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Assessment of physical traits of rat offspring derived from mothers exposed to dioxin

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ABSTRACT

Negative effects of dioxin action are associated with limited abilities of their bio-degradation along with continuously increasing production and long-term bio-accumulation of those toxins in living organisms. Dioxins penetrate through placenta to fetus indicating indirect toxic effects on offspring of mothers exposed to the action of these toxins. During lactation a significant part of dioxins is excreted from organism with milk, which contributes to further accumulation of those compounds and multiple exceeding of maximal permissible dose of dioxins in newborns feeding with mother's milk. The aim of the study was to determine how a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) administered to females 3 weeks before getting pregnant affects the parturition duration, labour delay, number and weight of offspring. The studies were performed on rat offspring deriving from primigravida females from the Buffalo strain, which 3 weeks before getting pregnant were administered with a single dose of TCDD. The obtained results revealed that labours in females exposed to dioxin effects were characterized by significant temporal range and their end occurred 3 weeks later compared to females from the control group, which gave birth within a very narrow temporal range ending within 2 days. Offspring obtained from females exposed to the TCDD action was smaller in number and was characterized by smaller rearing and smaller birth weight even after the first month of life; however weight gain in both groups was similar and it was twelve-fold increase.

1. Introduction

Dioxins are by-products formed in the process of production and combustion of pesticides, herbicides, solvents, and plastics, occurring particularly in industrial districts. The contamination is mainly pollution of environment by heavy metal elements and a group of chloro-organic compounds including dioxins (TCDD). It was found that particularly large amounts of dioxins are cumulated in fatty tissue. It is associated with the fact that dioxins are lipophilic compounds that bond to sediments and organic substance in the environment and so they have an affinity for accumulation in fatty tissue of humans and animals (Piskorska-Pliszczyńska, 1999). Dioxins are metabolized in liver and excreted through bile ducts (Van den Berg et al., 1994). Dioxins accumulated in fatty tissue of humans and animals are eliminated depending on life span and metabolism level characteristic of particular species, e.g., in humans the process of dioxin elimination lasts for about 9 years. Elimination of dioxins from organism also depends on the level of toxicity, that is, on a number of chlorine atoms occurring in dioxin congeners (Schlatter, 1991). Their half-life may be subject to shortening by excretion of TCDD with milk during lactation (Van den Berg et al., 1989).

Dioxins penetrate into the organisms of humans and animals mainly through the alimentary tract, via consumption of animal products with high fat content. Therefore dioxin infections may also apply to organisms, in whose natural environment dioxins do not occur; instead, the infection takes place as a result of consumption of contaminated meat, such as that of a high-fat fish. It has been proved by numerous studies which indicated presence of significant amount of dioxins in milk and fatty tissue of female Eskimos living in non-industrial environment, yet feeding on large amounts of contaminated meat and fat of fish and sea mammals (Całkosiński et al., 2005).

Dioxins are characterized by diverse activity depending on sex. The studies on rats proved that females are more susceptible to effects of the dioxins compared to males. It is the result of

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anti-oestrogenic mechanism of reaction with oestrogen receptor and, because of that, it comes to disturbances in gonad functioning (Chaffin and Hutz, 1997; Ivens et al., 1992). The presence of dioxins in female organisms contributes to decreased reproduction; those compounds also affect the development of foetus through placenta. Among offspring of polar bears, increased mortality was observed, as well as immunological deficits and disorders in sex hormone balance, e.g., hermaphroditism (Całkosiński, 2008; Sonne et al., 2006). Dioxin also affects the health of offspring, since a large amount of dioxins is eliminated from organism with mother's milk during lactation (Heimler et al., 1998; Pluim et al., 1992). In infants it contributes to multiple (about 50–70 times) exceeding of maximal dose per body weight (Patandin et al., 1999).

In the 1990s, there was an increase in infertility and abortion in the area of industrial districts, which may be associated with pollution of natural environment by the group of chloro-organic compounds including dioxins (Grochowalski et al., 2001). A significant part in disturbance of reproduction in humans and animals is ascribed to the effects of dioxins, classified as xenobiotics, which reveal hormone-like activity. This results from binding of dioxins with aryl hydrocarbon receptor (AhR), which leads to endocrine disorders. Studies on rat male and female gonads revealed inhibition of oogenesis and spermatogenesis, which is the cause of infertility (Całkosiński et al., 2004b). Biological effects of dioxin action associated with disorders of synthesis of hormones whose precursor is cholesterol result in liver function impairment. TCDD binding with AhR receptor, which reveals similarity to oestrogen receptor, impairs steroidal balance (Całkosiński et al., 2005).

Dioxins as xenobiotics can imitate activity of hormones; therefore, they are called eco-oestrogens, environmental oestrogens, or endocrine modulators. Long-lasting exposure to hormone-like compounds as well as to some xenobiotics found in rain and drinking water, soil, and animal food is associated with functional disorders in the development of animals and, in early stages of foetal life, it causes permanent and irreversible effects (Colborn et al., 1993).

The aim of this study was to determine how a single TCDD dose administered to females 3 weeks before getting pregnant affects the number, birth weight, and body mass of offspring during the 1st month of life.

2. Material and methods

2.1. Animals

The studies were conducted on rat offspring deriving from 10 primigravida females and 10 males aged 4 months, weighing 180–200 g, from the Buffalo strain, and coming from the same breed. All animals received human care in compliance with the Guide for the Care and Use of Laboratory Animals (NRC 2011). All experiments were performed according to guidelines for the experimentation on animals. The study was approved by a Local Ethics Council for Animal Experiments (permission number: 38/2009). The rats were bred and kept in air-conditioned rooms characterized by 15-cycle change of air per hour, in polystyrene cages of dimension 60 cm × 40 cm × 40 cm, in constant temperature 22 °C and humidity 55%, and a 12-h light-day cycle with five individuals in a cage. Two pregnant females were kept in each cage.

The females were divided into two groups with five females in each group: C – control group of mothers, and D – a group of mothers, which 3 weeks before getting pregnant were administered with dioxin. Offspring of mothers was also divided into groups: C_I – 1st generation offspring deriving from females from control group which were not exposed to dioxin action, D_I – 1st generation offspring deriving from females, which were administered

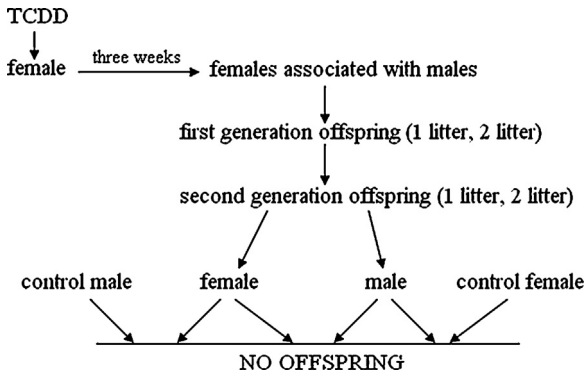

Fig. 1. Schematic diagram showing the rats flow through the study stages.

with TCDD 3 weeks before getting pregnant, D_{II} – 2nd generation offspring deriving from females from the group D_I, and D_{III} – 3rd generation offspring deriving from females from the group D_{II}. The studies were conducted according to diagram shown in Fig. 1.

2.2. TCDD administration

At the first stage of the research, the females from the group D, 3 weeks before mating with males, were hypodermically administered with a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin dissolved in DMSO at a dose of 5 μg/kg b.w. The females from particular groups were mated with males not exposed to effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, randomly selected and coming from the same breed. At the next stage of the research, observations were carried out concerning the ability of females to get pregnant in each group, and deviations in pregnancy length (from the moment of mating with a male until the moment of labour) from the correct value (normally 21–23 days) were checked as well. After delivery, litter size (N), vitality, and weight of the whole litter were determined. Weight measurements were made using basic analytical balance (0–200 g, accuracy 1 mg).

2.3. Experimental design

In order to assess the vitality of offspring, swimming and tail tests were conducted, which were performed in accordance with generally accepted and established procedures. Swimming tests consisted of putting rats into a cylinder-shape container of 40 cm height and 18 cm in diameter, filled up with water of temperature 25 °C for 5 min. The test measured 'idle time', i.e., time when a rat did not react and stopped swimming. Immobility was determined at the 0–3 pts. Swimming intensity scale; no swimming – 0 pt., slight swimming – 1 pt., average swimming – 2 pt., intensive swimming – 3 pt.

Test of tail consisted of putting rats in a cylinder with a hole for the tail, which was then put into a test-tube filled with water of temperature 58 °C to check unconditioned reflex. Time was measured from the moment of putting tail into water until the moment of unconditioned reflex (pulling the tail out of the test-tube by rat). The longer the reaction time was, the lesser the vitality and activity of the rat. In order to determine reaction dynamics, measurements of reaction times in the tail test in each individual were conducted three times with intervals of 60 s. Thirty days after birth, weight measurements were made again for individuals in litters C_I and D_I; their size and vitality were evaluated.

In the second part of the experiment, when the females from the litter of mothers exposed to TCDD effects (group D_I) reached sexual maturity (on 40th day of life), they were mated with randomly selected males of the same age, coming from the same breed and not exposed to dioxins. Next, the same measurements were made