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Effects of Indoleacetic Acid and Kinetin on Lipid Peroxidation Levels in Various Rat Tissues

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Abstract: This study was carried out to investigate whether plant growth regulators (PGRs) could affect lipid peroxidation (Malondialdehyde = MDA level) in erythrocyte, muscle, liver, heart and kidney tissue. Eighteen Sprague-Dawley albino rats were divided into three experimental groups: control and PGR-treated (indoleacetic acid and kinetin). Seventy-five ppm of PGRs, indoleacetic acid (IAA), and kinetin were administered orally to 6 rats *ad libitum* for 25 days. The hormone treatments caused different effects on the level of malondialdehyde (MDA) to those of the control rats. According to the results, while liver and kidney MDA levels were increased significantly by IAA administration in rats, kinetin did not affect the MDA levels in erythrocyte and other tissues. It was concluded that IAA might affect lipid peroxidation in animals at subacute treatment.

Key Words: Plant growth regulators, Malondialdehyde, in vivo

İndolasetik asit ve Kinetinin Sıçanların Çeşitli Dokularında Lipid Peroksidasyon Düzeyi Üzerine Etkisi

Özet: Bu çalışmada, subletal konsantrasyonda laboratuar şartlarında sıçanların kas, karaciğer, kalp, böbrek ve eritrosit malondialdehit (MDA) düzeyi üzerine indolasetik asit (IAA) ve kinetinin etkilerinin araştırılması amaçlanmaktadır. Sıçanlar deney süresince içebildiğince 75 ppm'lik IAA ve kinetin uygulamasına 25 gün maruz bırakıldı. Hormon uygulamalarının çeşitli dokulardaki MDA düzeyi üzerine farklı etkilere neden oldukları gözlendi. Sonuçlara göre; karaciğer ve böbrek MDA düzeyi IAA tarafından önemli derecede arttırılırken, kinetin eritrosit ve doku MDA düzeyi üzerinde etkisiz bulundu. Sonuç olarak; IAA'nın subkronik uygulamalarda toksik etkilere sahip olduğu görüldü.

Anahtar Sözcükler: Bitki büyüme düzenleyicileri, Lipid peroksidasyonu, in vivo

Introduction

Lipid peroxidation is an autocatalytic mechanism leading to the oxidative destruction of cellular membranes (1). Their destruction can lead to cell death and also to the production of toxic and reactive aldehyde metabolites called free radicals. Of these free radicals, malondialdehyde (MDA) is the most important (2).

Today, because the effects of plant growth regulators (PGRs) on plants are well known, they are used widely in agriculture. Information on the toxic effects of these chemicals on animals is limited. This subject has therefore recently attracted the interest of many researchers.

Many chemicals are currently used in agriculture, and PGRs are among those most widely employed. On the other hand, some of these are endogenous plant hormones that are most likely included in the diet of all herbivorous and omnivorous animals. Pollution of the environment by these substances may soon exceed that by insecticides (3).

The effects of various PGRs on insects have already been investigated in several studies (4-7), but reports concerning vertebrates are very limited (8,9). In the literature, it is reported that PGRs cause increases in the number of splenic plaque-forming cells and circulating white blood cells, hematocrit values, and thymus weight in young deer mice (10). El-Mofty and Sakr (11) found that Gibberellin A_3 (GA_3) induced liver neoplasm in Egyptian toads, and 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 (12). On the other hand, it is reported that fecundity, longevity and egg viability

have been changed in different insects by PGR treatment (13,14). Some PGRs, the effects of which we investigated in another study (15), affect the carbonic anhydrase izoenzymes of erythrocytes in humans and bovines. While indoleacetic acid (IAA) increased the activation of both bovine carbonic anhydrase and human carbonic anhydrase-II, kinetin was found to have no effect on bovine or human carbonic anhydrase-I or human carbonic anhydrase-II isozymes *in vitro*. The effects of IAA and kinetin on human serum enzymes *in vitro* were also investigated. IAA was found to inhibit AST and activate amylase, CPK and LDH. Kinetin inhibited muscle creatine kinase (CK-MB), while it activated AST and ALT (16). On the other hand, in a previous study we found that the levels of AST, LDH and CPK were increased significantly by IAA and kinetin (17).

In order to achieve a more rational design of PGRs, it is necessary to clarify the mechanism of their toxic effects in detail, as well as their structural toxicity. For this purpose, the treatment of PGRs *in vivo* was done orally because the effect of chemicals represents a well characterized *in vivo* toxicity model system.

Materials and Methods

Materials: Technical grade PGRs were supplied by the Sigma Chemical Co. (St. Louis, MO, USA).

Animals: Rats (Sprague-Dawley albino) weighing 150-200 g were provided by the Medical School of Yüzüncü Yıl University, and were housed in 3 groups, each containing 6 rats. All animals were fed with a group wheat-soybean meal-based diet *ad libitum* in stainless steel cages during the experiment, and received human care according to the criteria outlined in '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 animals were housed at 20 ± 2 °C in a daily light/dark cycle.

Treatment of rats: This investigation was performed on male rats. A dosage of IAA and kinetin was used. The rats were given drinking water containing 75 ppm of IAA and kinetin *ad libitum* for 25 days. Seventy-five milligrams of each PGR was dissolved in 1 ml of 1 N NaOH, and then diluted with tap water to obtain a 75 ppm dose of drinking water. For the control rats, only 1 ml of NaOH was added to 1000 ml of tap water. The control rats were only given tap water.

At the end of the treatment, the rats were anesthetized with diethyl ether and sacrificed, after blood and tissue samples had been obtained. The blood samples were obtained by cardiac puncture using syringes for the determination of MDA levels. Blood samples drawn using ethylenediaminetetraacetic acid (EDTA) as an anticoagulant were immediately put into ice-chilled siliconized disposable glass tubes. The MDA level was measured in these blood samples.

The tissues were dissected and put in petri dishes. After the tissues had been washed with physiological saline (0.9% NaCl), samples were taken and kept at -87 °C until analysis. The tissues were homogenized for 5 min in 0.115 M potassium chloride (KCl) solution (1:5 w/v) using a glass-porcelain homogenizer (20 KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000 xg for 15 min. All processes were carried out at 4 °C. Supernatants were used to determine the MDA.

Biochemical analysis: The MDA concentration in erythrocyte and tissue samples was determined using the method described by Jain et al., (18), based on thiobarbituric acid (TBA) reactivity. Briefly, 0.2 ml erythrocyte packets or supernatant obtained from tissues, 0.8 ml phosphate buffer (pH 7.4), 0.025 ml butylated hydroxytoluene (BHT) and 0.5 ml 30% trichloroacetic acid (TCA) were added to the tubes and mixed. After 2 h incubation at -20 °C, the mixture was centrifuged (400 xg) for 15 min. After this, 1 ml of supernatant was taken and added to each tube, and then 0.075 ml of 0.1 mol EDTA and 0.25 ml of 1% TBA were added. These tubes with Teflon-lined screw caps were incubated at 90 °C in a water bath for 15 min and cooled to room temperature. The optical density was measured at 532 and 600 nm in a spectrophotometer (Novaspec II Pharmacia-Biotech, Biochrom Ltd., UK) for erythrocytes, and the optical density was measured at 532 MDA concentration in tissues.

Analysis of data: The data were expressed as mean ± standard deviation (SD). For statistical analysis the SPSS/PC+ package (SPSS/PC+, Chicago, IL, USA) was used. For all parameters, means and SD were calculated according to standard methods. The Mann-Whitney U-test was employed to determine differences between the means of the treatment and control rats. The significance level was considered $p \leq 0.05$ for all tests (19).

Results

The results of the experiment showed that treating rats with IAA and kinetin hormones produced some changes in the levels of MDA in erythrocyte, muscle, liver, heart and kidney in comparison to those of the control rats. According to the results, exposure to subacute administration of IAA caused significant increases in the level of MDA in the liver and kidney, but did not significantly affect the other tissues. While the level of kidney and liver MDA was increased significantly by IAA, kinetin did not affect the MDA levels in erythrocyte and other tissues. In order to establish the significance of increases in MDA levels in different tissues after exposure to IAA for 25 days, the data obtained were subjected to the Mann Whitney-U test.

Discussion

The first aim of this study was to investigate whether IAA and kinetin could affect lipid peroxidation in rats. The data collected were all taken at the same time in the experiment. As shown in the Table and Figure 1, treatment with IAA increased the production of lipid peroxides. Also as shown in Figure 2, IAA and kinetin did not affect the levels of MDA in rat erythrocytes.

Research has shown that the toxicological or biological effects of PGRs differ, and that the dose-effect relationship changes with different organisms. For example, kinetin increases DNA in the nuclei of a fibroblastic cell culture at low doses, but at higher doses it causes foamy and vacuolized cytoplasm in these cells (11). There are no studies examining the effect of IAA and kinetin *in vivo* on MDA concentrations in rat erythrocyte, muscle, liver, heart and kidney. It was therefore impossible to compare our results. However, Candeias et al., (20) investigated the peroxidation of liposomes by a haem peroxidase and hydrogen in the presence of IAA and derivates. They found that these compounds can accelerate lipid peroxidation up to 65-

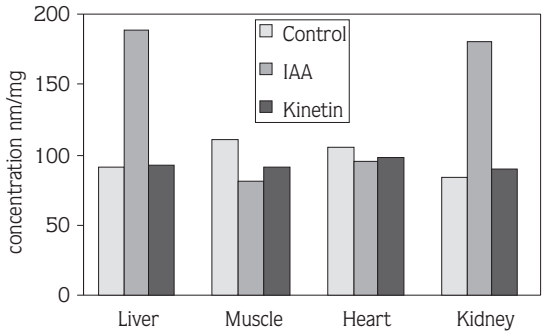

Figure 1. The level of lipid peroxidation in different rat tissues

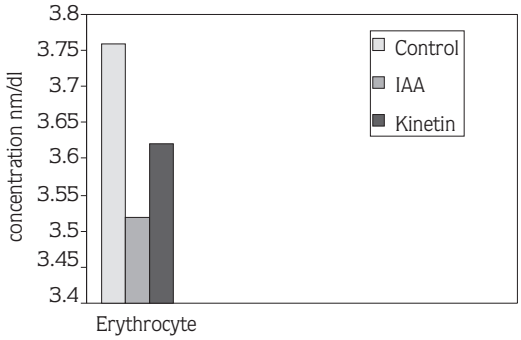

Figure 2. The level of lipid peroxidation in rat erythrocytes

fold, and this is attributed to the formation of peroxyl radicals that may react with the lipids, possibly by hydrogen abstraction. Consequently, that study agrees with our results.

The present study indicates that IAA possesses toxic effects. This is evidenced from our observation that, following PGR treatment *in vivo*, the concentration of MDA in liver and kidney increased in comparison to that of the control rats. In addition, PGRs exerted different effects on rat liver and kidney functions, causing on MDA increase. The reasons for such effects of PGRs are not understood at present, although it can be said that the chemicals tested have an effect on tissues. However, it is conceivable that IAA, being toxically like other pesticides, might be interacting primarily with the liver and kidney tissue cells, resulting in lipid peroxidation synthesis

Table 1. Lipid peroxidation levels in different rat tissues (Mean ± SD).

TISSUE	CONTROL (n = 6) X ± SD	IAA (n = 6) X ± SD	KINETIN (n = 6) X ± SD
LIVER (nm/mg)	91.02 ± 3.03	188.82 ± 12.68a	91.74 ± 16.07
MUSCLE (nm/mg)	111.2 ± 34.3	81.4 ± 12.2	90.3 ± 8.3
HEART (nm/mg)	105.2 ± 19.2	95.3 ± 25.5	97.54 ± 9.93
KIDNEY (nm/mg)	84.14 ± 14.35	180.66 ± 20.49b	89.06 ± 12.36
ERYTHROCYTE (nm/dl)	3.760 ± 0.513	3.520 ± 0.460	3.620 ± 0.733

a: $p < 0.05$, b: $p < 0.05$

induced by free radicals originating from stressed conditions in rats. Further studies are required to correlate the *in vivo* damage to liver and kidney MDA.

Conclusion

It is postulated that liver and kidney MDA might offer a marker of choice for monitoring the biotoxicity of direct-acting compounds such as PGRs. It is impossible to forbid the use of these kinds of chemicals, which are used against harmful insects and cause production losses under present-day conditions. However, the necessity of using regulators should be decreased by improving plant resistance to diseases and unfavorable conditions. Such species can be developed with the aid of biotechnological and plant-improving procedures.

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