JOURNAL OF PREVENTIVE MEDICINE
2007; 15: 63-69

ANTIOXIDANT STATUS IN PROTECTED EXPERIMENTAL HERBICIDE INTOXICATION

Felicia Gradinariu, Al. Maftei, Doina Popa

Institute of Public Health, Iaşi, România

Abstract: This study investigates the possible protective effect of dietary supplements and their efficacy in rat experimental 6 months intoxication with 2,4-dichloro-phenoxyacetic acid herbicide (2,4-D). The protecting tested agents were: *metaspar* (M), a drug containing methionine, aspartic acid, B1, B6 and PP vitamins and *eurovita* (E),.a mix of multiminerals and multivitamins. All tested substances were administered in food, twice a week, dissolved in sunflower oil. Animals 10/group were fed respectively with: standard food (control), equivalent vehicle (vehicle control), toxic (2,4-D), *metaspar* protected group (2,4-D+M) and *eurovita* protected group (2,4-D+E). After 6 months they were sacrificed, blood and liver were analyzed with standard methods and commercial kits for total and reduced glutathione, erythrocyte superoxide dismutase (SOD) activity and serum thiobarbiuric acid reactive substances (TBARS) (lipoperoxides). The results showed the enhancement of antioxidant defense in protected groups, without clear-cut differences between the two tested compounds.
Key words: 2,4-dichlorophenoxyacetic acid, protection, amino acid, lipid peroxidation, herbicide

Rezumat: Studiul de faţă investighează efectele posibil protectoare ale unor suplimente nutritive şi eficacitatea lor in intoxicaţia experimentală cu acidul 2,4-diclor–fenoxiacetic pe şobolani, timp de 6 luni. Substanţele protectoare testate au fost *Metaspar* (M), un preparat ce conţine metionină, acid aspartic şi vitaminele B1, B6, PP şi *Eurovita* (E) un amestec polivitaminic cu multiminerale. Toate substanţele au fost administrate în hrană, de două ori pe saptămână, dizolvate în ulei de floarea soarelui. Animalele au fost grupate câte 10 pe lot şi au primit hrană obişnuită (lotul martor), hrană cu cantitatea echivalentă de ulei (martor cu ulei), toxic (2,4-D), protecţie prin *Metaspar* (2,4-D+M) şi respectiv protecţie prin *Eurovita* (2,4-D+E). După 6 luni animalele au fost sacrificate şi s-au determinat prin metode standard şi cu ajutorul kit-urilor comerciale, glutationul hepatic redus şi total, precum şi activitătea superoxid dismutazei eritocitare şi lipoperoxizii serici (substanţele care reacţionează cu acd tiobarbituric TBARS). Rezultatele obţinute au evidenţiat o intensificare a protectiei antioxidante la loturile care au primit substanţe de protecţie, fără însă a se înregistra diferenţe nete între cei doi compuşi testaţi.
Cuvinte cheie: acid 2,4-diclor–fenoxiacetic, protecţie, aminoacid, lipoperoxizi, erbicid

INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a selective, systemic auxin-type herbicide most widely and successfully used throughout the world, due to its efficacy and to its biodegradability in the soil. Its intensive use has led to the emergence of resistant weeds and might give rise to several toxicological problems when present in concentrations above those recommended (1). Sources of exposure to 2,4-D are during manufacturing, conditioning and in agriculture during spraying the fields.

Exposure to pesticides is recognized as an important environmental risk factor associated with development of cancer. Epidemiological studies, although sometimes contradictory, have linked phenoxyacetic acid herbicides with non-Hodgkin's lymphoma and soft tissue sarcoma (2). Working in areas with high use of 2,4-D was associated with gastric cancer in California Hispanic farm workers employed in the citrus fruit industry (3).

Long time cytotoxic effects of phenoxyacetic herbicides in humans were detected recently. New Zealand Vietnam war veterans checked for sister chromatid exchange (SCE) in blood showed significantly high frequencies compared to controls, demonstrating that they were exposed to a clastogenic compound which continues to exert an observable genetic effect even after decades, this being attributable to their service in Vietnam. During the war, thousand of tones of defoliant agents were used for military purposes in tropical forests (4).

Substantial dermal exposure to 2,4-D could led occasionally to systemic features including mild gastrointestinal irritation and progressive mixed sensorimotor peripheral neuropathy. Mild, transient gastrointestinal and peripheral neuromuscular symptoms have occurred after occupational inhalator exposure (5).

In spite of the well known efforts of the European Community of reducing the production of pesticides and that of herbicides, 2,4-D still remains the most extensively used herbicide. In summer 2006, the European Commission adopted the long awaited *Thematic Strategy on the Sustainable Use of Pesticides* which tries to harmonize the legislation of EU members in this respect (6).

AIM

Because very often protective devices are neglected, the risk of exposure and intoxication during working time and even post-exposure to 2,4-D still exist. That is why we intended to test possible antidotes to counteract 2,4-D harmful effects. In an experimental model we evaluated the effects and efficacy of two compounds in 2,4-D chronic intoxication, by assessing some markers of oxidative stress. It is postulated that one of the mechanisms of toxic injury of 2,4-D is via the enhancement of oxidative stress (7). The high sensitivity of peroxidative reactions in fish have recommended their use as markers for pollution monitoring (8).

MATERIALS AND METHODS

Male Wistar white rats, weighting 120±20g from the Biobreeding of the Institute of Public Health Iasi (Romania) were used. They were housed in plastic cages, 10/cage, in identical conditions of temperature (18-20 ^{o}C) and humidity (40-60%). All tested compounds were administered in food, twice a week, dissolved in sunflower oil in doses of 24 mg/kg body weight for 2,4-D (representing 1/16.7 of oral DL_{50}), and 1 tablet/kg body weight for each protecting agent. The protecting substances were *Metaspar* (M) and *Eurovita* (E), both marketed in pharmacies as alimentary supplements.

One capsule of *Metaspar* contains a mixture of vitamin B1 (1.50 mg), B2 (1.50 mg), B6 (6.00 mg), PP (3.00 mg), methionine (62.50 mg) and aspartic acid (62.50 mg). *Eurovita* is also a vitaminic complex, each tablet containing vitamin A (1500 µg), vitamin C (60 mg), vitamin D3 (10 µg), vitamin E (20.13 mg), vitamin K (25 µg), vitamin B1 (1.5 mg), vitamin B2 (1.7 mg), vitamin B6 (2000 µg), vitamin B12 (6 µg), biotin (30 µg), folic acid (400 µg), pantothenic acid (10 mg), niacin (20 mg), Ca (162 mg), P (125 mg), I (150 µg), Mg (100 mg), Mn (2.5 mg), Mo (25 µg), Zn (15 mg), Se (20 µg), Cr (25 µg), Cu (2000 µg), Fe (18 mg), Cl (36.3 mg)l, K (40 mg), Ni (5 µg), Wa (10 µg), Si (2 mg), Sn (10 µg) B (150 µg). Animals 10/group were fed respectively with: standard food (control), equivalent vehicle (vehicle control), toxic (2,4-D), *metaspar* protected group (2,4-D+M) and *eurovita* protected group (2,4-D+E). After 6 months they were sacrificed, blood and liver were analyzed with standard methods and commercial kits for liver total and reduced glutathione expressed as mg/100 g of fresh tissue (9), eryhrocyte superoxide dismutase (SOD) activity expressed as U/ml of whole blood (10) and serum thiobarbituric acid reactive substances (TBARS) expressed as nmol of 1,1,3,3-tetraethoxy-propane/ ml serum (11).

RESULTS AND DISCUSSIONS

The hepatic metabolism of 2,4-D involves the activation of the mixed function oxidase-system which converts the compound to a powerful arylating agent detoxified by glutathione-S-transferases. The process might be accompanied by the depletion of hepatic glutathione reserves (12). In the 2,4-D exposed group liver reduced and total glutathione statistically decreased after 6 months of herbicide ingestion ($t=6.20$, p,0.001). Conversely, the two markers increased significantly in protected groups compared to toxic group ($t=5.30$ for M, respectively 4.80 for E protected groups, $p<0.001$) as show in figure 1. This augmentation shows the protecting effects of the tested compounds which counteracted the deleterious effects of the herbicide, by their supplement in vitamins and essential amino acids.

Whole blood SOD activity raised in toxic exposed group compared to controls ($t=2.20$, $p<0.05$) (fig.2). The increases of the enzyme activity demonstrate organisms' effort to balance the deleterious effect of 2,4-D, which enhances lipidperoxidative processes. The lower SOD activity level in protected groups could be explained by the increased capacity of the liver to neutralize herbicide toxic effects.

In vivo studies on fish have shown that 2,4-D induces proliferation of hepatic peroxisomes, which are important sites for beta-oxidative fatty acid metabolism and peroxidative detoxification. The agents causing such peroxisomal proliferation have been often associated with reproductive and developmental toxicity and hepatocarcinogenesis (13). That is why we consider that any protection against the hepatic toxicity of 2,4-D could be beneficial in exposed subjects.

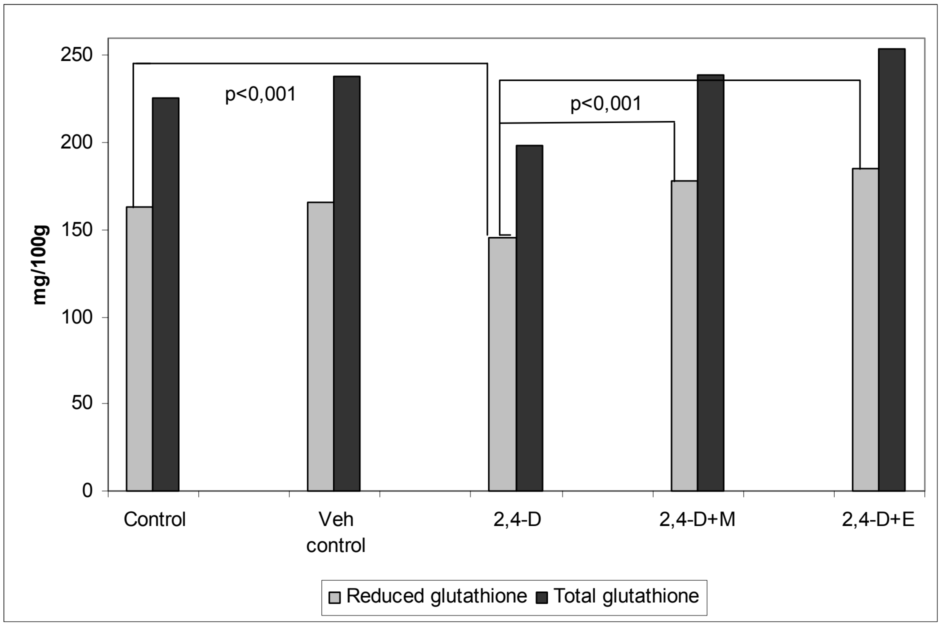

Fig. 1. Liver reduced and total glutathione after 6 months of 2,4-D ingestion

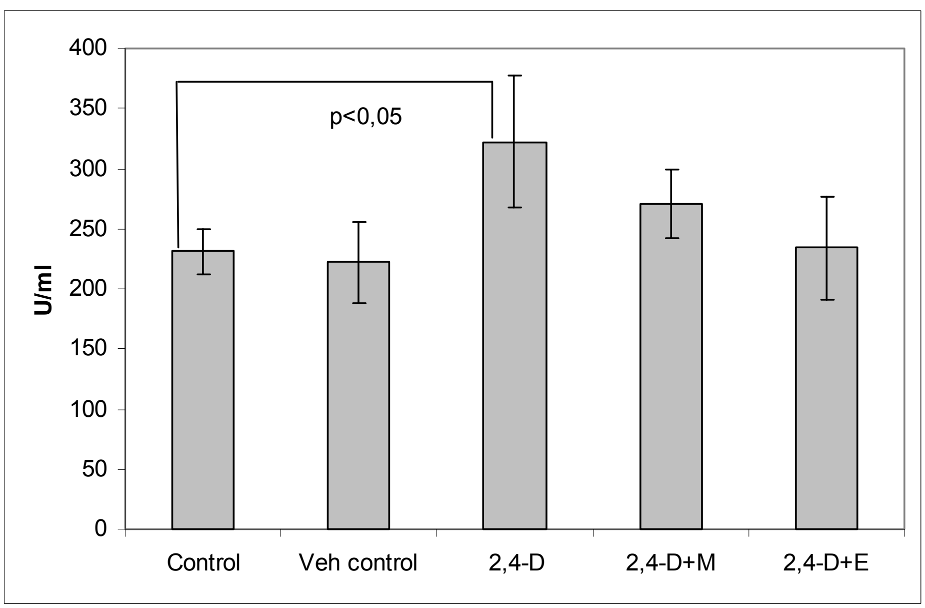

Fig. 2. Blood SOD activity after 6 months of 2,4-D ingestion

SOD and total glutathione had a positive variation in both protected groups, but without straightforward statistical significance. This shows the link between antioxidant defense and detoxifying mechanisms. The lack of clear-cut correlation might be due to the low number of cases (animals/ group).

Serum TBARS did not changed significantly within experimental groups, showing only a moderate increase in 2,4-D group.

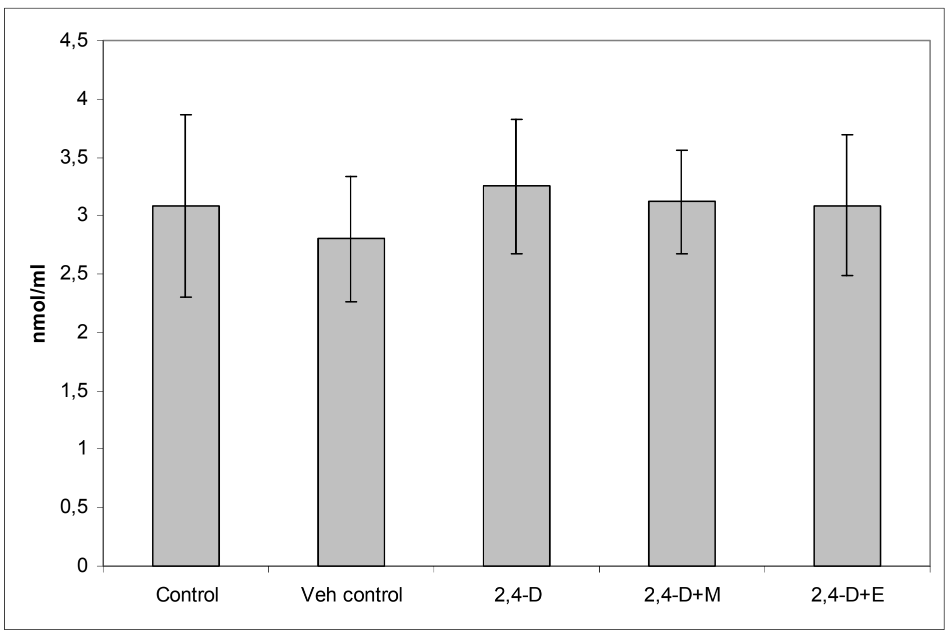

Fig. 3. Serum TBARS level in experimental groups

Similar protection against oxidative stress produced by 2,4-D *in vitro* was recently observed, the compounds exhibiting such properties being melatonin (14) and quercetin (15).

In other recent studies the attention was drawn towards the factors which could influence the transdermal absorption of 2,4-D. Experimental researches have demonstrated that skin penetration of the herbicide is enhanced by moisturizing agents (16) and also by alcohol ingestion (17). These findings underline the influence of life-style upon health consequences of occupational exposure to different noxious substances and reinforce the recommendation of the use of dietary supplements in such conditions.

CONCLUSIONS

As shown by the variation of SOD activity, and that of reduced and total glutathione, antioxidant defense is better acting in protected groups. Both protectors had benefic effects in counteracting chronic 2,4-D toxicity. The appropriate dosing of protecting agents is necessary each time to obtain best protection. Our results confirm the recommendation of using dietary supplements in occupational chronic herbicide exposure.

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