 45, 60 and 90 minute incubation times are 0.85, 2.77, 2.78, 2.95, 3.22 and 3.53, respectively. An lsd test ($\alpha = 0.05$) after a completely randomized factorial analysis of variance showed significant differences in the means at incubation times of 15, 45, 60 and 90 minutes.

with male rats treated with TCDD (25 µg/kg single i.p.). The plasma membrane from the liver of the treated animals (sacrificed at day 10) showed a significantly lower level of ^{125}I-LDL binding compared to controls.

It is known that the LDL-receptor interaction results in the bound LDL being internalized through an endocytotic process, and eventually metabolized within the hepatocyte (19). Therefore, the reduced binding of LDL to its receptor in TCDD-treated animals could mean a reduction in LDL metabolism. This possibility was tested by using primary hepatocytes (200,000 cells per test) to study ^{125}I-LDL uptake. The results (Fig. 2) indicate decreased uptake of LDL in hepatocytes from treated guinea pigs and are in good agreement with the prior results in Table 2. Reduced receptor activity at the cell surface can explain the decreased internalization in treated hepatocytes since LDL binding is integral to LDL internalization and metabolism (20).

DISCUSSION

One of the most conspicuous signs of TCDD effects in the guinea pig is hyperlipidemia (7,21). This phenomenon may appear to be unusual because serum LDL and VLDL levels are elevated without changes in the levels of HDL and free fatty acids (17). However, recent results on this subject indicate that the LDL receptor may have a dual role to degrade both LDL and VLDL remnants (22). In the case of a strain of rabbit lacking the LDL receptor (i.e., "the Watanabe rabbit"), LDL degradation is hindered but also VLDL production rates are stimulated due to an inability of this strain's receptor to degrade VLDL remnants (22). Thus a decrease in LDL receptors could explain the simultaneous increase in VLDL and LDL levels.

Evidence obtained in the current work that support this hypothesis are: (a) all binding tests with isolated hepatocytes, hepatic plasma membranes and liver slices indicate the level of LDL binding in the TCDD-treated animals at the hepatocyte surface is signficantly reduced; (b) this effect was significant at an effective dose of 1 μg/kg, but less significant at an ineffective dose of 0.01 μg/kg; (c) such an effect was not observed in the guinea pigs treated with an almost lethal dose of DDT (200 mg/kg daily for 10 days), a compound known to have many toxic hepatic effects; and (d) a similar effect is observable with the rat.

On the other hand, one could raise another critical question whether the reduction of LDL receptor activity is the result of hyperchlosterolemia rather than the cause for it (e.g., in rabbit studies increased dietary cholesterol is known to reduce LDL receptor numbers). There are three factors that give support to the causal relationship of the LDL receptor in the hypercholesterolemia of TCDD-treated animals. First, guinea pig liver, unlike its counterparts of the rat and mouse, does not show any overt sign of toxic lesions even at lethal doses (5,6); i.e., many effects observed in rat liver could be a result rather than a cause of TCDD toxicity, while those observed in the guinea pig liver have more chance to be directly related to the cause of TCDD's action. Second, a similar reduction in the LDL receptor activity is observed in TCDD-treated rats which show higher serum levels of HDL, but not LDL or VLDL. This clearly indicates that at least in the rat the cause for the reduction in LDL receptor activity is not due to down regulation resulting from accumulation of its immediate ligand (LDL) in the serum. In this respect, the rat is unusual in that its serum contains a high titer of HDL which apparently serves as a lipoprotein-cholesteryl ester pool (23). Indeed, unlike the rabbit, increases in dietary cholesterol in rats do not result in the immediate increase in serum LDL (24). Third, there are a number of precedents, demonstrating increases of serum LDL levels as a result of reductions in LDL receptor activities. A good example of such a cause-effect relationship is the genetic disorder in human known as familial hypercholesterolemia (FH) in which serum LDL levels are elevated as a result of lower LDL receptor numbers.

A potentially serious implication of the current finding is that there is a possibility that TCDD causes a similar effect in humans. For instance, Oliver (25) has reported cases of hypercholesterolemia in humans who have been exposed to TCDD three years prior to examination. Pazderova and coworkers (26) have also reported that about one-half of the 55 workers involved in 2,4,5-T production showed increased levels of serum cholesterol and phospholipids. Walker and Martin (27) have examined eight workers who showed chloracne due to occupational exposure to TCDD containing products, and found serum triglyceride and cholesterol levels higher than age-matched controls (n = 100). Though these epidemiological data do not provide an absolute proof for a causal relationship, it points to need of further studies in this area.

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