BRIEF COMMUNICATIONS AAEM

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INCREASED URINARY EXCRETION OF THIOETHERS AS A MARKER FOR DETECTING EXPOSURE TO HERBICIDE CONTAINING 2,4-DICHLOROPHENOXYACETIC ACID DIMETHYLAMINE – EXPERIMENTAL STUDY ON MICE

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Abstract: The possibility that urinary thioethers concentration might be a marker for detecting exposure to herbicide containing 2,4-dichlorophenoxyacetic acid dimethylamine (2,4-DMA) was investigated in animals. Mice were treated with the herbicide containing 2,4-DMA consecutively for 4 days. Urinary concentrations of thioethers related either to body weight or creatinine concentration in urine in the group of animals treated with herbicide were significantly higher compared to control group. Results suggest that thioethers determination in urine might be a noninvasive and simple method for detecting exposure to herbicide containing 2,4-DMA.

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Key words: 2,4-dichlorophenoxyacetic acid dimethylamine, herbicide, thioethers, excretion, urine, exposure, mice.

INTRODUCTION

Herbicides are widely used in agriculture, especially in developed agricultural regions [14]. One of them is 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin which promotes uncontrolled plant growth. The International Agency for Research on Cancer classified herbicide 2,4-D as an agent possibly carcinogenic to humans (group 2B) [25]. The epidemiological evidence for an association between exposure to 2,4-D and non-Hodgkin's lymphoma is suggestive. There is little evidence of an association between use of 2,4-D and soft tissue sarcoma or Hodgkin's disease, but it is not clear whether it is specifically related to 2,4-D [10]. Biological monitoring is increasingly being used to detect whether workers have been exposed to and have absorbed the chemical [13]. 2,4-D is rapidly eliminated unchanged in urine [13, 22]. However, 2,4-D was also excreted as an unidentified acid-labile conjugates, via urine in human (10-15%) [20, 22] and via bile in rat (about 3%) [8]. For biological monitoring of 2,4-D exposure, typically used is a radioimmunoassay (RIA) in urine [12].

The exact mechanisms related to the toxic effects of 2,4-D remain obscure [19]. Palmeira *et al.* reported that 2,4-D initiated the process of cell death by decreasing cellular glutathione [17, 18]. Our previous study showed that concentrations of thioethers in urine of agricultural workers were significantly higher after occupational exposure to the herbicide containing 2,4-dichlorophenoxyacetic acid dimethylamine (2,4-DMA) [15]. The aim of the present study was to investigate, using an animal model, the possibility that urinary thioethers concentration might be a marker for detecting exposure to herbicide containing 2,4-DMA.

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MATERIALS AND METHODS

Materials. Albino BALB/C mice, 2-4 months old (Pasteur Institute, Novi Sad, FR Yugoslavia) which were kept in a natural dark-light cycle and fed with standard diet (Veterinarian Institute Zemun, FR Yugoslavia) and water *ad libitum* were used. Herbicide "Monosan herbi" containing 2,4-DMA as active compound, i.e. 2,4-D in the form of dimethylamine salt (464 g/l 2,4-D, 200 g/l dimethylamine and rest water) was obtained from ICN Galenika Co. (Zemun, FR Yugoslavia) for use in experiment, since human exposure to herbicide occurs not only to the active principles but to all chemicals present in a commercial formulation. 2,4-D in "Monosan herbi" is of technical quality. Impurities in total are up to 4.8% and include: 2-chlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, 2,6-dichlorophenoxyacetic acid, 2,4,6-trichlorophenoxyacetic acid, bis-(2,4-dichlorophenoxyacetic acid), 2,4-dichlorophenol, 4-chlorophenol and perchloroetilen.

Experimental design. The animals were divided into experimental and control groups, each with 5 mice. The experimental group of animals was treated on 4 consecutive days with herbicide "Monosan herbi" containing 2,4-DMA as active compound (30 mg/kg of 2,4-D in the form of dimethylamine salt, per body weight i.p. on each day), whereas the animals of the control group received saline solution. The used dose was comparable with occupational exposure of humans, as in our previous study it was found that agricultural workers were occupationally exposed to this herbicide for 2–4 days, 5–6 hours per day by spraying application [15]. The mice received for 4 days of the treatment one i.p. LD_{LO} (lowest published lethal dose) of 2,4-D in the form of dimethylamine salt, equal to 120 mg/kg [21]. 24 hours after the last injection, urine samples were collected during 8 hours for determination of total thioethers concentration [24]. Mice metabolic cages were used to obtain a good separation between urine and faeces. The determination of thioethers was based on the method of Ellman [6]. Free SH groups react with 5,5'-dithio-bis(2-nitrobenzoic acid) - DTNB to a photometrically measurable thiol. The mercapturic acids and other thioethers were converted into the corresponding thiophenols by alkaline hydrolysis. Protein was precipitated with perchloric acid. After centrifugation, sodium hydroxide was added to the supernatant. Alkaline hydrolysis was carried out in closed polypropylene tubes at 100°C for 120 minutes. After cooling, the hydrolysate was neutralized in the cold (10°C) to pH 7.2-7.8 with 5N hydrochloric acid and buffered with triethanolamine hydrochloride. Ellman assay and DTNB were added to hydrolyzed urine samples and the increase of absorbance was measured at 405 nm on a Unicam Ultraviolet Spectrophotometer SP 1800 [24]. Concentrations of thioethers were related to creatinine concentration in urine [11] and animal body weight.

Table 1. Concentration of thioethers in urine related to body weight (mmol/g b. w.) and to concentration of creatinine in urine (mmol/mmol creat.) in control group and animals treated with herbicide 2,4-DMA.

Group	mmol/g b. w. (Mean ± SD)	mmol/mmol creat. (Mean ± SD)
Experimental	0.17 ± 0.11[a]	1.02 ± 0.64[a]
Control	0.05 ± 0.03	0.28 ± 0.12

[a] $p < 0.05$ compared to control

Statistical analysis. The statistical significance of the results was analyzed by Student t-test, and $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The results obtained for the measured parameters in the urine of the animals are presented in Table 1.

Urinary excretion of thioethers related either to animal body weight or creatinine concentration in urine were significantly higher in the experimental group compared to the control group.

Exposure to certain electrophilic agents reacting with reduced glutathione (GSH) increases total thioethers detected after alkaline hydrolysis of urine. GSH conjugation results in the formation of cysteine conjugates, pre-mercapturic acids, mercapturic acids and other thioethers which are excreted in urine [4, 9]. Toxic mechanism of herbicide 2,4-D in animal cells is still poorly understood. 2,4-D rapidly depletes intracellular GSH and protein thiols in isolated rat hepatocytes. The lack of *in vivo* studies with 2,4-D precludes any comparison with *in vitro* data [18].

There are many different influences that can change urinary thioethers excretion: diet [1, 2], tobacco smoke [3, 7, 23] and mostly various alkilating agents [5, 9, 26]. But, in this experiment, diet factors were kept under strict control and there were no other influences such as tobacco smoke or other xenobiotics. The only treatment was with herbicide containing 2,4-DMA. On this basis, we concluded that difference in thioethers excretion between the two compared groups was the consequence of the treatment.

It is important to recognize that N-nitrosamines can occur as a toxic impurity in amino formulations of 2,4-D (N-nitrosodimethylamine, N-nitrosodiethanolamine) [27, 28]. Results of biological monitoring of workers exposed to N-nitrosodiethanolamine in the metal industry showed that high exposure subjects had a higher mean value of urinary thioethers than low-exposure and control subjects [16]. Because we have used a commercial herbicide in the present work, it cannot be excluded that nitrosamines, or some other constituents of the formulation, might influence to some degree the results. In this test, we took into account all possible alkilating agents which could be present in commercial herbicide, apart from 2,4-D.

In our study in agricultural workers, urinary thioethers concentrations related to creatinine concentration in urine were significantly higher after occupational exposure for 2-4 days to the same 2,4-D based commercial herbicide "Monosan herbi" [15]. The results of our present study using the animal model confirmed the results obtained in the previous study in agricultural workers, that thioethers determination in urine samples seems to be a suitable noninvasive and simple method for detecting exposure to herbicide containing 2,4-DMA. This assay, compared to RIA, is much cheaper, and thus more suitable for routine usage and with a broader spectrum not only for 2,4-D, but also for other alkilating agents which can occur during occupational exposure in agriculture.

However, this study is preliminary. Long term research is needed to elucidate the mechanisms of increased urinary thioethers excretion after exposure to this herbicide.

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