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Neurotoxic and immunotoxic effects of Indole-3-butyric acid on rats at subacute and subchronic exposure

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ABSTRACT

This study was carried out to investigate the neurotoxic and immunotoxic effects of Indole-3-butyric acid (IBA), a plant growth regulator (PGR), on rats at subacute and subchronic exposure. The neurotoxic effects of IBA were evaluated by measuring the activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Biomarkers selected for immunotoxic monitoring were the activities of adenosine deaminase (ADA) and myeloperoxidase (MPO) in various tissues of rats exposed to 25 and 50 ppm dosages of IBA for 20 and 45 days. Results showed that the administrations of IBA decreased AChE and BChE activities in some tissues of the rats treated with both dosages and periods of IBA. With regard to the immunotoxic effects, ADA activity significantly decreased whereas MPO activity increased after subacute and subchronic exposure with both dosages in most of the tissues of rats compared with controls. The observations presented led us to conclude that the administrations of IBA at subacute and subchronic exposure decreased AChE, BChE and ADA activities whereas increased MPO activity in various tissues of rats. This may reflect the potential role of these parameters as useful biomarkers for toxicity of IBA.

1. Introduction

Many chemicals are currently used in agriculture, and plant growth regulators (PGRs) are among those widely employed. Indole-3-butyric acid (IBA) is a naturally occurring plant growth regulator of the auxin class, affecting cell enlargement, division, and differentiation (Mickel, 1978). As a result of the industrial usage, this agrochemical is consumed by non-target organisms (Cokuggras and Bodur, 2003). Although PGRs are used for pest control and giving rise to product on a wide variety of crops, little is known about the biochemical or physiological effects in mammalian organisms. However, there are some studies about endogenous PGRs including IBA. Furukawa et al. (2004) indicated that indole acetic acid (IAA) might induce the neuronal apoptosis in the S phase and lead to microencephaly. de Melo et al. (2004) determined that incubation for 24 h in the presence of IAA (1 mM) showed increase in the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase in rat neutrophils and lymphocytes. John et al. (1979) observed that IAA possesses teratogenic effects in gestation mice and rats at 500 mg/kg/day. Ozmen et al. (1995) observed that Abcisic acid (ABA) and Gibberellic acid (GA_3) was effective on sexual differentiation and some physiological parameters of laboratory mice. El-Mofty and Sakr (1988) found that GA_3 induced liver neoplasm in Egyptian toads, and they suggested that the tumors could be diagnosed as hepatocellular carcinomas. GA_3 also induces microabscesses and hydropic degeneration in the liver and mononuclear inflammatory infiltration in the kidneys of laboratory mice, but not tumors. In a study, IAA effect investigated on human serum enzymes *in vitro*, it was found that IAA inhibited aspartate aminotransferase (AST) but activated amylase, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) (Celik and Kara, 1997). Also, it was reported that while the levels of LDH and CPK increased significantly by IBA, the levels of AST, LDH and CPK were increased significantly by IAA after subacute exposure with 100 ppm dosages (Celik et al., 2002). IAA was found to be linear-mixed type inhibitor for human serum BChE, and uncompetitive inhibitor for the horse serum BChE enzyme (Cokuggras and Bodur, 2003). Further, PGRs may induce oxidative stress, leading to the generation of free radicals and cause lipid peroxidation as one of the molecular mechanisms involved in PGR-induced toxicity (Celik and Tuluce, 2006; Tuluce and Celik, 2006; Candeias et al., 1995; Celik et al., 2007, 2006a,b).

Despite the reasons mentioned in above paragraphs, little is known regarding the IBA effects on nervous and immune system of vertebrata. In order to achieve a more rational design of IBA, it is necessary to clarify the mechanism of neurotoxicity and immunotoxicity for IBA. To this end, the treatments of IBA were

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done orally because the effect of chemicals represents a well characterized *in vivo* toxicity model system. The enzymes were chosen due to their important role for nerve, immune systems and important role during detoxification in the degradation and bioactivation of IBA. As it is known, ChE activity which is expressed as the total of AChE and BChE is sensitive to pollutants. Therefore, it is commonly used for ecotoxicological risk assessment and environmental pollutants monitoring studies. Also, ADA and MPO are essential for the proper functioning of the vertebrates' body immune system. This study was approved by The Ethic Committee of Yüzüncü Yıl University. Further, the research was supported by the University Grant Commission of Yüzüncü Yıl University.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine iodide (ATCh), butyrylthiocholine iodide (BTCh), 5-50-dithiobis (2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), potassium dihydrogenphosphate (KH_2PO_4) and IBA were from Sigma (USA). All other chemicals used were of the best analytical grade.

2.2. Animals

Rats (Wistar) of 4 months of age and of average weighing approximately 200–250 g were provided by the animal house of the Sciences Faculty of Yüzüncü Yıl University, and were housed in 3 groups. The animals were housed at 20 ± 2 °C in daily light/dark cycle. All animals were fed a group wheat–soybean-meal-based diet and water *ad libitum* in stainless cages, and received human care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institutes of Health. The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments.

2.3. Treatment of rats

This investigation was performed on male rats. The animals were housed for a minimum of 2 weeks to 'acclimatize' before being dosed with the substance. The rats were exposed to 25 and 50 ppm IBA *ad libitum* 20 days for subacute and 45 days for subchronic applications as drinking water. 25 and 50 mg of the IBA were dissolved in 1 ml of 1N NaOH, and then were diluted with tap water to 1000 ml to obtain 25 and 50 ppm dosages. For the control rats, only 1 ml of 1N NaOH was added to 1000 ml of tap water. Because the PGRs are photoactive compounds the drinking water containing IBA was prepared and refreshed every day in amber bottle. Since all rats have the same physiologic characters, daily water consumption of all groups of rats was approximately 25 ± 3 ml during the tests. Consequently, the PGRs intake amount of each rat was about 2.1 ± 0.3 mg/day.

At the end of the treatments, the rats were anesthetized by the inhalation of diethyl ether and the rat's liver, lungs, heart, brain, kidney, muscle and spleen tissue samples were obtained. The tissues were dissected and put in Petri dishes. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at −78 °C until analysis. For obtaining tissue supernatants, the tissues were homogenized for 5 min in 50 mM ice-cold KH_2PO_4 buffer solution (pH 7.0) (1:10 w/v; 0.5 g tissue + 5 ml buffer solution) using a glass-porcelain homogenizer (20 kHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at $7000 \times g$ for 15 min. All processes were carried out at 4 °C. Supernatants were used to determine enzyme activities.

2.4. Biochemical analysis

Acetylcholinesterase (E.C. 3.1.1.7) and butyrylcholinesterase (E.C. 3.1.1.8) activities were measured on Shimadzu UV-1201 spectrophotometer using acetylthiocholine and butyrylthiocholine as substrate, respectively, by the method described by Ellman et al. (1963). ADA (EC 3.4.5.5) was assayed by the method described by Giusti (1974). MPO (EC 1.11.1.7) was assayed by the method described by Bradley et al. (1982).

2.5. Analysis of data

All data were expressed as mean ± standard deviation (S.D.). The statistical analyses were made using the Minitab 13 for windows packet program. Means and standard deviations were calculated according to the standard methods for all parameters. One-way ANOVA statistical test was used to determine the differences between means of the treatments and the control group accepting the significance level at $p \leq 0.05$.

3. Results

Following the exposure to 25 and 50 ppm dosages of IBA, the effects of IBA administration on the rats were evaluated as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) for neurotoxic, and adenosine deaminase (ADA) and myeloperoxidase (MPO) activities for immunotoxic in the tissues samples from the control and treated rats. It was observed that AChE activity significantly decreased in some tissues of rats treated with 25 and 50 ppm dosage of IBA at subacute and subchronic exposure (Table 1). The BChE decreasing in the liver and kidney, treated with 25 ppm IBA, and liver, heart, and kidney tissues of rats treated with 50 ppm IBA were significant at subacute period. Also, the liver BChE activity with both dosages and the kidney BChE activity treated with 50 ppm IBA decreased significantly at subchronic period (Table 2). With regard to the immunotoxic effects, ADA activity significantly decreased (Table 3) whereas MPO activity increased in almost all the tissues (Table 4) after subacute and subchronic periods with both dosages compared with controls.

Table 1
Effects of subacute and subchronic treatment of IBA on AChE enzyme (U/g tissue) of rats.

Period	Tissue	Control	25 ppm	50 ppm
		$X \pm$ S.D.	$X \pm$ S.D.	$X \pm$ S.D.
Subacute	Liver (U/g)	1.38 ± 0.19	1.22 ± 0.28[a]	1.19 ± 0.19[a]
	Lungs (U/g)	1.88 ± 0.31	1.71 ± 0.29	1.05 ± 0.32[b]
	Heart (U/g)	1.96 ± 0.07	1.87 ± 0.12	1.80 ± 0.25
	Muscle (U/g)	2.29 ± 0.11	2.13 ± 0.23	2.04 ± 0.31
	Spleen (U/g)	2.40 ± 0.24	2.06 ± 0.21[b]	2.05 ± 0.60
	Kidney (U/g)	1.18 ± 0.07	1.16 ± 0.17	0.83 ± 0.08[c]
	Brain (U/g)	2.29 ± 0.17	2.27 ± 0.09	2.24 ± 0.20
Subchronic	Liver (U/g)	1.38 ± 0.19	1.67 ± 0.13	1.70 ± 0.23
	Lungs (U/g)	1.88 ± 0.31	2.14 ± 0.22	1.96 ± 0.28
	Heart (U/g)	1.96 ± 0.07	2.17 ± 0.30	2.29 ± 0.6
	Muscle (U/g)	2.29 ± 0.11	2.04 ± 0.14[a]	1.92 ± 0.33[a]
	Spleen (U/g)	2.40 ± 0.24	2.33 ± 0.24	2.31 ± 0.10
	Kidney (U/g)	1.18 ± 0.07	0.88 ± 0.04[d]	0.93 ± 0.06[d]
	Brain (U/g)	2.29 ± 0.17	2.23 ± 0.23	2.18 ± 0.07

Each value represents the mean ± S.D.
[a] Significantly different from control rats at 0.034 (one-way ANOVA).
[b] Significantly different from control rats at 0.02 (one-way ANOVA).
[c] Significantly different from control rats at 0.001 (one-way ANOVA).
[d] Significantly different from control rats at 0.043 (one-way ANOVA).