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Neurotoxicity and oxidative stress induced by gibberellic acid in rats during late pregnancy and early postnatal periods: Biochemical and histological changes

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ABSTRACT

Gibberellic acid (GA_3) is an endogenous plant growth regulator used worldwide in agriculture; however, little is known about its biochemical and physiological effects on mammals. This study investigated possible neurotoxic effects of GA_3 on the cerebrum and cerebellum of suckling rats. Female Wistar rats were given daily 200 ppm GA_3 in drinking water from the 14th day of pregnancy until day 14 after delivery. Acetylcholinesterase activity in both cerebellum and cerebrum was inhibited after treatment with GA_3. Neurotoxicity was demonstrated by a significant increase in malondialdehyde level and a decrease in the antioxidant enzyme activities of catalase, superoxide dismutase, glutathione peroxidase in the cerebrum and cerebellum of suckling pups. A significant decline of glutathione content and vitamin C was also observed. The biochemical parameters were correlated histologically with an abnormal development of the external granular layer and a loss of Purkinje cells in the cerebellum of GA_3-treated suckling rats.

1. Introduction

The mammalian brain is very sensitive to oxidative damage due, in part, to its high oxygen requirement and abundance of oxidizable substrates such as polyunsaturated fatty acids and catecholamines (Somani et al., 1996; Chong et al., 2005). Moreover, according to Ali et al. (2004) the abundance of redox active transition metal ions, like iron in the brain, makes it more susceptible to oxidative stress via the metal-catalysed formation of reactive oxygen species (ROS). Oxidative stress has also been shown to be linked to a functional decline in neurodegenerative diseases such as Parkinson's, Alzheimer's and amyotrophic lateral sclerosis (Lin and Beal, 2006).

Many chemicals like plant growth regulators (PGRs) can induce oxidative stress and provoke several diseases. Previous studies of Celik et al. (2002a,b) demonstrated that phytohormones were present in the diet of herbivorous and omnivorous animals. Excessive and chronic plant growth regulators (PGRs) consumption through diet is known to produce organ damages, including the brain. In fact, indole acetic acid (IAA) was found to be a linear-mixed type inhibitor of human serum BChE and an uncompetitive inhibitor of the horse serum butyrylcholinesterase (BChE) enzyme (Cokuggras and Bodur, 2003). Moreover, it has been shown by Furukawa et al. (2004) that IAA induces neuronal apoptosis in S phase and leads to microencephaly. According to previous studies (Candeias et al., 1995; Celik and Tuluce, 2006; Tuluce and Celik, 2006; Celik et al., 2007) PGRs, as well as other xenobiotics, may induce oxidative stress, leading to the generation of free radicals and causing lipid peroxidation.

Gibberellic acid (GA_3) is the most commonly used PGR in agriculture in many countries, including Tunisia in order to enhance fruit growth like date palm (Ben Abdallah et al., 2000) and some vegetables such as pepper (Arous et al., 2001) and olive (Chaari-Rkhis et al., 2006). Moreover, GA_3 plays an important role in many cellular processes, in that it promotes stem elongation, overcomes dormancy in seeds and buds involved in parthenocarpic fruit development, flowering, and the mobilisation of food reserves in grass seed germination (Salisbury and Ross, 1992).

GA_3 has also been shown to cause alarming toxicity to mammalian systems, particularly in the breast, lung (El-Mofty et al., 1994), kidney and liver (Ustun et al., 1992) of adult mice. In addition, GA_3 administration by gavage for 22 months induces carcinogenic effects in adult Swiss Albino mice (El-Mofty et al., 1994). According to Ozmen et al. (1995) treatment with GA_3 affects sexual differentiation and some physical parameters in laboratory mice.

Recent reports indicate that this PGR may induce oxidative stress, leading to the generation of free radicals and causing cells damage in many organs, including the heart, kidney, stomach and

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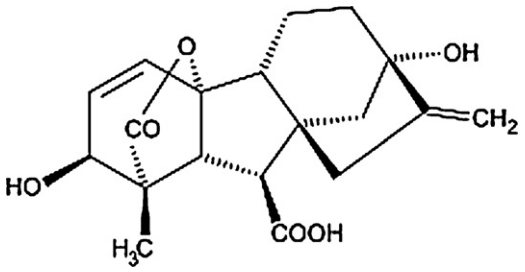

Fig. 1. Chemical structure of gibberellic acid (GA_3).

spleen of adult rats (Celik and Tuluce, 2006) and the liver of GA_3 treated suckling rats (Troudi et al., 2010). However, no information is available regarding the neurotoxicity effect of this compound on the level of oxidative stress in suckling pups whose mothers were treated with GA_3. Therefore, the aim of this study was to examine the possible neurotoxic effects of GA_3 exposure on the cerebrum and cerebellum of suckling rats.

2. Materials and methods

2.1. Chemicals

GA_3 in powder was purchased from Sigma Chemical Co. (St. Louis, France). All the remaining chemicals were of high commercially available grade (Fig. 1).

2.2. Animals

Experiments were performed on 'Wistar' male and female rats weighing 170 ± 10 g. They were purchased from the Central Pharmacy (SIPHAT, Tunisia). Food (standard diet, supplied by SICO, Sfax, Tunisia) and water were available *ad libitum*. Animals were acclimatized for one week to laboratory conditions: a photoperiod of 12-h light/12-h dark cycle, a minimum relative humidity of 40% and a room temperature of 22 ± 2 °C. Female rats were allowed to mate in the ratio of two females to one male. The first day of pregnancy was confirmed by the presence of viable sperms in the vaginal smear.

2.3. Experimental design

Pregnant female rats were randomly divided into two groups of six each: group 1 served as controls and group 2 received 200 ppm of GA_3 (equivalent to 55 mg/kg) through drinking water from the 14th day of pregnancy until day 14 after delivery. The GA_3 dose chosen in our experiment represented 1/100 of LD 50. The dose used in our study (200 ppm of GA_3) was chosen because it provoked oxidative stress without lethal effects in suckling rats whose mothers were treated with GA_3. The day of parturition was considered as day zero of lactation. No delay was observed in the delivery of the treated group. Pups were counted and weighed and each litter was reduced to eight pups (4 males and 4 females if possible) to ensure standardized nutrition and maternal care (Fishbeck and Rasmussen, 1987).

During the course of treatment, daily fluid consumption, body weight gain and feed consumption were recorded periodically. Each lactating rat treated with GA_3 ingested 10.04 mg GA_3/day (Table 2). This dose is similar to the amount released in the air by pulverisation (Personal communication of plant Biotechnology laboratory, Sfax Faculty of Science, Tunisia).

On day 14 of lactation at 8:00 am, litters were separated from mothers. Four hours later, milk was accumulated in breast. Then, 10 mIU/ml of oxytocin was injected intraperitoneally to anesthetized dams. The expelled milk was aspirated from teats and stored at −20 °C until analysis. Milk tinged with blood was not aspirated, thus avoiding the introduction of blood into the milk collected (Heil et al., 1999).

After milk collection, 96 pups (controls and treated rats) and 12 dams were anesthetized with chloral hydrate by intra-abdominal injection. The body weights of pups and their mothers were recorded and blood samples were collected by aortic puncture in dams and by brachial artery in pups. They were then sacrificed by decapitation to avoid stress. Plasma was drawn from blood after centrifugation at 2200 × *g* for 15 min. Stomach contents of suckling rats were taken and weighed. All samples were stored at −20 °C until analysis.

Cerebrum and cerebellum of control and GA_3-treated suckling pups were collected, cleaned and weighed. Some samples were rinsed, homogenized (10%, w/v) in phosphate buffer (pH 7.4) and centrifuged. The resulting supernatants were used for biochemical assays. Others were immediately fixed in 10% formalin solution for histological studies. From the dams, milk, blood, cerebrum and cerebellum were collected in order to determine their GA_3 contents.

The experimental procedures were carried out according to the general guidelines on the use of living animals in scientific investigations (Council of European Communities, 1986) and approved by the Ethical Committee of the Faculty of Science of Sfax.

2.4. Determination of GA_3 in plasma, cerebrum, cerebellum and stomach content

The extraction of GA_3 from cerebrum, cerebellum and stomach contents was carried out according to the method of Ünyayar et al. (1996) with minor modifications. Briefly, one gram of each sample was taken and 60 ml of methanol/chloroform/2 N ammonium hydroxide mixture (12:5:3, v/v/v) were added. Each extract was treated with 25 ml of distilled water. The chloroform phase was discarded. The water phase was adjusted to pH 2.5, 7 and 11, respectively. Then 15 ml of ethyl acetate was added at each of the three steps. After 1 hour of incubation at 70 °C, ethyl acetate was evaporated at 45 °C using a rota-evaporator system. The extract was dissolved with 2 ml of methanol and filtered. The absorbance of samples and standard GA_3 was measured at 254 nm using a spectrophotometer.

2.5. Determination of acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was measured immediately in tissue homogenates according to the method of Ellman et al. (1961), using acetylthiocholine iodide as a substrate. The reaction mixture was composed as follows: phosphate buffer (0.1 M; pH 8) and 0.01 M DTNB. The hydrolysis rate of acetylthiocholine iodide is measured at 412 nm through the release of the thiol compound which, when reacted with DTNB, produces the colour-forming compound TNB. The reaction was initiated by adding 0.075 M acetylthiocholine iodide. Activities were expressed as micromole of substrate/min/mg protein.

2.6. Estimation of lipid peroxidation

Lipid peroxidation was determined in cerebral and cerebellar homogenates by the method of Draper and Hadley (1990) following a reaction of thiobarbituric acid (TBA) with malonyldialdehyde (MDA) formed owing to lipid peroxidation. After the incubation of the homogenates with TBA at 95 °C during 10 min, the pink colour produced by this reaction, was determined spectrophotometrically at 532 nm.