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TCDD, Dietary Iron and Hepatic Iron Distribution in Female Rats

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a prototype for a large group of halogenated aromatic hydrocarbons, and is the most potent of these compounds. It is an unwanted by-product in the manufacture of some groups of herbicides as 2,4,5-trichlorophenoxyacetic acid, and occurs during the incineration of some types of municipal wastes. TCDD is an environmental pollutant with exceptional toxicity for certain mammalian and avian species. The liver is one of the principal target organs affected by TCDD in the rat and other laboratory species (Poland et al., 1985).

TCDD induces many functional, biochemical and pathological changes, including altered lipid metabolism in the liver. Chronic oral administration of TCDD to rats and mice results in hepatic porphyria (Goldstein et al., 1973). Depletion of body iron stores is effective therapy for porphyria, and iron deficiency protects mice against porphyria caused by TCDD (Rowley and Sweeney, 1984). Non-heme iron in the liver supports the hepatotoxic effects of dioxins (Jones et al., 1981). However, the mechanisms involved are unknown. Ferrous iron plays an important role in the initiation of lipid peroxidation (Morehouse et al., 1984). Iron is sequestered in healthy cells in ferritin as ferric iron (Fe^{3+}) to protect against iron toxicity (Munro and Linder, 1978). A proposed mechanism for the production of liver injury in chronic iron overload is that organelle damage leading to cell death occurs as a result of membrane lipid peroxidation initiated and promoted by intracellular iron (Bacon et al., 1985). The presence of iron in subcellular fractions *in vitro* may catalyze lipid peroxidation and produce membrane damage (Hultcrantz et al., 1982).

Stohs et al. (1983) have provided evidence for the occurrence of hepatic lipid peroxidation after TCDD administration. The purpose of this study was to determine if TCDD induced lipid peroxidation was associated with an increase in the iron content of liver and its subcellular fractions. The effect of TCDD administration on the iron content of whole homogenate, microsomes, mitochondria, and cytosol of livers of female rats fed defined diets containing deficient, normal and excessive levels of iron for 17, 24 and 31 days was investigated.

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MATERIALS AND METHODS

Female Sprague-Dawley rats, weighing 140-160 gm, were obtained from Sasco Co. Inc., Omaha, NE. The animals were given free access to water and laboratory chow. They were maintained at a temperature of 21°C, with lighting from 6:00 a.m. to 6:00 p.m. daily. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was obtained from Dr. James D. McKinney, NIEHS. Groups of rats were fed defined diets containing deficient (6 ppm), normal (35 ppm) and supplemented (120 ppm) levels of iron for 17, 24 and 31 days. The iron was added as ferrous sulfate. The three diets were prepared by Teklad, Madison, WI, and are identified as TD85131, TD85278 and TD85279 diets, respectively. Half of each group of animals was treated with 40 µg TCDD/kg/day P.O. in corn oil on days 9, 8 and 7 prior to sacrifice. The other half of each group received the corn oil vehicle. All animals were killed by cervical dislocation between 6:00-7:00 a.m. to eliminate possible effects due to diurnal variation.

Livers were perfused *in situ*, and microsomal, mitochondrial and cytosolic fractions were isolated by differential centrifugation in 0.05 M Tris chloride-0.15 M KCl buffer, pH 7.4. For iron determination, whole homogenate and subcellular fractions were digested in concentrated nitric acid at 130-140°C until clear (approx. 15 min) using a modification of the procedure of Goldstein et al. (1973). Iron content was assayed with a Perkin-Elmer Model 2380 atomic absorption spectrophotometer with $FeCl_3$ (Fischer Scientific Company) as the standard. The results were expressed as nmol iron/mg protein. Protein concentrations of whole homogenate, microsomes, mitochondria and cytosol were determined according to the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

A significant decrease in iron content of whole homogenate occurred after female rats were maintained on an iron deficient (6 ppm) diet for 17 days (Fig. 1). Partial adaptive recovery occurred by day 31. A small but steady increase occurred with time in the iron content of the whole liver in animals on the high iron (120 ppm) diet. The iron content of whole liver in rats fed the iron deficient and iron supplemented diets were -26.3% and +13.4%, respectively, relative to the control (35 ppm) diet. Small but insignificant changes in iron content of whole liver were observed in TCDD treated rats fed the 3 diets for 17, 24 and 31 days (Fig. 1). The percentage changes in iron content after 31 days on the control, iron deficient and iron supplemented diets following TCDD administration relative to animals which did not receive TCDD were +4.5%, -2.1% and +5.3%, respectively.

The iron content of hepatic microsomes after feeding the 3 diets for 17, 24 and 31 days is presented in Fig. 2. No change in iron content occurred with time in microsomes from untreated animals fed the control diet. A marked decrease was observed in the iron content of microsomes from rats fed the iron deficient diet. The

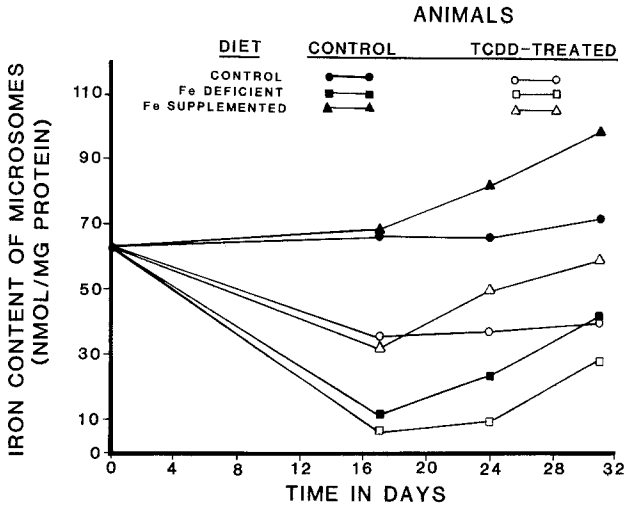

Figure 1. Iron content in whole liver homogenate. Female rats were fed control, iron deficient and iron supplemented diets for 17, 24 or 31 days. TCDD treated animals received 40 μg TCDD/kg P.O. on days 9, 8 and 7 prior to sacrifice. Control animals received the corn oil vehicle. Values are the mean ± S.D. of 4-6 animals, and the data are expressed as nmol/mg protein.

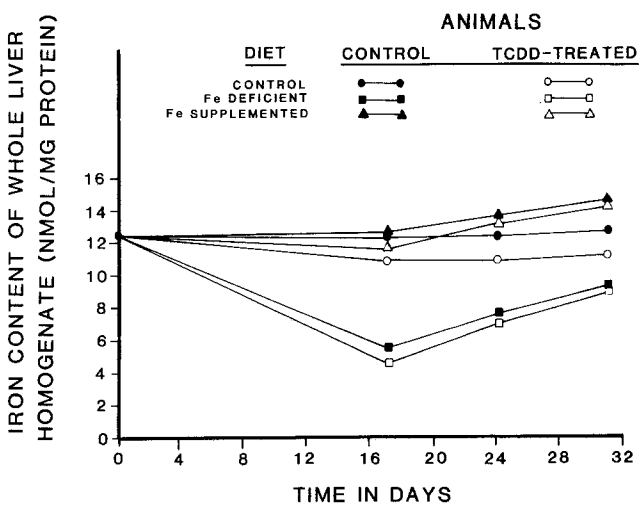

Figure 2. Iron content of hepatic microsomes. Animals were fed the diets for 17, 24 or 31 days. TCDD treated animals received 40 μg TCDD/kg P.O. on days 9, 8 and 7 prior to sacrifice. Control animals received the vehicle. Values are the mean ± S.D. of 4-6 animals, and the data are expressed as nmol/mg protein.

greatest decrease (89.5%) occurred after 17 days on the diet. The iron content of hepatic microsomes of rats fed the iron deficient diet increased between days 17 and 31, presumably due to greater iron sequestration by these animals. A steady increase in iron content of microsomes occurred with time when animals were fed the iron supplemented diet. The percentage changes for the deficient and supplemented diets relative to the control diet after 31 days were -42.5% and +41.0%, respectively. The effect of administering TCDD on microsomal iron content in animals on the 3 diets is given in Fig. 2. TCDD treatment resulted in a significant decrease in microsomal iron content of rats on all 3 diets. After 31 days, decreases of -44.0%, -31.1% and -41.8% were produced by TCDD administration in animals fed the control, iron deficient and iron supplemented diets, respectively.

The iron content of hepatic mitochondria after feeding the 3 diets for 17, 24 and 31 days is given in Fig. 3. No change occurred in mitochondrial iron content with time in rats fed the control (35 ppm) diet. However, after 31 days mitochondrial iron content decreased by 18.4% in animals given the iron deficient (6 ppm) diet and increased by only 2.3% in animals on the iron supplemented (120 ppm) diet relative to the control diet. When rats on the 3 diets were administered TCDD, significant increases in iron content of mitochondria occurred (Fig. 3). In TCDD treated rats fed the control, iron deficient and iron supplemented diets, after 31 days the hepatic mitochondrial iron contents relative to animals which did not receive TCDD increased by 46.2%, 28.1% and 71.4%, respectively. Thus, the greatest increase in mitochondrial iron after 31 days on the diets was observed in TCDD treated animals fed the iron supplemented diet.

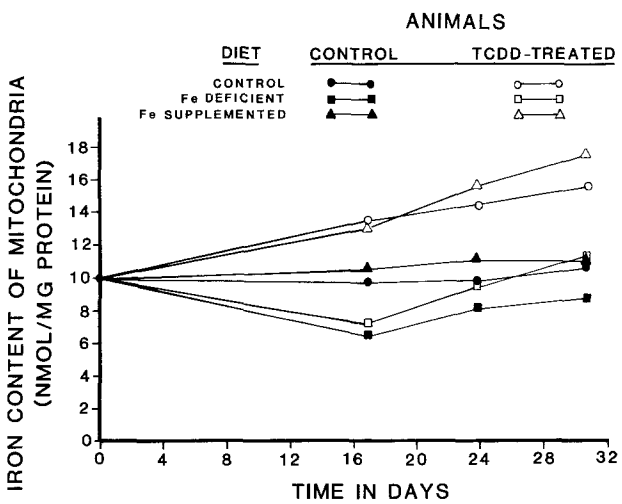

Figure 3. Iron content of hepatic mitochondria. Animals were fed the diets for 17, 24 or 31 days. TCDD treated animals received 40 µg TCDD/kg P.O. on days 9, 8 and 7 prior to sacrifice. Control animals received the corn oil vehicle. Values are the mean ± S.D. of 4-6 animals and the data are expressed as nmol/mg protein.

The effect of dietary iron on the iron content of hepatic cytosol is presented in Fig. 4. No change occurred in the iron content of cytosol with time in animals on the control diet. A 15.6% decrease in cytosol iron content occurred in animals on the iron deficient diet, and a 5.6% increase occurred in cytosol iron content in animals fed the iron supplemented diet relative to the control diet. When rats fed the control diet for 31 days were treated with TCDD, a small (5.9%) but non-significant increase in iron content of cytosol occurred (Fig. 4). TCDD treatment of rats fed the iron deficient diet for 31 days also resulted in a small (11.8%) but non-significant increase in cytosol iron content. A significant increase (18.6%) in the iron content of the cytosol fraction occurred as a result of TCDD treatment of rats fed the iron supplemented diet for 31 days.

Ferrous iron is believed to play a catalytic role in the formation of hydroxyl radical by reacting with H_2O_2 via the Fenton reaction, and thus enhances the process of lipid peroxidation. An increase in hepatic microsomal malondialdehyde (MDA) after TCDD administration might be due to an increase in iron content since iron is known to be associated with lipid peroxidation (Thomas et al., 1985). Therefore, we determined the effect of TCDD administration on iron distribution in whole homogenate, and mitochondria, microsomes and cytosol in livers of female rats fed defined diets containing deficient, normal (control), and excessive (supplemented) levels of iron for 17, 24 and 31 days.

The results suggest that TCDD induced hepatic lipid peroxidation is not due to an increase in hepatic iron content. The increase in

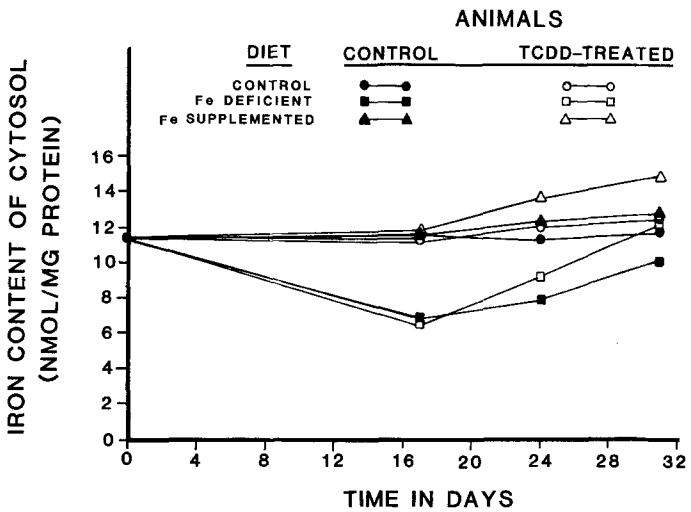

Figure 4. Iron content of hepatic cytosol. Animals were fed the diets for 17, 24 or 31 days. TCDD treated animals received 40 μg TCDD/kg P.O. on days 9, 8 and 7 prior to sacrifice. Control animals received the corn oil vehicle. Values are the mean ± S.D. of 4-6 animals, and the data are expressed as nmol/mg protein.

MDA production by microsomes following TCDD administration can not be attributed to an increase in microsomal iron content, as demonstrated in Fig. 2. However, TCDD may result in the liberation of iron from bound or storage forms in microsomes, thus making more iron available for participation in the process of lipid peroxidation. Rowley and Sweeney (1984) have studied the release of iron from ferritin by hepatic microsomes from control and TCDD-treated rats. Iron release correlated most closely with cytochrome P-450 concentration. TCDD is a well known inducer of cytochrome P-450. Thomas and Aust (1986) have shown that large amounts of ferritin are associated with microsomes prepared by conventional differential centrifugation. The iron associated with ferritin may be involved in microsomal lipid peroxidation.

Goldstein et al. (1973) reported an increase in the total iron content (μg/g) of mouse liver 17-30 days following the administration of up to 150 μg TCDD/kg. The present study does not confirm these observations in rats after TCDD treatment. The reason for these differences is not known, but may be associated with dose of TCDD, time after TCDD administration, species of experimental animal, or manner in which the data was reported.

Iron is causally related to the changes in MDA production (Hultcrantz et al., 1984). However, under physiological conditions and in healthy cells, iron is sequestered in ferritin as a nontoxic oxyhydroxide complexed with phosphate, and release of iron from ferritin requires reduction (Munro and Linder, 1978). TCDD may promote microsomal iron reduction (Rowley and Sweeney, 1984). As a result of structural disorders or the toxic effects of xenobiotics, iron may be released and reach sites to which it normally has little or no access.

Reactive oxygen species or free radicals may interact with cellular constituents and initiate peroxidation of the unsaturated fatty acids localized in the lipids of membranes. TCDD induced hepatic lipid peroxidation may occur through enhanced H_2O_2 formation (Stohs et al., 1984) or a decrease in H_2O_2 elimination (Stohs et al., 1986). TCDD is a potent inducer of microsomal cytochrome P-450 isozymes (Jaiswal et al., 1985). NADPH-cytochrome c reductase or cytochrome P-450 may produce superoxide anion and H_2O_2. Furthermore, TCDD has been shown to inhibit the H_2O_2 metabolizing enzyme selenium-dependent glutathione peroxidase (Stohs et al., 1986). Thus, H_2O_2 accumulation may occur by several possible mechanisms. Iron may be reduced to the ferrous state either by superoxide anion or directly by microsomal cytochrome P-450 reductase (Morehouse et al., 1984).

Free forms of iron may initiate membrane lipid peroxidation by the formation of perferryl ion (FeO_2^{2+}) or by the formation of a ternary free radical complex between polyunsaturated fatty acids, ferrous ions and oxygen (Bacon et al., 1985). The rate of lipid peroxidation is highly dependent on the presence of iron and the $Fe^{2+} \rightleftharpoons Fe^{3+}$ redox system, but not on the total amount of iron (Hultcrantz et al., 1984). A TCDD-mediated increase in the availability of H_2O_2 and/or other reactive oxygen species in

association with iron could result in peroxidation of the fatty acid components of membrane phospholipids, and may contribute to abnormalities of cellular function which are dependent on intact organelle membrane structure (Bacon et al., 1985).

The rate of iron-promoted lipid peroxidation may depend on the site of binding of iron in the cell, and also on the nature of the iron. It is unlikely that excess protein-bound storage iron is the biochemical form of iron responsible for microsomal lipid peroxidation since the data clearly indicate that no increase in microsomal iron content occurred upon TCDD administration to rats fed the 3 diets (Fig. 2). However, TCDD treatment may result in an increase in free iron, and thus produce an increase in hepatic microsomal lipid peroxidation. Sweeney et al. (1979) suggested that TCDD-induced porphyria may be secondary to a progressive increase in lipid peroxides. An iron deficiency protects against TCDD-induced porphyria (Rowley and Sweeney, 1984), providing further evidence for a relationship between some forms of TCDD toxicity, iron and lipid peroxidation.

An increase in iron associated with mitochondria was observed. Mitochondria may function in a compensatory mechanism, participating in sequestration, recycling and storage of excess iron in the cell between various cell compartments. The release of iron from mitochondria depends on many factors such as the phospholipid content of the mitochondrial inner membrane, the nature of iron chelators, and enzymes present in mitochondria as compared to microsomes (Ulvik and Romslo, 1979).

In summary, TCDD treatment produces significant alterations in iron content of microsomes and mitochondria. The increase in microsomal lipid peroxidation induced by TCDD may be due to an increase in microsomal free iron rather than an increase in microsomal total iron content. Further studies on the effect of TCDD on the various forms of iron in microsomes as well as other subcellular fractions is warranted.

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