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Perinatal exposure to glyphosate-based herbicide alters the thyrotrophic axis and causes thyroid hormone homeostasis imbalance in male rats

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ABSTRACT

Glyphosate-based herbicides (GBHs) are widely used in agriculture. Recently, several animal and epidemiological studies have been conducted to understand the effects of these chemicals as an endocrine disruptor for the gonadal system. The aim of the present study was to determine whether GBHs could also disrupt the hypothalamic-pituitary-thyroid (HPT) axis. Female pregnant Wistar rats were exposed to a solution containing GBH Roundup®Transorb (Monsanto). The animals were divided into three groups (control, 5 mg/kg/day or 50 mg/kg/day) and exposed from gestation day 18 (GD18) to post-natal day 5 (PND5). Male offspring were euthanized at PND 90, and blood and tissues samples from the hypothalamus, pituitary, liver and heart were collected for hormonal evaluation (TSH—Thyroid stimulating hormone, T3—triiodothyronine and T4—thyroxine), metabolomic and mRNA analyses of genes related to thyroid hormone metabolism and function. The hormonal profiles showed decreased concentrations of TSH in the exposed groups, with no variation in the levels of the thyroid hormones (THs) T3 and T4 between the groups. Hypothalamus gene expression analysis of the exposed groups revealed a reduction in the expression of genes encoding deiodinases 2 (*Dio2*) and 3 (*Dio3*) and TH transporters *Slco1c1* (former *Oatp1c1*) and *Slc16a2* (former *Mct8*). In the pituitary, *Dio2*, thyroid hormone receptor genes (*Thra1* and *Thrb1*), and *Slc16a2* showed higher expression levels in the exposed groups than in the control group. Interestingly, *Tshb* gene expression did not show any difference in expression profile between the control and exposed groups. Liver *Thra1 and Thrb1* showed increased mRNA expression in both GBH-exposed groups, and in the heart, *Dio2*, *Mb*, *Myh6* (former *Mhca*) and *Slc2a4* (former *Glut4*) showed higher mRNA expression in the exposed groups. Additionally, correlation analysis between gene expression and metabolomic data showed similar alterations as detected in hypothyroid rats. Perinatal exposure to GBH in male rats modified the HPT set point, with lower levels of TSH likely reflecting post-translational events. Several genes regulated by TH or involved in TH metabolism and transport presented varying degrees of gene expression alteration that were probably programmed during intrauterine exposure to GBHs and reflects in peripheral metabolism. In conclusion, the role of GBH exposure in HPT axis disruption should be considered in populations exposed to this herbicide.

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1. Introduction

To date, most studies concerning endocrine disruptors (EDs) have addressed the action of these compounds on the hypothalamic-pituitary-gonadal axis, showing varying degrees of interference (Bellingham et al., 2010; Rhind et al., 2010). However, only in recent years, studies have been published demonstrating interference in the hypothalamic-pituitary-thyroid (HPT) axis (Doerge and Chang 2002; Herbstman et al., 2008; Langer et al., 2009; Leung et al., 2010; Pearce and Braverman 2009; Pearce et al., 2010; Sathyapalan et al., 2011; Turyk et al., 2007), which is responsible for the precise secretion of thyroid hormones (TH) in the bloodstream.

THs are essential for the proper functioning of the body, taking part in various physiological processes, such as differentiation and tissue proliferation, training and maintaining the stability of the nervous system and metabolic balance. TH production is coordinated by the HPT axis, which is auto regulated by a negative feedback mechanism exerted by the active form of TH, triiodothyronine (T3). Hormonal action happens mainly through nuclear receptors (TH receptors isoform alfa and beta—THRa and THRb). The control of intracellular availability of T3 depends on the entrance of tetraiodothyronine (T4) into the cell using TH transporters (MCT8 and OATP1C1). Inside the cell, the T4 to T3 conversion is catalyzed by a 5′-deiodinase, that remove a molecule of iodine out of T4. There are three types of deiodinases (type I, II and III), these three enzymes are differently expressed in the various tissues, D1 and D2 are TH-activating enzymes, and D3 the inactivating one. Since thyroid produces mostly T4, the intracellular conversion mechanism is the most important source of peripheral T3 (Ortiga-Carvalho et al., 2016).

EDs are exogenous compounds with potential to alter hormonal regulation and the normal endocrine system (Casals-Casas and Desvergne, 2011; Colborn et al., 1993; Vandenberg et al., 2012). This interference may occur in hormonal production, release and metabolism (Tabb and Blumberg, 2006). Factors such as doses and period of exposure could interfere with the effects of this chemical in the endocrine system (Diamanti-Kandarakis et al., 2009; Schug et al., 2011; Vandenberg et al., 2012). Endocrine-disrupting agents comprise a wide variety of chemical classes, including pesticides, herbicides, detergents, repellents, flame-retardants and other compounds used in the plastics industry (Casals-Casas and Desvergne, 2011; De Coster and van Larebeke, 2012).

The studies reporting disruption of the HPT axis suggested alteration in various points of the HPT axis (Langer et al., 2009), such as thyroid hormones synthesis, action, peripheral concentration and thyroid hormone metabolism (Doerge and Chang, 2002; Herbstman et al., 2008; Langer et al., 2009; Leung et al., 2010; Pearce and Braverman, 2009; Pearce et al., 2010; Sathyapalan et al., 2011; Turyk et al., 2007). Some EDs have some definition about their mechanism of action, perchlorate is recognized and used in the past to treat thyrotoxicosis crisis for its antithyroidal effects, due to the inhibition of iodine uptake by the sodium iodide symporter (NIS) (Tonacchera et al., 2004). More recently, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers or flame retardants (PBDEs) and Bisphenol A (BPA) were described with affinity with thyroid hormone receptor (THRs), acting as antagonist to T3 action (Boas et al., 2012; Freitas et al., 2011; Gilbert et al., 2012; Iwasaki et al., 2002; Kitamura et al., 2005a; Miyazaki et al., 2004; Moriyama et al., 2002; Sun et al., 2009). PCBs and PBDEs can also interact with the transthyretin (TTR) displacing T4 from this binding protein. Finally, pesticides, PCBs and PDBEs interfere with thyroid hormone hepatic clearance (Fini et al., 2007; Jagnytsch et al., 2006; Kitamura et al., 2005b).

Glyphosate-based herbicides (GBH) are one of the most studied EDs and have been used in agriculture for over 4 decades; however, in the last twenty years, with the introduction of genetically engineered glyphosate-tolerant crops, GBH application in agriculture has increased, and these compounds are currently the most used herbicide worldwide (Myers et al., 2016; Romano et al., 2012). Consequently, there is an increasing concern about the damage these compounds may cause to human health. Recently, several animal and epidemiological studies have contributed to this field of knowledge (Myers et al., 2016). Currently, GBHs are proposed as safe and non-toxic herbicides and were classified in 1991 as class E, non-carcinogenic substances (Pieniazek et al., 2003). The potential damages caused by these substances apparently depend on the applied concentration (Casals-Casas and Desvergne 2011; Marrs et al., 1989). However, even at a lower concentration than that recommended by the herbicide manufacturer and lower that the concentration detected in food, glyphosate is lethal to cells *in vitro* (Gasnier et al., 2009).

Glyphosate was already detected in the urine of children, mothers and fathers, in concentration up to 5.0 ng/ml in the urine of non farm individuals and up to 11 ng/ml in the urine of farm individuals, these results may be linked to the environment and also to exposure due to diet (Curwin et al., 2007). McQueen et al. (2012) showed in their study the exposure of pregnant women to glyphosate is less than 2% of the acceptable daily intake and estimates that only 15% of this amount crosses the placenta (McQueen et al., 2012). The placenta is very important for intrauterine development controlling growth and substance exchange between the fetus and the mother (Aris and Leblanc, 2011). Richard et al. (2005) have shown that in a nontoxic (bellow the recommended) of GBH was still toxic for human placental cell line and also it was disturbing to estrogen production (Richard et al., 2005). Benachour et al. (2007) showed study that GBH has a toxic and endocrine disrupting effect in human embryonic cells (in lower nontoxic concentration), in placental derived cells and fresh human placental cells was also sensitive to lower doses of GBH, showing endocrine disruptor characteristic of this substance, affecting human reproduction and fetal development (Benachour et al., 2007).

Furthermore, in 2015, the WHO International Agency for Research on Cancer reclassified GBHs as “probably carcinogenic to humans” (Guyton et al., 2015). In addition, studies in the gonadal axis have shown that glyphosate interferes with the activity of aromatase, leading to changes in reproductive development in rats (Romano et al., 2012, 2010) and demonstrating the potential for endocrine disruption through GBH. However, there are few data available concerning the potential for GBH-mediated interference of the Hypothalamic-Pituitary-Thyroid (HPT) axis. In 2015, the Office of Pesticide Programs from the U.S. Environmental Protection Agency published a report from the Endocrine Disruptor Screening Program Tier 1 Assessment, that did not detected any evidence of glyphosate disrupting the thyroid pathway. This report was based in experiments with female and male pubertal assays, and amphibian metamorphosis, and used the glyphosate technical, not the commercial formulation (GBH) (Akerman and Blankinship, 2015).

Therefore, the aim of the present study was to verify the potential impact of a commercial GBH on the HPT axis to establish the risk for endocrine system disruption. Increasing awareness of this potential risk could stimulate more studies to establish plans for public health prevention and promotion.

2. Material and methods

2.1. Animals, experimental design and treatment

Female and male Wistar rats (*Rattus norvegicus*) were mated in monogamous couples. For this study, 24 adult female were used (8