The effect of 2,4-dichlorophenoxyacetic acid on some antioxidant enzymes in kidneys of the second cross offsprings mice*

H. RamazanYılmaz*, Eşref Yüksel**, Yusuf Türköz***

*Süleyman Demirel University, Faculty of Medicine, Department of Medical Biology and Genetics, Isparta.
**Gazi University, Faculty of Arts and Sciences, Department of Biology, Ankara.
***İnönü University, Faculty of Medicine, Department of Biochemistry, Malatya.

Özet

2,4-Diklorofenoksiasetik asit'in ikinci çapraz yavruların böbreğinde bazı antioxidant enzimlerin aktiviteleri üzerine etkisi

2,4-diklorofenoksiasetik asit (2,4-D) tarımda geniş yapraklı yabani otların kontrolünde kullanılan sistemik bir herbisittir. Fakat, bazı çalışmalar, 2,4-D'in karsinojenik, mutajenik, klastogenik ve nörotoksik bir ajan olduğunu belirtmektedirler. Bu çalışmanın amacı, 2,4-D'in ikinci çapraz yavruların (*Mus musculus*) böbreğinde antioksidan enzimlerden süperoksit dismutaz (SOD), glutatyon peroksidaz (GSH-Px) ve katalaz (CAT) enzimlerinin aktiviteleri üzerine etkilerini incelemektir. Dişi fareler, 2,4-D ve kontrol gruplarına ayrıldı. 2,4-D anne farelere LD_{30} dozu olan 338 mg/kg vücut ağırlığı dozundan 1/100 seyreltilerek üç günde bir intraperitoneal (i.p.) olarak verildi. Kontrol grubuna da etanol aynı yolla verildi. Bu işlemler iki nesil boyunca yapıldı. İkinci çapraz yavruların böbreğinde, enzim aktiviteleri spekrofotometrik olarak ölçüldü.

CAT aktivitesi, 2,4-D grubunda, kontrol grubuna göre anlamlı olarak azaldı ($p<0.001$). SOD ve GSH-Px aktivitelerinde ise anlamlı bir fark gözlenmedi. Sonuç olarak, ikinci çapraz yavruların böbreğindeki CAT aktivitesinin 2,4-D'in toksisitesinden etkilendiği söylenebilir.

Anahtar Kelimeler: 2,4-D, süperoksit dismutaz, glutatyon peroksidaz, katalaz, *Mus musculus*, böbrek.

Abstract

2,4-Dichlorophenoxyacetic acid (2,4-D) is a systemic herbicide used to control a wide variety of broad-leaved weeds in agriculture. But, several studies have been suggested that 2,4-D is a carcinogenic, mutagenic, clastogenic and neurotoxic agent. The aim of the present study was to investigate the effect of 2,4-D on some kidney antioxidant enzymes activities such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in the second cross offsprings (*Mus musculus*). Female mice were divided into two equal groups: 2,4-D group and ethanol control group. Two the chemicals used were applied intraperitoneally. 2,4-D was administrated to maternal mice as a 1/100 dilution of 338 mg/kg body weight (LD_{30}) in 3 day intervals.

In the control group it was administrated by similar way. Same processes were repeated over two generations. Spectrophotometric method was used to determine the activities of enzymes in the kidney of the second cross offsprings. The activity of CAT was found to be significantly lower in 2,4-D group compared to control ethanol group ($p<0.001$). There was no significant difference in the SOD and GSH-Px activities between the 2,4-D and control groups. The results obtained suggest that the CAT activity of kidney in the second cross offsprings was affected from 2,4-D toxicity.

Key words: 2,4-D, superoxide dismutase, glutathione peroxidase, catalase, *Mus musculus*, kidney.

*This work was presented as a poster at the 2nd International Meeting on free radicals in healt and disease May 8-12, 2002, Istanbul, Turkey.

Introduction

The discovery of herbicides has had a revolutionary impact on the modern agricultural industry. The use of these compounds in agriculture either to eliminate pests or to modify crop growth has also toxicological consequences. 2,4-dichlorophenoxyacetic acid (2,4-D) is may be the most widely used herbicide worldwide. At low doses it acts as a plant auxine analog, promoting growth, in a manner similar to indole acetic acid. However at high doses it is lethal to broad leaved weeds (1). There is currently renewed interest in 2-4-D, mostly owing to reports about the potentiality of this herbicide as toxicant to animals and humans. It is taken by three classical routes: inhalation, ingestion and dermal absorpsion. It is readily absorbed by cells and its elimination rate and time, however, varies greatly among species. In all animal species, excretion is largely via urine as in non-metabolized form (2). In humans, 2,4-D has a half- life of

Corresponding Address:
Dr. H.Ramazan YILMAZ
Süleyman Demirel University Faculty of Medicine Department of Medical Biology and Genetics 32260 Isparta,
Tel : +90-246-211-3330 - Fax: +90-246-237-1165
E-mail: hramazany@yahoo.com

approximately 18-20 h and 75-90% of absorbed dose is excreted within 4 days. Dogs, for example, having poor excretion of organic acids compared to rodents and humans, have an oral LD_{50} dose about 100 mg/kg body weight, while in rodents this is in the range of 300-1000 mg/kg body weight (3).

2,4-D has been reported as a typical uncoupler of the respiratory chain-oxidative phosphorylation, thus, affecting a broad spectrum of anabolic reactions through inhibiting ATP synthesis (4). This was confirmed by Palmeira et al (5) and Oakes and Pollak (6) who showed that 2,4-D caused a decrease in membrane potential in mitochondria. Also, when incubated with 2,4-D, the hepatocites' level of ADP, AMP and NAD^+ increased while there was a simultaneous depletion of NADH and ATP. It has been suggested that such herbicides are hepatoxic and initiate the process of cell death by decreasing NADH, ATP, and also glutathione, an important intracellular free radical scavenger (7).

The evidence suggests that 2,4-D does not exhibit the gene-damaging potential of a classical mutagene and overall evidence for its mutagenicity remains equivocal. While 2,4-D caused sister chromatide exchange in cultured human lymphocyte cells, same effect was not observed *in vivo* in rats, mice, hamsters and humans. However, positive mutagenic effects have been noted in *in vivo* tests of mouse bone marrow at doses 100 mg/kg and 300 mg/kg body weight which are near LD_{50} dose (3).

It has been reported that 2,4-D can induce mitotic and meiotic irregularities in plant cells *in vivo* and *in vitro*. A concentration higher than 5 mg/L was reported to induce chromosome aberrations like spindle failure and chromosome mutations(8). In animals high doses were reported to cause a decrease in protein and nucleic acid synthesis and also cause a decrease in activities of some enzymes (1).

The aim of the present study was to investigate the effect of 2,4-D on some kidney antioxidant enzymes activities such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), in the second cross offsprings (*Mus musculus*).

Materials and Methods

Animals: Swiss Albino mouse (*Mus musculus*) was routinely produced in our laboratories. They were given free access to food and water. Adult females weighing 18-22 g were used. For mating, 2 females and one male were held in a stainless steel cage for 2 days. Then, the male was taken from the cage.

2,4-D Application: 2,4-D was dissolved in 70% ethanol before the application. All the females were injected (Just after the separation of males) intraperitoneally with 2,4-D at doses of 3.38 mg / kg body weight (9) in three days intervals until their sacrifice. When their pregnancies were apparent, females was taken into a separate cage. Following birth the offspring were kept together until 35th day. At this time the males and females were separated each other. 2,4-D applications to female parents were continued until 55th day of birth. These applications were continued for two generations.

The Control: The control group was given 70% ethanol.

In each generation, at least 6 mating pairs were established for 2,4-D experimental group. On the other hand, minimum 5 mating pairs were the control groups.

Enzyme Studies: After the mice were sacrificed by cervical dislocation the kidneys were removed. Tissue samples to be processed for the determination of antioxidant enzyme activities were homogenized (1:3, w/v) in phosphate saline buffer (pH 7.4). After homogenization, homogenate samples were sonicated for 1.5 min bursts for 30 s. All procedures were performed at 0-4ºC. Homogenate samples were centrifuged at 22.000 *g* for 17 min at 4ºC, in a microcentrifuge (Ole Dich 157. MP). The supernatant was frozen at -40 ºC until the time of assay. Supernatant fraction was used for enzyme activities and protein determination.

Superoxide dismutase activity in supernatant was measured according to the method of Sun et al.(10) by determining the reduction of nitro blue tetrazolium (NBT) by superoxide anion produced with xanthine / a xanthine oxidase system. One unit of SOD was defined as the amount of protein that inhibits the rate of NBT reduction by 50%. Results were defined as units per milligram protein (U/mg protein).

Glutathione peroxidase activity in supernatant was measured according to Paglia and Valentine (11) by monitoring the oxidation of reduced nicotinamide adenine dinucleotid phosphate (NADPH) at 340 nm. Enzyme units were defined as the number of micromoles of NADPH oxidised per minute. Results were defined as miliunits per milligram protein (mU/mg Protein).

Catalase activity in supernatant was determined according to the method of Aebi (12) by monitoring the initial rate of disappearance of hydrogen peroxide (initial concentration 10 mmol) at 240 nm in a spectrophotometer. Results were reported as rate constant per second per milligram protein (k/mg protein).

Protein was measured by method of Lowry et al.(13).

Statistical Methods: The Mann-Whitney U test was used for statistical analysis. The results were

expressed as mean ± SEM. P values <0.05 were regarded as statistically significant.

Results

The results of the experiment are shown in the Table 1.

Table 1 : Effect of 2,4-D on enzymes activities

Groups	N	SOD (U/mg protein)	GSH-Px (mU/mg Protein)	CAT (k/mg protein)
Control	6	7.87 ± 0.7	156 ± 15	0.24 ± 0.007
2,4-D	6	6.32 ± 0.5	141 ± 11	0.14 ± 0.010*

Each value represents mean ± SEM. N is the numbers of animals used.

*Significantly different from controls. Mann-Whitney U test

CAT activity decreased in the 2,4-D group compared to the control group ($p<0.001$). There was no significant difference in the SOD and GSH-Px activities between the 2,4-D and control groups.

Discussion

2,4-D is plant growth regulator, specially acting as synthetic auxin or plant hormone (14). It strongly decreases the GSH/GSSH ration, induces lipid peroxidation, decreases ATP, NADH, and protein thiols and promotes cell death (5,7,15). The decrease in NADH leads to decrease of activity of protective enzymes, such as glutathione reductase, further increasing the susceptibility of cell to death (7,15). Intracellular reduced glutathione (GSH) plays an important role in detoxification generates a variety of reactive free radicals as well as reactive oxygen species (ROS) that will modifiy and destroy biologically important constituents of living body such as lipids, protein, and DNAs (16).

Cellular defense systems including antioxidative enzymes, such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and non-enzymatic scavengers, such as glutathione, vitamin A and E protect the organism against oxyradical damage such as DNA strand breaks, protein oxidation and the induction of lipid peroxidation (17). An increase of the activated form of molecular oxygen species due to overproduction and /or to inability to destroy them may lead to damage in the DNA structure and thus, may cause mutations, chromosomal aberrations and carcinogenesis. Free radicals may also stimulate cell growth by damaging specific genes that control the growth rate and differentiation during the promotion phase (18).

In the present study, we showed that 2,4-D causes a decrease of CAT activity in the offsprings of the second cross maternal mice ($P<0.001$). In 2,4-D group, SOD and GSH-Px activities were not found significantly different compared to control group. Contradictory to our results, Kawashima et al (19) showed that 2,4-D did not affect catalase activity. In a histopatologic study, it was shown that rodents exposed to 2,4-D have exhibited lesions in the proximal convoluted tubes of the kidney and enlargement of the liver (20). In another study, Oruc and Uner (21) observed increased activities of glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR), GSH-Px and unchanged activity of SOD in the liver of Oreochromis niloticus after 2,4-D exposure. In a different study, it was shown that a single dose application of 2,4-D did not change G6PD activity after 24 and 48 h while G6PD activity was found elevated after 72 h (22).

As a results, it is possible that an increased rate of ROS production may inhibit the action of CAT antioxidant enzyme, or alternatively may cause the decreased expression of this antioxidant enzyme.

References

1. Turkula TE, Jalal SM. Induced clastogenicity in white rats by the herbicide 2,4-D. Cytologia 1987;52:275-81.
2. Kelley M, Vessey DA. Interaction of 2,4-dichlorophenoxyacetate (2,4-D) and 2,4,5-trichlorophenoxyacetate (2,4,5-D) with the acyl-CoA: amino acid N-acetyltransferase enzymes of bovine liver mitochondria. Biochem Pharmacol 1986;35:289-95.
3. Ibrahim MA, Bond GG, Burke TA, Cole P, Dost FN, Enterline PE, et al. Weight of the evidence on the human carcinogenicity of 2,4-D. Environ Health Perspect 1991;96:213-22.
4. Rodriguez EM, Schuld M, Romano L. Chronic histopathological effects of parathion and 2,4-D on female gonads of Chasmagnathus granulata (decapoda, brachyura). Fed Chem Toxicol 1994;32:811-8.
5. Palmeira CM, Moreno AJ, Madeira VMC. Interactions of herbicides 2,4-D and dinoseb with liver mitochondrial bioenergetics. Toxicol Appl Pharmacol 1994b;127:50-7.
6. Oakes DJ, Pollak JK. Effects of a herbicide formulation, Tordon 75 D, and its individual components on the oxidative functions of mitochondria. Toxicology 1999;136:41-52.
7. Palmeira CM, Moreno AJ, Madeira VMC. Metabolic alterations in hepatocytes promoted by the herbicides paraquat, dinoseb and 2,4-D. Arch Toxicol 1994a;68:24-31.
8. Pavlica M, Papes D, Nagy B. 2,4-Dichlorophenoxyacetic acidcauses chromatin and chromosome abnormalities in plant cells and mutation in cultured mammalian cells. Mutat Res 1991;263:77-81
9. Dere E, Yanikoğlu A. The effect of endosulfan and 2,4-D on some enzyme activities on the livers and kidneys of Swiss-Albino (Mus musculus). Doğa-Tr Biology 1993;17:163-173
10. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:479-500.
11. Paglia, D, Valentine, WN. Studies on the quantitive and qualitative charecterization of erythrocyte glutathione per-

oxidase. J Lab Clin Med 1967;70:158-63.
12. Aebi H: In Bergmeter HU(ed). Methods in Enzymatic Analysis, Weinheim, Verlag Chemie 1982;3:273-82.
13. Lowry OH, Rosenbrough NJ, Farr AL, Rondall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193,265-275.
14. Stevens JT, Summer DD. Herbicides. In:Hayes WJ, Laws ER (eds) Handbook of pesticide toxicology, vol 3, Acedemic Press, New York, 1991;1317-91.
15. Palmeira CM, Moreno AJ, Madeira VMC. Thiols metabolism is altered by the herbicides paraquat, dinoseb and 2,4-D: A study in isolated hepatocytes. Toxicol Lett 1995;81:115-23.
16. Yamazaki S, Ozawa N, Hiratsuka A, Watabe T. Photogeneration of 3ß-Hydroxy-5α-cholest-6-ene-5-hydroperoxide in rat skin: evidence for occurrence of singlet oxygen in vivo. Free Rad Biol Med 1999;27:3/4,301-8.
17. Winzer K, Becker W, Van Noorden CJF, Köhler A. Short-time induction of oxidative stress in hepatocytes of the European flounder (Platichthys flesus) Mar Environ Res 2000;50:495-501.
18. Akyol Ö, Arslanoğlu R, Durak İ. Activities of free radical and DNA turn-over enzymes in cancerous and non-cancerous human brain tissues. Redox Report 1995;1:255-9.
19. Kawashima Y, Katoh H, Nakajima S, Kozuka H, Uchiyama M. Effects of 2,4-dichloropenoxyacetic acid and 2,4,5-trichlorophenoxy acetic acid on peroxisomal enzymes in rat liver. Biochem Parmacol 1984;33:241-5.
20. Bucher, N. Effects of 2,4-dichlorophenoxyacetic acid on experimental animals. Proc Soc Exp Biol 1946;63:204-5.
21. Oruc EO, Uner N. Combined effects of 2,4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of Oreochromis niloticus. Comp Biochem Physiol C Toxicol Pharmacol 2000;127:291-6.
22. Oruc EO, Uner N. Marker enzyme assessment in the liver of cyprinus carpio (L.) exposed to 2,4-D and azinphosmethyl. J Biochem Mol Toxicol 2002;16:182-8.