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Effect of tocopherol and acetylsalicylic acid on the biochemical indices of blood in dioxin-exposed rats

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ABSTRACT

New sources of dioxins and increased dioxin concentrations in the environment, coupled with their increased bioavailability along the food chain and accumulation in adipose tissues, contribute to various adverse long-term biological effects. The purpose of the study was to determine whether tocopherol protects the CNS by decreasing the pro-inflammatory influence of free radicals generated by TCDD; whether acetylsalicylic acid inhibits the production of inflammatory mediators; and whether the combined administration of tocopherol and acetylsalicylic acid to TCDD-exposed rats has a potential CNS-protective effect. The study included 117 rats divided into 8 groups: 75 female and 12 male Buffalo rats aged 8–10 weeks, weighing 140–160 g; as well as 30 female rats aged 6 weeks and weighing 120 g, which were the offspring of females from each study group. In the experiment, the following substances were used: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), dosed at 5 μg/kg BW and 12.5 μg/kg BW, diluted in a 1% DMSO solution at the concentration of 1 μg/ml; α-tocopherol acetate, dosed at 30 mg/kg BW, in 0.2 ml of oil solution; and acetylsalicylic acid, 50 mg/kg BW, suspended in 0.5 ml of starch solution, administered orally using a feeding tube. Pleurisy was induced by an injection of 0.15 ml of 1% carrageenin solution. The use of tocopherol reduces the adverse effects of the inflammatory reaction induced by TCDD. Administering tocopherol improves protein metabolism by reducing protein catabolism, and raises γ-globulin fraction levels. Combined acetylsalicylic acid and tocopherol suppress catabolic processes accompanying inflammation.

1. Introduction

New sources of dioxins and increased dioxin concentrations in the environment, coupled with their increased bioavailability along the food chain and accumulation in adipose tissues, contribute to various adverse long-term biological effects (Gaborek et al., 2001; Mai et al., 2007; Rayne et al., 2005; Rayne, 2005; Sorg et al., 2009; Tang et al., 2004a, 2004b). As the natural environment is increasingly polluted with 2,3,7,8-tetrachlorodibenzodioxin derivatives due to the limited biodegradation capabilities, the substances accumulate in living organisms. The long-term biological effects of dioxins observed in the second generation are related to their xenoestrogenic activity. Dioxins exhibit affinity for estrogen receptors and can impair the reproductive capacity

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(Całkosiński et al., 2003, 2004a,b; el-Demerdash et al., 2004; Jung et al., 2010). Dioxins are eliminated from the body directly, with milk and through epidermal lipids produced by sebaceous glands, and indirectly, in metabolized forms, with urine and feces (Geusau et al., 2001a,b). Higher concentrations of dioxins have been found in highly industrialized areas. There, the substances accumulate, enter the food chain, and reach its final stage, i.e. humans or carnivore mammals (Basselin et al., 2011; Helgason et al., 2010; Li et al., 2006; Oehme et al., 1995a,b).

Dioxins and other toxins (cytostatics) damage the liver and impair acute-phase protein synthesis, changing the nature and dynamics of inflammatory response (Całkosiński et al., 2005, 2009a,b, 2011a,b). Dioxin-induced changes in liver metabolism impair cholesterol and estrogen metabolism (Całkosiński, 2005), increase the activity of liver enzymes such as AspAT and AlAT, and decrease the activity of antioxidant enzymes (Całkosiński, 2008; Turkez et al., 2012). Moreover, increased susceptibility to infection, neurological disorders and hormonal disorders have been observed (Akahoshi et al., 2006; Aly and Domenech, 2009; Clements et al., 2009; Haarmann-Stemmann et al., 2009; Ishida et al., 2010; Ozeki et al., 2011; Sato et al., 2008; Stevens et al., 2009; Stockinger et al., 2011; Teraoka et al., 2009; Tsukamoto et al., 1995). Dioxins act directly and indirectly, also impairing cholesterol metabolism and sex hormone balance in the offspring of dioxin-poisoned mothers (Całkosiński, 2005, 2008; Jin et al., 2008, 2010; Kakeyama et al., 2008). Long-term exposure to dioxins contributes to many adverse health effects, as the substances are immunotoxic (Stevens et al., 2009; Stockinger et al., 2011; Ivens et al., 1992; Marshall and Kerkvliet, 2010; Singh et al., 2011), neurotoxic, hepatotoxic and teratogenic.

Because of the proinflammatory action of dioxins, it can be assumed that in dioxin poisoning, as in other inflammatory reactions, the serum concentration of leptin stimulated by TNF-α and IL-6 increases (Ferrante et al., 2014; Koenig et al., 2014; Linden et al., 2005; Pina et al., 2015). This leads to increased oxidative stress, loss of appetite, and ultimately, cachexia (Anubhuti, 2008; Fowler et al., 1973; Lensu et al., 2006; Linden et al., 2010), which, combined with the dioxin-induced hepatic cell damage, impairs plasma protein synthesis (Całkosiński, 2008). Consequently, cachexic symptoms occur, including muscle atrophy, increased urea and creatinine concentrations, and ultimately, ascites (Linden et al., 2005).

The molecular action of dioxins involves activating the transcription of genes encoding xenobiotic-metabolizing enzymes, including various molecular forms of cytochrome P-450 (CYP), especially the CYP1A1 family (Strucinski et al., 2011). The induction of CYP1A1 family genes is controlled by the cytoplasmic AhR (aryl hydrocarbon receptor) receptor and the nuclear ARNT (aryl hydrocarbon receptor nuclear translocator) protein, present in most human and animal cells (Palut et al., 2002). TCDD has also been found to stimulate the COX-2 cyclooxygenase. Tocopherol blocks the AhR receptor and may protect estrogen receptors against dioxin availability (Całkosinski et al., 2013). As to acetylsalicylic acid, it reduces inflammation by blocking COX-2. Acetylsalicylic acid weakens the effects of dioxins by blocking the AhR receptor contributing to CYP effects (Całkosiński, 2008).

A study by Moon et al. (2008) showed that a single dose of TCDD increased the levels of corticotropin-releasing hormone, arginine vasopressin and pro-opiomelanocortin in the brain. This was correlated with the rats' lowered food and water intake during the 14 days following TCDD administration (Moon et al., 2008). The resulting metabolic disorders affect central nervous system function.

So far, there are no reliable study results on the effect of low doses of TCDD on humans. As has been mentioned above, dioxins are slow-acting but effective poisons, and the resulting disorders and immune deficiencies may only become manifest in subsequent generations (Całkosiński et al., 2003, 2004a; Jung et al., 2010; Oehme et al., 1995a,b; Clements et al., 2009; Singh et al., 2011; Ding et al., 2011).

2. Materials and methods

2.1. *Animals*

The study included 117 rats: 75 female and 12 male Buffalo rats aged 8–10 weeks, weighing 140–160 g; as well as 30 female rats aged 6 weeks and weighing 120 g, which were the offspring of females from each study group described below. All experiments were performed in compliance with guidelines for the experimentation on animals. The study was approved by Local Ethics Council for Animal Experiments (permission no.: 38/2009).

The animals were kept in air-conditioned rooms, with 15 air changes per 1 h, at 22 °C, 55% humidity, in a 12/12 h light cycle. The animals were kept in polystyrene cages (6 rats per cage) with free access to water and to a balanced feed for laboratory animals, Labofeed H. Rats were randomized into the following study groups:

- 1 – K – control group of 6 females in which no substances were administered; histological, ultra-structural, and immunohistochemical analyses were performed;
- 1A – K + Z – control group of 6 females in which pleurisy was induced, and test samples were taken at 120 h of inflammation;
- 1B – K + TCDD – 3 females used as a positive control; administered a single IM dose of TCDD, 12.5 μg/kg BW;
- 2 – TCDD + Z – 6 females administered IM TCDD 3 weeks before the experiment; after the 3 weeks, pleurisy was induced, and test samples were taken at 120 h of inflammation;
- 3 – TCDD + T + Z – 6 females administered IM TCDD 3 weeks before the experiment; for the following 3 weeks, they were administered a daily SC dose of α-tocopherol acetate, 30 mg/kg BW; after the 3 weeks, pleurisy was induced, and test samples were taken at 120 h of inflammation;
- 4 – TCDD + ASA + Z – 6 females administered IM TCDD 3 weeks before the experiment; for the following 3 weeks they were administered a daily oral dose of acetylsalicylic acid, 50 mg/kg BW; after the 3 weeks, pleurisy was induced, and test samples were taken at 120 h of inflammation;
- 5 – TCDD + T + ASA + Z – 6 females administered IM TCDD 3 weeks before the experiment; for the following 3 weeks, they were administered a daily SC dose of α-tocopherol acetate,