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An acute exposure to glyphosate-based herbicide alters aromatase levels in testis and sperm nuclear quality

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ABSTRACT

Roundup is the major pesticide used in agriculture worldwide; it is a glyphosate-based herbicide. Its molecular effects are studied following an acute exposure (0.5%) of fifteen 60-day-old male rats during an 8-day period. Endocrine (aromatase, estrogen and androgen receptors, Gper1 in testicular and sperm mRNAs) and testicular functions (organ weights, sperm parameters and expression of the blood–testis barrier markers) were monitored at days 68, 87, and 122 after treatment, spermiogenesis and spermatogenesis. The major disruption is an increase of aromatase mRNA levels at least by 50% in treated rats at all times, as well as the aromatase protein. We have also shown a similar increase of Gper1 expression at day 122 and a light modification of BTB markers. A rise of abnormal sperm morphology and a decrease of the expression of protamine 1 and histone 1 testicular in epididymal sperm are observed despite a normal sperm concentration and motility.

1. Introduction

Exposure of human and mammalian populations to environmental and industrial contaminants represents a growing concern due to the impact of these pollutants on developmental and reproductive functions (Mathur and D'Cruz, 2011). There is an ongoing international debate as to the potential reproductive toxicity of glyphosate-based herbicides (GBH) such as Roundup. These are used worldwide in agriculture, including on edible Roundup-tolerant GMOs, and are major pollutants of rivers and surface waters (Cox, 1998; IFEN, 2007). Consequently, mammals and humans could be exposed to herbicide residues by agricultural practices, food and water (Acquavella et al., 2003). The presence of pesticide residues and/or their metabolites has been reported in the urine of families living in farms and nonfarm households (Curwin et al., 2005, 2007). Increased levels of glyphosate were also found

Abbreviations: BTB, blood–testis barrier; GBH, glyphosate-based herbicide; P450, arom aromatase; ROS, reactive oxygen species.

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in the urine of the farmer's son who lived at some distance from the sprayed fields. The contamination occurred through contact with the father (Mesnage et al., 2012). Recently, a much-debated study about the health effects of GBH exposure over a two-year period in adult rats (Séralini et al., 2013) revealed pathologies in females (increased rates of premature death and mammary tumors) and in males (increase in liver congestions and necrosis and kidney nephropathies). Paternal exposure to pesticides is increasingly recognized as causing birth defects due to pesticide-mediated alterations in germ cells (Ngo et al., 2009).

During the last two decades, the relevance of estrogens in the male gonad has been well documented (Carreau and Hess, 2010), suggesting that the androgen/estrogen balance is essential for normal sexual development and reproduction in mammals. The androgen/estrogen balance is controlled by the cytochrome P450 aromatase (aromatase, CYP19 or P450 arom), a key enzyme responsible for the irreversible bioconversion of androgen into estrogens (Simpson et al., 1994). P450 arom levels are crucial in various tissues including gonads (Sipahutar et al., 2003), and are implicated in numerous physiological functions (Simpson et al., 1997). The use of transgenic models has brought us valuable information about the involvement of P450 arom in the molecular mechanisms underlying the androgen/estrogen balance. P450 arom-deficient male mice initially fertile became infertile as the result of a disruption of spermatid production (Robertson et al., 1999). Over-expressing mice P450 arom was characterized by an elevation of estrogen production resulting in both histological and functional abnormalities in male phenotypes (Li et al., 2001). The modifications of the androgen/estrogen balance could induce pathologies such as hormone-dependent cancers for which several classes of P450 arom inhibitors have been designed (Séralini and Moslemi, 2001).

It has been hypothesized that human exposure to environmental xenoestrogens could have adverse effects on reproductive health in adult life (Stillerman et al., 2008; Meeker, 2012). Environmental contaminants could interfere with male reproductive functions consecutively to an alteration of gene expression pertinent for spermatogenesis, for the disturbance of steroidogenesis, the induction of ROS production, or the modification of the BTB integrity (Mathur and D'Cruz, 2011), or for several of these parameters. Effects of GBH as endocrine disruptor has been recently suggested in in vitro cellular models and in vivo studies consecutively to low dose exposure. Glyphosate alone could inactivate aromatase at low doses and its formulations inhibited sex steroid receptors in human placental and embryonic cells in vitro (Richard et al., 2005; Benachour et al., 2007; Gasnier et al., 2009). Until now, most studies have been carried out at low doses. Globally, the main consequences of GBH chronic exposures on reproductive function consisted of a modification of the androgen/estrogen balance and a disruption of the testicular structure (Dallegrave et al., 2007; Oliveira et al., 2007; Romano et al., 2009, 2011). A chronic treatment with a sublethal concentration of GBH in mature male New Zealand white rabbits resulted in a decrease of body weight and an alteration of sperm parameters (Yousef et al., 1995). Subchronic studies in F344 male rats treated orally with glyphosate at a very high dose of 25,000 ppm revealed a significant decrease in epididymal sperm counts (Chan and Mahler, 1992). Concerning the impact of an acute and transitory exposure of GBH, studies about reproductive functions are scarce. However, acute exposures of farmers during field treatments are frequent.

It has become imperative to reinvestigate and validate the reproductive effects of an acute exposure to GBH and to delineate the possible mechanisms implicated in the regulation of the androgen/estrogen balance (estrogen receptors ESR1 and ESR2, androgen receptor, Gper1 and P450 arom) at a molecular level in testis and epididymal sperm at different times after the end of treatment: immediately after the treatment (d68), after one cycle of spermiogenesis (19 days, d87) and after one cycle of spermatogenesis (54 days, d122). As described for Bisphenol A, we have also investigated molecular markers of the blood–testis barrier integrity (connexin-43, occludin, claudin and N-cadherin), Sertoli cell junctional proteins being important for spermatogenesis.

2. Materials and methods

2.1. Chemicals

The environmental product used for this study was Roundup Grand Travaux Plus (GT$^+$, approval 2020448, Monsanto), a commercial formulation of the GBH, composed of 450 g/L glyphosate, 607 g/L isopropylamine salt and adjuvants such as Polyoxyethylamine (POEA).

2.2. Animals and experimental design

Thirty sexually mature 60-days-old Sprague-Dawley (SD) male rats (Janvier, Le Genest Saint Isle, France) were fed and housed under standard conditions (photoperiod of a 12 h dark/light cycle and controlled room temperature) in the CURBE department (University Center of Biological Experimental Resources, Caen, France). All the procedures were performed in accordance to the French Government Regulations (Veterinary Health and Animal Protection, Ministry of Agriculture). Each group was randomized and animals had access to plain water and standard diet. Fifteen male SD rats were subjected to an acute exposure of GBH at a 0.5% dose, similar to those found in water after agricultural practices. GBH was diluted in a deionized water suspension and administered in drinking water for a short period (8 days from postnatal day (PND) 60–68) (GBH+). The water's consumption was followed every 2 days for one week before the experiment and during the protocol period. The fifteen untreated rats used as controls (GBH−) were fed and housed in the same conditions but with deionized water without added Roundup. Five GBH+ rats and five GBH− rats were systematically euthanized at three different periods after the end of treatment: immediately after the treatment (d68), after one cycle of spermiogenesis (19 days after treatment, d87 in our experiment) and after one cycle of spermatogenesis (54 days after treatment, d122 in our experiment).

2.3. Tissue collection and organ weights

Testes and epididymes were removed and weighed. Absolute weights and relative weights of testes and epididymis reported