OXIDATIVE STRESS IN THE TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

S.J. Stohs, M.A. Shara, N.Z. Alsharif, Z.Z. Wahba, and Z.A.F. Al-Bayati

Creighton University School of Pharmacy and Allied Health

Omaha, Nebraska 68178 USA

ABSTRACT: TCDD induces the production of an oxidative stress in rodents as evidenced by: enhanced lipid peroxidation; increased DNA damage; decreased membrane fluidity; altered calcium homeostasis; and decreased non-protein sulfhydryl and NADPH contents in liver. Possible sources of reactive oxygen species include mitochondria, microsomes, and macrophage. Oxidative stress may contribute to many of the tissue damaging effects of TCDD.

INTRODUCTION: The mechanism of toxicity of TCDD and its bioisosteres involves binding to the Ah receptor, interaction of this complex with chromatin and the ultimate production of a pleiotropic response. Characteristic toxic effects, teratogenesis, mutagenesis, and tumor promotion occur. However, the post-translational mechanisms involved in the production of these effects are not known. We have hypothesized that an oxidative stress may play a central role in the toxic manifestations of TCDD [Stohs et al., 1990].

EXPERIMENTAL: Female Sprague-Dawley rats were treated with 100 μg TCDD/kg orally or the vehicle. The animals were killed on days 0, 1, 3, 5, 7, 9 or 12. Lipid peroxidation was determined as the content of thiobarbituric acid reactive substances [TBARS] [Alsharif et al., 1990a]. Calcium content was estimated by atomic absorption spectrometry following digestion of tissues in concentrated nitric acid [Al-Bayati et al., 1988]. Non-protein sulfhydryl content was determined spectrophotometrically using Ellman's reagent [Sedlack and Lindsay, 1968]. NADPH content was estimated by the enzymatic cycling spectrophotometric method of Giblin and Reddy [1980]. Membrane fluidity studies were performed by steady state fluorescence spectroscopy [Alsharif et al., 1990a]. DNA damage was measured as single strand breaks by the alkaline elution procedure [Wahba et al., 1989]. The results for each time point were derived from 8-12 control animals and 4-8 treated animals.

RESULTS AND DISCUSSION: The data are presented as percent of control values in Figures 1-5. Time-dependent increases in lipid peroxidation of 400-500% occurred in mitochondrial (Fig. 1), microsomal (Fig. 2), and nuclear (Fig. 4) membranes with maximum increases occurring 5-6 days post-treatment. Smaller increases in lipid peroxidation were observed in plasma membranes (Fig 5). Large increases in lipid peroxidation in microsomal membranes preceded the maximum increases in lipid peroxidation in mitochondrial and nuclear membranes.

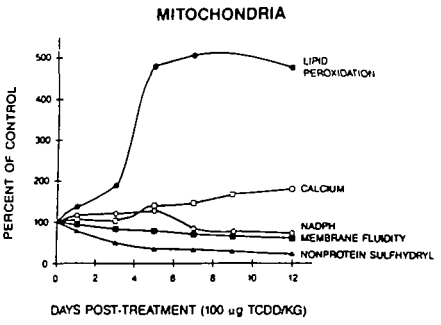

Figure 1

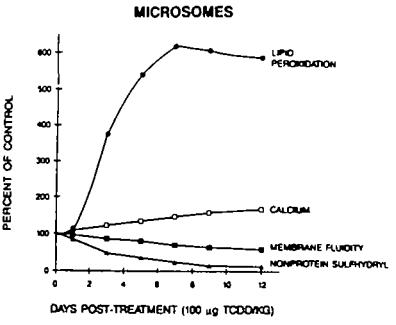

Figure 2

Gradual decreases in non-protein sulfhydryl content of membrane fractions were observed, with decreases of approximately 50% and 80% occurring in mitochondrial (Fig. 1) and microsomal (Fig. 2) fractions 3 and 12 days, respectively, after treatment with TCDD. Decreases in membrane fluidity paralleled decreases in non-protein sulfhydryl content with decreases of 30-40% by day 12 post-treatment in microsomes (Fig. 2), mitochondria (Fig. 1), and plasma (Fig. 5) membranes. A sharp increase in the incidence of DNA single strand breaks was observed in hepatic nuclei and appeared to follow the increase in lipid peroxidation (Fig. 3). Increases of 70-80% occurred in the calcium content of mitochondria (Fig. 1) and microsomes (Fig. 2) 9-12 days after TCDD administration. An increase in mitochondrial calcium was not observed until 5 days post-treatment while a significant increase in microsomal calcium content was observed by day 3. A small but significant increase in cytosolic calcium was present by day 12 (Fig. 4). Cytosolic (Fig. 4) and mitochondrial (Fig. 1) NADPH content increased 40-60% 5 days after TCDD administration, but decreased to approximately two-thirds of control values by day 12.

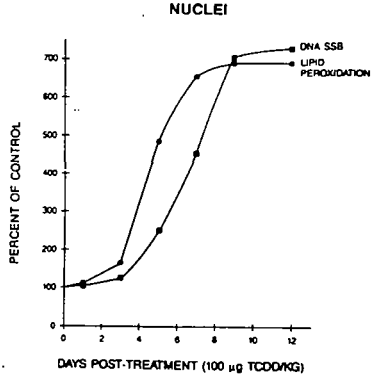

Figure 3

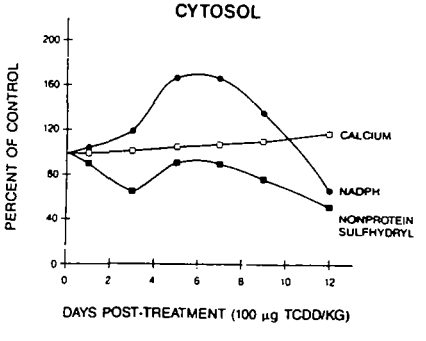

Figure 4

The results demonstrate that TCDD induces an oxidative stress as demonstrated by enhanced lipid peroxidation, increased DNA and membrane damage, decreased non-protein sulfhydryl and NADPH contents, and increased calcium content. Similar changes were observed in hepatic mitochondria and microsomes with respect to lipid peroxidation, membrane fluidity and non-protein sulfhydryl content.

A greater lag time existed in mitochondria with respect to the onset of lipid peroxidation as well as the increase in calcium content which may be related to the ability to generate NADPH. Figure 6 depicts probable interrelationships associated with TCDD-induced oxidative tissue damage. The proposal takes into consideration oxidative damage to lipids, DNA and proteins with subsequent alterations in membrane fluidity, calcium homeostasis, and NADPH content.

The precise mechanisms involved in the production of reactive oxygen species by TCDD are not known. However, several possible sources exist. Alsharif et al. [1990b] have demonstrated that the administration of TCDD to rats results in a marked increase in superoxide anion production by peritoneal exudate cells. The studies of Wahba et al. [1989] suggest that both mitochondria and microsomes may produce reactive oxygen species which result in the formation of DNA damage. Stohs et al. [1986] demonstrated that hydrogen peroxide and superoxide anion as well as hydroxyl radical may be involved in TCDD-induced microsomal lipid peroxidation by using inhibitors and free radical scavengers. Furthermore, iron is required for TCDD induced microsomal lipid peroxidation [Al-Bayati and Stohs, 1987] and DNA damage [Wahba et al., 1989]. Nohl et al. [1989] also observed that mitochondria may be a source of superoxide anion and hydrogen peroxide in response to TCDD.

The results clearly demonstrate that TCDD produces an oxidative stress. In general, concerted changes occur in the indicators of oxidative stress in the liver in response to TCDD. The evidence indicates that the TCDD induces the formation of reactive oxygen species which results in the changes that are reflected in the parameters which have been assessed in this study. Since oxidative stress is well known to be deleterious to many biological molecules and produce a broad range of untoward effects, many of the toxic manifestations of TCDD may be mediated through this mechanism.

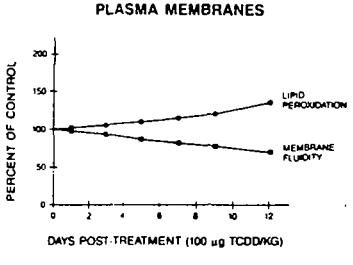

Figure 5

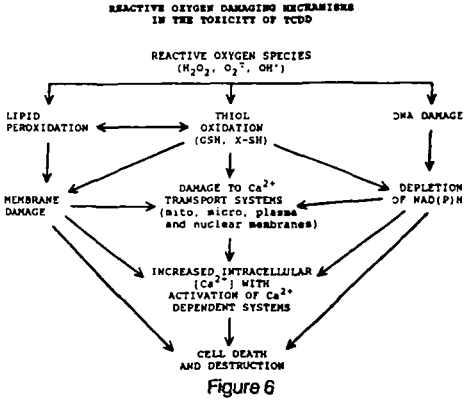

Figure 6

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