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Effects of Indole-3-Acetic Acid and Kinetin on the Adenosine Deaminase Activity in Various Tissues of Rats

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The effects of a sub-lethal concentration of some plant growth regulators on heart, kidney and muscle adenosine deaminase activity in female rats (Sprague-Dawley) were investigated under laboratory conditions. Thirty-six Sprague-Dawley albino rats were divided into three experimental groups: control and plant growrh regulator treatment [indole-3-acetic acid and kinetin]. 100 ppm each of plant growrh regulators, indole-3-acetic acid and kinetin were administered orally to 12 rats *ad libitum* for 25 d continuously. The hormone treatment caused different effects on the heart adenosine deaminase activity in comparison to those of the control rats. According to results, while the heart adenosine deaminase activity was decreased significantly by indole-3-acetic acid and kinetin, these plant growrh regulators did not affect the adenosine deaminase activity of kidney and muscle tissues. In conclusion, these chemicals have toxicological effects on the animals in subacute treatment.

Key Words: Plant growth regulators, Adenosine deaminase, Rat.

INTRODUCTION

Adenosine deaminase (ADA) is widely distributed in human tissues, which may contribute to the maturation of the immune system. Especially, ADA plays an important role in the proliferation and differentiation of lymphoid cells and seems to be critical in different stages of the maturation process[1, 2]. ADA has been found in high levels in the spleen, lungs and lymph nodes and in low levels in the myocardium, muscles, kidneys, bladder and liver[3]. ADA is present in all cell types, but the amount of enzyme differs widely amongst tissues. The highest ADA levels in humans are found in lymphoid tissues[4].

Contamination of the environment by plant growth regulators (PGRs) may cause several physiological changes in mammals. Interactions of agrochemicals with mammalian tissues are of great importance and interest. Many chemicals are currently used in agriculture, and plant growth regulators are among those widely used. The toxic effects of these chemicals on animals are limited; therefore, this subject has attracted the interest of many researchers recently. As a result of the agricultural usage, these agrochemicals are consumed by many non-target organisms. Even though they are consumed in small amounts, these plant growth regulators can still affect animals.

The effects of various PGRs on insects have already been investigated in several studies[5–7], but reports concerning vertebrates are very limited. In the literature, it is reported that PGRs cause increase in the number of splenic plague forming cells and circulating white blood cells, hematocrit values and thymus weight in young deer mice[8]. El-Mofty and Sakr[9] found that Gibberellin A_3 (GA_3) induced liver neoplasm in Egyptian toads and they suggested that the tumours could be diagnosed as hepatocellular carcinomas. While indole-3-acetic acid increased the activation of both bovine carbonic anhydrase and human carbonic anhydrase-II, kinetin was found to be effective neither on bovine nor human carbonic anhydrase-I and human carbonic anhydrase-II isozymes *in-vitro*[10]. The effects of indole-3-acetic acid (IAA) and kinetin were also investigated on human serum enzymes *in-vitro*. IAA was found to inhibit AST and activate amylase, CPK and LDH. Kinetin inhibited muscle creatin kinase (CK-MB), while it activated AST and ALT[11]. On the other hand, in a previous study, the levels of AST, LDH and CPK were increased significantly by IAA and kinetin[12]. In another study, while liver and kidney MDA levels were increased significantly by IAA administration in rats, kinetin did not affect the MDA levels in erythrocyte, muscle, liver, heart and kidney tissues[13].

The aim of this study is to evaluate the effects of the plant hormones IAA and kinetin on the specific activity of ADA enzyme in heart, kidney and muscle of rats (Sprague-Dawley) in order to assess its possible toxicity. The treatments of PGRs were done orally because the effect of plants represents a well characterized *in-vivo* effect on the model system.

EXPERIMENTAL

Indole-3-acetic acid (IAA) and kinetin, sodium hydroxide, hydrochloric acid and phenol red were obtained from Merck, Germany. Adenosine, dipotassium hydrogen phosphate, sodium hypochloride, ammonium sulphate and potassium chloride were obtained from Sigma Chemical Co., USA. Alkaline hypochlorite was prepared from commercial bleaching powder and sodium hydroxide.

Animals: Rats (Sprague-Dawley albino) weighing 150–200 g were provided by the animal house in the Medical School of Yuzunzu Yil University and were housed in 3 groups, each group containing 12 rats. The animals were fed a standard laboratory diet purchased from Van Animal Feed Factory (Van, Turkey) and they had access to food *ad libitum* during the experiments. Animals were housed at 20 ± 2°C.

PGR Treatment: The investigation was performed on female rats. A dosage of IAA and kinetin was used. Rats were exposed to 100 ppm of IAA and kinetin *ad libitum* during the tests for 3 weeks. 100 mg of PRGs were dissolved in 1 mL of 1 N NaOH and then were diluted with tap water to obtain a 100 ppm dose. For control rats, only 1 mL of NaOH was added into 1000 mL of tap water.

Analysis: At the end of the treatment, rats were anesthetized and then sacrificed. 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 –20°C until analysis. The tissues were homogenized for 5 min in 0.115 M ice-cold potassium chloride solution (1 : 5 w/v) using a glass-porcelain homogenizer (20

KHz frequency ultrasonic, Jencons Scientific Co.) and then centrifuged at 7000 × g for 15 min. All processes were carried out at 4°C. Supernatants were used to determine ADA.

ADA was assayed by the method described by Giusti[14]. The ADA assay is based on indirectly measuring the formation of NH_3 produced when ADA acts in excess of adenosine. The release of ammonia was determined colorimetrically at 630 nm after the development of an intense blue colour with hypochlorite and phenol in an alkaline solution. Results were expressed as units per gram tissue (U/g tissue) and calculated as mean standard deviation (SD).

Analysis of Data: All statistical analyses were carried out using Minitap for Windows statistical software. The Mann-Whitney U-test was employed to test differences between means of the treatments and the control rats. The significance level $p \leq 0.05$ was used for all tests.

RESULTS AND DISCUSSION

The results of the experiment showed that the treatment of female rats with IAA and kinetin hormones produced some changes in the ADA activity in heart, kidney and muscle in comparison to those of control rats (Table-1 and Fig. 1). To find out the significance of decrease in ADA activity on exposure to IAA and kinetin for 25 d, the data obtained have been subjected to Man Whitney U-test. According to the results, while the heart ADA activity was decreased significantly by IAA and kinetin, these PGRs did not affect the ADA activity of other tissues.

TABLE-1
EFFECTS OF 100 ppm OF IAA AND KINETIN ON THE ADA ACTIVITY IN HEART, KIDNEY AND MUSCLE OF RATS (MEAN ± SD)

Parameters	Control (X ± SD)	IAA (X ± SD)	Kinetin (X ± SD)
Heart (U/g)	0.0054 ± 0.00134	0.00122 ± 0.00054*	0.00214 ± 0.00087*
Kidney (U/g)	0.0046 ± 0.00207	0.00340 ± 0.00152	0.00348 ± 0.00160
Muscle (U/g)	0.0026 ± 0.00207	0.00300 ± 0.00071	0.00320 ± 0.00130

*Statistically significant; $p \leq 0.05$

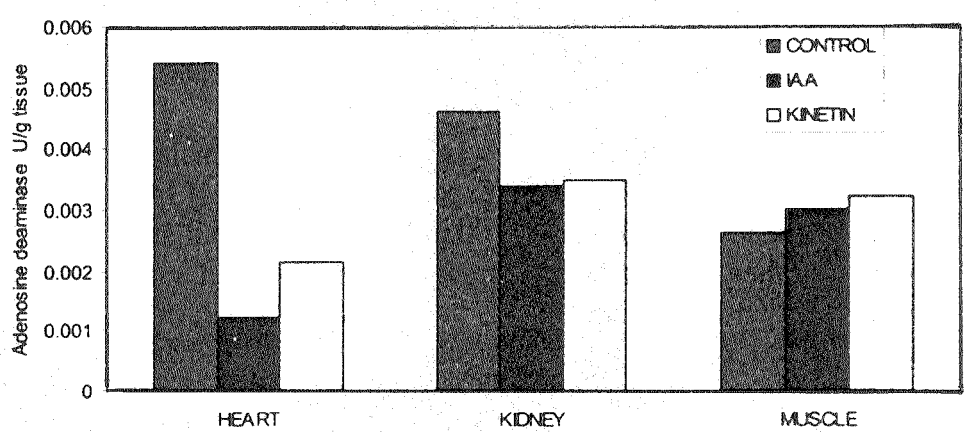

Fig. 1. Effects of 100 ppm of IAA and kinetin on the ADA activity in heart, kidney and muscle of rats

Researches show that the toxicological or biological effects of PGRs are divergent and also the dose-effect relationship changes in living organisms. For example, kinetin increases DNA in the nuclei of a fibroblastic cell culture at low doses, but at high doses kinetin causes foamy and vacuolated cytoplasm in these cells[15]. So far, no study examining the effect of PGRs *in-vivo* has been made on the ADA activity in heart, kidney and muscle of rats. Because of this, we could not have the chance to compare our results with the previous results.

Low levels of ADA have been found in the heart. A possible toxic effect may have been developed in the heart by the IAA and kinetin treatment in the present study. Lack of ADA and the consequent accumulation of adenosine are associated with absence of T cells, decrease in B cells and some hepatotoxicity[16]. Indeed, the inhibition of ADA activity in a number of cell types and organs has been shown to result in a massive accumulation of Ado[17-21]. On the other hand, Green and Chan[22] suggest that the immune deficiency disease associated with lack of ADA may be the result of pyrimidine starvation.

Cytotoxic effects of an enzyme deficiency are caused by either a lack of the enzyme's product or the buildup of the enzyme's substrate. In the case of ADA deficiency, there are normal levels of both inosine and 2′-deoxyinosine, due to the presence of alternate bypass pathways in the purine catabolic pathway. However, in the ADA deficiency, there are high levels of both adenosine and 2′-deoxyadenosine[23]. When ADA is absent, deoxyadenosine is converted to dATP[24]. The most potent way to elevate Ado concentrations in biological systems is to block ADA activity with inhibitors because the suppression of the deamination reaction inhibits a major step in the pathway of ATP degradation in most cells. In human heart, 70% of ATP catabolism proceeds *via* the ADA reaction[25]. Inhibition of ADA is a potent way to increase the tissue half-life of Ado. Accumulations of dAdo (deoxyadenosine) and dATP (deoxyadenosine triphosphate), which is the phosphorylated end-product of dAdo seems to be instrumental in the harmful effects of ADA deficiency. These accumulations result in the inactivation of an enzyme called S-adenosylhomocysteine hydrolase (SAH), whose substrate accumulation inhibits certain methylations of nucleic acids, proteins, and lipids. High levels of dAdo are also responsible for an inhibition of ribonucleotide reductase, causing an imbalance of the deoxynucleotides (dNTP). This imbalance, in addition to the enzyme inhibitions, leads to impairment of DNA synthesis and repair in T lymphocytes[26].

Inhibition of the enzyme adenosine deaminase by IAA and kinetin, as well as the inhibition of adenosine transport and metabolism by IAA and kinetin, might increase the levels of adenosine. This increase may indicate depressed cell-mediated immunity. Adenosine deaminase is essential for the proper functioning of the human body's immune system. The presence of abnormal levels of enzymes in tissues is used in clinical practice to indicate whether or not tissue damage or certain other changes have occurred and to suggest which organ has been affected. Because ADA is the major enzyme responsible for the degradation of Ado, the inhibition of its activity should represent one of the best ways to increase accumulation of Ado in tissues under stress conditions.

Conclusion

It can be postulated that determination of ADA might offer a marker of choice for monitoring immune system deficiency and biotoxicity of direct acting compounds such as PGRs. In order to achieve a more rational design of PGRs, it is necessary to clarify the mechanism of PGR's toxic effect in detail and its structural toxicity. The fact that ADA levels remained low suggests an immune system deficiency. Furthermore, determination of ADA isoenzymes may be helpful in the assessment of tissue degeneration caused by the effect of PGRs.

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