Pesticide Biochemistry and Physiology 106 (2013) 38–44

Contents lists available at SciVerse ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Chronic low level metribuzin exposure induces metabolic alterations in rats

F.Z. Chiali [a], H. Merzouk [a,*], S.A. Merzouk [b], A. Medjdoub [a], M. Narce [c]

[a] Laboratory of Physiology and Biochemistry of Nutrition, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria
[b] Department of Technical Sciences, Faculty of Engineering, University Abou-Bekr Belkaïd, Tlemcen 13000, Algeria
[c] INSERM UMR 866, "Lipids Nutrition Cancer", University of Burgundy, Faculty of Sciences, 6 Boulevard Gabriel, Dijon 21000, France

ARTICLE INFO

Article history:
Received 8 September 2012
Accepted 30 March 2013
Available online 8 April 2013

Keywords:
Metabolism
Metribuzin
Organs
Oxidative stress
Rat

ABSTRACT

In this work we evaluated the *in vivo* effects of chronic metribuzin exposure at doses that mimic human exposure through diet. Male and female rats were fed a potato diet containing metribuzin at low doses (D1, 1.3 mg/kg or D2, 13 mg/kg) for 3 months. Plasma biochemical parameters (glucose, lipid, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST)), as well as lipid and protein contents, markers of oxidative stress in different organs (liver, adipose tissue, muscle, intestine) were determined.

Our results showed that exposure to metribuzin induced a significant reduction in body weight, food intake and adverse alterations in biochemical parameters such as an increase in plasma glucose, triglyceride, urea, creatinine, ALT and AST levels. Total protein and lipid contents of organs were also altered with a concomitant presence of oxidative stress.

In conclusion, chronic metribuzin exposure is associated to underfeeding and has adverse effects on organ functions leading to physiological impairment even at low concentrations. The nutritional management appears of the first importance.

1. Introduction

Metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one) is an herbicide used on vegetable crops to selectively control broadleaf weeds and grassy weed species. Metribuzin is commonly applied to soybeans, potatoes, lentils, peas and tomatoes.

Exposure of the general population to this pesticide may occur through consumption of foods treated with pesticides. The presence of pesticides in food is a great public concern since they may have both acute and chronic effects on health [1,2]. Chronic exposure to pesticides is associated with an increased frequency of neurodegenerative diseases, cancer, endocrine, immunological abnormalities, adverse reproductive and developmental effects [1,3–6]. Several authors have attempted to investigate the mechanism of pesticide toxicity by using animal models.

Different doses have been used to observe the subchronic and the chronic effects of metribuzin in rats. Studies of metribuzin toxicity, for instance, have reported effects on body weight, liver enzyme activities and histopathological changes. Wistar rats, exposed to metribuzin through their diet at 37.5 mg/kg for 3-months, exhibited a significant reduction in body weight gain, and increased liver and thyroid weights [7]. Another 2-year feeding study using a higher dose (70 mg/kg) of metribuzin reported histopathological changes in the liver, and adrenal and thyroid glands in males and in female rats [8]. The highest concentrations of metribuzin and/or its metabolites are found in the liver followed by other tissues (fat, muscle, plasma) [9,10].

Evidence indicates that glucuronidation and sulfation do not play a major role in metabolism or excretion. In contrast, conjugation with reduced glutathione (GSH) followed by conversion to mercapturic acid derivatives appears to play a major role in detoxification and excretion [11].

The GSH content of the liver decreased progressively with increasing doses of metribuzin [10]. There is evidence that at very high levels, or in the absence of non-protein sulfhydrils (e.g., glutathione), metribuzin metabolites can bind to proteins. GSH, a sulphydryl containing tripeptide (gamma glutamyl cystenyl glycine), is an important intracellular antioxidant which plays a central role in the defense against oxidative damage and toxins. Alterations in GSH contents are observed in several pathologies, including, cancer, neurodegenerative disorders and aging [12].

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, bovine serum albumin; MDA, malondialdehyde; NOAELs, dose with no-observed adverse effect levels; LD 50, lethal dose 50; GSH, reduced glutathione; CV, coefficient of variation; TBA, thiobarbituric acid; HPLC, high-performance liquid chromatography; ROS, reactive oxygen species.

* Corresponding author.
E-mail address: hafidamerzouk_2@hotmail.com (H. Merzouk).

0048-3575/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pestbp.2013.03.011

Oxidative stress, which affect cellular function and modify cell signaling, is considered to play a major role [13,14]. Some studies have shown that herbicides induce oxidative stress in plants [15,16]. We have previously reported that *in vitro* metribuzin exposure induced oxidative stress, reflected by high hydroperoxides (lipid peroxidation marker) and carbonyl proteins (protein oxidation marker) and low GSH levels in human and rat lymphocytes [17].

However, the *in vivo* effects of low doses of metribuzin on the different organs and the molecular mechanism of metribuzin toxicity are still not clear. To the best of our knowledge, there are no reports in the literature of the induction of intracellular oxidative stress in different organs by metribuzin.

The aim of our work was to evaluate *in vivo* the chronic effects of metribuzin at low doses. We tested the hypothesis that low metribuzin doses increase metabolic alterations and oxidative stress in rat tissues. We then tried to examine how low metribuzin residues in food can still have an impact on the health. Potatoes are the most vegetables consumed in Algeria. Furthermore, in order to mimic the consumer's exposure to metribuzin through food, we chose to give rats a potato diet containing metribuzin at low doses (dose with no-observed adverse effect levels (NOAEL) and lethal dose 50 divided by 100 (LD 50/100)) [18]. We examined the *in vivo* effects of chronic exposure through diet (3 months) on serum biochemical parameters (glucose, lipid, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotrasferase (AST)), as well as lipid and protein contents, markers of oxidative stress in target organs (liver, adipose tissue, muscle, intestine) in male and female rats.

The results of this study should increase our understanding of the long-term effects of low dose of metribuzin and the physiological changes that occur in rats.

2. Materials and methods

2.1. Preparation of the diet

Potatoes used in this study were provided from untreated parcels by any pesticide located in Tlemcen area (INRAA, ALGERIA). Potatoes were washed, peeled and cut into pieces. They were put in a pot, cover with water and boiled for 10 min. After removing water, potatoes were mashed. Potato puree was prepared every day and was mixed to standard chow (ONAB, Algeria) (w/w) with corn oil (1 ml for 7 g puree and 7 g standard chow) to meet the nutritional requirements for rats. This mixture constituted the control diet (Table 1).

To prepare experimental diets, appropriate concentrations of metribuzin (Metribuzin 98% TECH; CAS No: 21087-64-9; purchased from China Leading Manufacturer and supplier by INRAA, ALGERIA) were dissolved in corn oil (1 ml) and added to 7 g of potato puree according to the rat weight. This puree was mixed to standard chow (w/w). The dose of mitribuzin given daily was adjusted each three days for changes in body weight of rats. Each diet was analyzed for metribuzin concentration using high-performance liquid chromatography (HPLC) to assure that the proper dose of metribuzin was delivered. Control diet was also analyzed to verify the absence of metribuzin.

Table 1
Composition of control diet.

	Control diet (C)
Energy sources (g/100 g)	
Protein	14
Carbohydrate	52
Fat	4
Cellulose	5
Vitamins mix	2
Minerals mix	3
Humidity	20
Total polyphenols (mg/100 g)	64
B carotene (mg/100 g)	1
α Tocopherol (mg/100 g)	5
Vitamin C (mg/100 g)	10
Energy content (kcal/100 g)	303
Fatty acid composition (% fatty acids)	
SFA	30
C18:1 n-9	12
C18:2 n-6	45
C18:3 n-3	8
C20:4 n-6	4

SFA: saturated fatty acids. The composition of the diet was analyzed by a private technical laboratory (GETALAB, Tlemcen, ALGERIA). Minor polyunsaturated fatty acids were not presented (<0.5%).

2.2. Animals and experimental protocol

All aspects of the experiment were conducted according to the guidelines provided by the ethical committee of the experimental animal care at Tlemcen University. Adult Wistar rats were obtained from Animal Resource Centre (Algeria). Female and male rats were housed individually in wood-chip-bedded plastic cages at a constant temperature (25 °C) and humidity (60 ± 5%) with a 12 h light/dark cycle. The rats had free access to water and were assigned to three dietary groups of equal average body weight. One group (control, $n = 8$ males and 8 females) fed the control diet (without metribuzin). The second and the third groups (D1 and D2, $n = 8$ males and 8 females) were fed the diets containing metribuzin at 1.30 mg/kg/day (D1, NOAEL) or at 13 mg/kg/day (D2, 1/100 LD 50) for 3 months. The rats received 14 g of each diet every day in the morning at 8H. In the afternoon, after controlling food consumption, control diet was given to all rats (14–30 g) until the next morning. This procedure helped to insure a consistent dosage over the course of the study.

The weight and food consumption of each animal were measured daily.

At the end of the experimental period (3 months) and after overnight fasting, rats were anaesthetized with intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight). The blood was drawn from the abdominal aorta into heparinized tubes, and plasma was used for biochemical determinations. After removal of plasma, erythrocytes were washed three times with 2 volumes of isotonic saline. Erythrocytes were lysed with ice-cold distilled water (1/4) and stored at 4 °C for 15 min. The cell debris was removed by centrifugation (2000*g* for 15 min). Erythrocyte lysates were assayed for antioxidant catalase activity and glutathione contents. Tissues (liver, gastrocnemius muscle, perirenal adipose tissue, intestine) were collected, and immediately placed on dry ice. An aliquot of each tissue was homogenized in an Ultraturrax homogenizer (Bioblock Scientific) for lipid extraction. A second aliquot of tissues was homogenized in 10 volumes of ice-cold 10 mmol/l phosphate-buffered saline (pH 7.4) containing 1.15% KCL. The homogenate was subjected to a 6000*g* centrifugation at 4 °C for 15 min. The supernatant fractions were collected and used for biochemical and redox marker determinations.

2.3. Chemical analysis

Plasma glucose was measured using the Trinder glucose kit (Sigma). Plasma triglyceride and cholesterol were measured using colorimetric enzymatic kits (Roche Diagnostics). For these enzymatic methods, the interassay CV (coefficient of variance) was in the range of 1.7–3%. Plasma creatinine and urea were measured using enzymatic colorimetric methods (Kits from BioAssay Sys-