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Effect of the herbicide glyphosate on liver lipoperoxidation in pregnant rats and their fetuses

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Abstract

Glyphosate is a post-emergence herbicide that acts on the synthesis of amino acids and other endogenous metabolites in plants. It is commonly used in agriculture, forestry, and nurseries for the control or destruction of herbaceous plants. Metabolic processes during development and pregnancy could be sensitive to changes induced by glyphosate such as lipid peroxidation. The present study has investigated the effects that 1% glyphosate oral exposure has on lipoperoxidation and antioxidant enzyme systems in the maternal serum and liver of pregnant rats and their term fetuses at 21 days of gestation. The results suggest that excessive lipid peroxidation induced with glyphosate ingestion leads to an overload of maternal and fetal antioxidant defense systems.

Keywords: Glyphosate; Herbicide; Lipoperoxidation; Liver; Pregnancy; Fetus; Rat

1. Introduction

Glyphosate (*N*-phosphonometil-glicine, also known by the trade names Roundup and Rodeo) is an organophosphate herbicide used to control undergrowth before seeding. This non-selective herbicide is widely used in agricultural applications, vegetation control in non-crop areas, and aquatic weed control in fish ponds, lakes, canals and slow running water [1].

Glyphosate exposure and metabolism in the liver can lead to excessive production of malonaldehyde (MDA) and oxidative stress through unregulated generation of reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radical, peroxyl radicals and singlet oxygen. Excessive ROS can compromise cellular integrity through oxidative damage of lipids, proteins, or DNA [2–4] and altered regulation of gene functions critical for development, differentiation, and aging [5].

ROS production in aerobic organisms is partly determined by the antioxidant substances and antioxidant defence enzymes. Examples of the latter include: superoxide dismutase, which eliminates superoxide anions; catalase that eliminates hydrogen peroxide; and glutathione peroxidase that eliminates organic hydroperoxides as hydrogen peroxide. Because the levels of these enzymes at term may reflect altered physiological states during pregnancy and gestation [6,7], the present study has investigated these enzymes as biomarkers for glyphosate induced lipoperoxidation in pregnant rats and their fetuses at term.

2. Materials and methods

2.1. Chemicals

All chemicals used here were of reagent grade and were obtained from Sigma and Merck Laboratories. Glyphosate is marketed in Argentina under the trade name Herbicygon and

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was obtained from M.F.L. S.R.L. The empirical formula is $C_3H_8NO_5P$.

2.2. Animals and diets

Animal protocols were conducted in compliance with national and institutional guidelines for the protection of animal welfare, using rats obtained from the National University of San Luis and acclimated for 1 week prior to experimentation. Wistar female virgin rats (210–230 g) at the proestrus stage were housed for one night with fertile males. Coitus was identified the following morning by sperm-positive vaginal smear and that day was designated day 1 of gestation. These dams were transferred to individual cages, housed at 22–25 °C with a 12-h light–dark cycle, and fed 20 g of a stock laboratory diet (Cargill) and fresh tap water (35 ml daily) ad libitum. The diet had the following ingredients: meat flour, bone and meat flour, fish meal, blood flour, soybean meal, toasted soybean, soy expeller, sunflower flour, cotton flour, peanut meal, animal fat, corn, wheat, sorghum, oat, barley, wheat bran, rice bran, gluten meal, vitamins A, E, B, D3, K3 and B12, niacin, pantothenic acid, choline, ascorbic acid, bone ash, salt, calcium carbonate, oyster, manganese oxide, zinc oxide, ferrous sulphate, copper oxide, sodium selenite, iodine, and cobalt. Body weight and food intake were measured daily. Rats were separated into two experimental groups ($n = 8$ rats per group). Group I, the control group, was provided with tap water. Group II, the experimental group, drank tap water laced with 1% glyphosate solution (w/v) for dosing at 0.4 ml glyphosate/20 ml water). Dams received the treatment from day 1 of gestation until euthanasia on day 21 of gestation (08.00–09.00 h). Following hysterectomy, each fetus was examined, weighed, and killed by decapitation. Maternal blood was collected from the heart and centrifuged to obtain the serum. Maternal and fetal livers were extirpated, washed in cold saline solution, and stored at −20 °C until analysis.

2.3. Serum assay

Thiobarbituric acid reactive substances (TBARS) of the pregnant rat serum were determined by 1,1,3,3-tetrametoxipropane (TMP) formation according to the method of Buege and Aust [7]. Briefly, to 1 ml serum was added 100 μl butylhydroxytoluene (BHT) and 1 ml 20% trichloroacetic acid (TCA). After setting 30 min on an ice bath the samples were centrifuged at 3000 rpm for 10 min. To 1 ml of the TCA-supernatant, 1 ml of TBA was added and samples were incubated at 100 °C for 60 min and cooled room temperature. Absorption was monitored in a Beckman spectrophotometer at 535 nm and 572 nm for baseline correction. MDA equivalents were calculated by the difference in absorption between the two wavelengths versus a standard calibration curve.

2.4. Tissue lipid peroxidation

Liver (0.3 g) was homogenized in an Ultra Turrax T25 machine with 3 ml of 30 mM phosphate-buffered saline (PBS), pH 7.4. The homogenate was centrifuged for 10 min at 2500 rpm. Lipid peroxidation was measured by the determination of TBARS [7]. Briefly, 100 μl of supernatant was mixed with 100 μl of 4% BHT and 100 μl 20% TCA, followed by 30 min incubation on ice. After centrifugation 100 μl of supernatant was mixed with 100 μl of 0.7% TBA and incubated at 95 °C for 60 min and cooling on ice. The organic layer was separated and absorbance was measured at 535 nm. The levels of lipid peroxidation products, mainly MDA, were determined spectrophotometrically as TBARS.

2.5. Assay of antioxidant enzymes

Liver (0.3 g) homogenate was mixed with 3 ml of 50 mM potassium phosphate buffer, pH 7.7 and centrifuged for 10 min at 2000 rpm. Glutathione peroxidase (GPx) activity was determined by monitoring NADPH oxidation at 340 nm in a reaction medium containing 0.2 mM glutathione (GSH), 0.25 units/ml yeast glutathione reductase, 0.5 mM *tert*-butyl hydroperoxide, and 50 mM phosphate buffer (pH 7.2). One unit of GPx activity was defined as the amount of GPx required to oxidize 1 μmol of NADPH per minute [8] (CAT) activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm. One unit of catalase activity was defined as the amount of enzyme required to degrade 1μ mol of H_2O_2 per min [9]. Superoxide dismutase (SOD) activity was measured using the xanthine/xanthine oxidase system as a superoxide source and monitoring the spectrophotometric oxidation of cytochrome *c* at 550 nm [10].

2.6. Statistical analysis

Significant differences among means were considered at an alpha level of $P < 0.05$ and identified by Student's test assuming homogeneous variance.

3. Results

3.1. Effect of glyphosate on body weight and liver weight of pregnant rats and fetuses

Food and water consumption was lower in the experimental group than the control group (Table 1). Maternal body weight gain was lower in glyphosate-treated dams, although fetal body weight was not significantly altered at term. Liver weight was lower in experimental dams, but not their fetuses (Table 1).

3.2. Effect of glyphosate on lipoperoxidation

Lipoperoxidation was higher in both the maternal and fetal livers in the glyphosate-treated group versus controls. Greater