Hepatotoxic and Immunotropic Effects of Herbicide 2,4-DA in Rats.

Alexander Zh. Gilmanov, Natalia A. Mufazalova, Guzel M. Abdullina, Shamil N. Galimov, Eugene K. Alekhin, Yury A. Medvedev, Edward G. Davletov, Natalia K. Basyrova and Roman D. Atnabaev.

Chairs of Farmacology and Biochemistry, Bashkir State Medical University, Ufa, 450000, Russia.

Introduction

The phenoxy herbicide 2,4-D contaminated with residual amount of dioxins seems to be one of the major chloroorganic pollutants of environment as reported[1,2]. Widely used in agriculture for control of weeds 2,4-D manufactured particular at Khimprom chemical plant in Ufa, Russia, can accumulate in soil, water and food and cause toxic action on organism and its functional systems of plant and agricultural workers and population living around, though the human intoxications are relatively rare. This action results to metabolic disorders, decrease of non-specific resistance, immunotoxic effects, pathology of endocrine organs etc[1,2]. As the dioxins themselves have hormone-like effect it can also influence upon metabolic pathways at intoxication.

The experimental acute intoxication of 2,4-D disrupted the serum levels of several enzymes and blood components which mainly reflect liver, muscle and kidney damage induced by herbicide[3]. We previously determined the changes of hormonal spectrum in rats in experimental 2,4-D action that generally reflect the disorders of endocrine function[4]. The aim of the present study was to determine the liver functional status by activity of some enzymes in serum and liver homogenate in conditions of intoxication by 2,4-D and its influence on blood cell immune properties in possible connection with hormonal disturbances for better understanding of the toxicity mechanisms.

Experimental Methods

The experiments were performed on 260 male rats of 180-230 grams body weight who received the water solution of 2,4-D-dimethylamine salt (2,4-DA) intragastrally during 4 weeks in total dose equivalent to LD_{50} for all period. Control animals were received sodium chloride solution. Upon the termination of the experiment rats were decapitated and blood sera examined for the activity of enzymes aspartate transaminase (SGOT, AST), alanine transaminase (SGPT, ALT) and γ-glutamyltranspeptidase (GGT). The liver homogenate was tested for the activity of NADP-dependent glucose-6-phosphate dehydrogenase (G6PDH),

succinate dehydrogenase (SDH), NAD-dependent malate dehydrogenase (MDH), glutamate dehydrogenase (GDH), and arginase as possible thyroid-dependent liver enzymes. The processes of microsomal oxydation were tested by hexenal-induced sleeping times. Together with it the tests of blood cell immune properties were performed, including latex stimulated Nitro Blue Tetrazolium test (NBT), quantity of blood polymorphonuclear cells (PNC, neutrophiles) and monocytes (MNC) and its fagocyte and fungicide (on *Candida albicans)* activity. All statistic data processing were performed using Student t-criterion.

Results and Discussion

The results of our study presented in fig. 1-4, testify the disorders of the functional liver status in rats developed in intoxication by 2,4-DA. In conditions of 28-day daily poisoning, the serum activity of ALT grew up to 151,2% of control level to the end of intoxication period, returning to normal values on day 60. The changes of AST activity were close to normal (from 85,0% to 109,1% of control level). These results showing slight hyperenzymaemia of hepatic cytosol enzymes but not mitochondrial, are in general concordance with data[5], qualifying 2,4-D as herbicide of small toxicological consequences. At the same time the serum activity of GGT was slowly but constrantly increased for all the period. Together with elevated LDH level[6] this can also reflect damage-repair processes in hepatocytes in conditions of 2,4-DA toxic action.

Fig.1. Serum activity of ALT, AST and GGT in rats poisoned by herbicide 2,4-DA

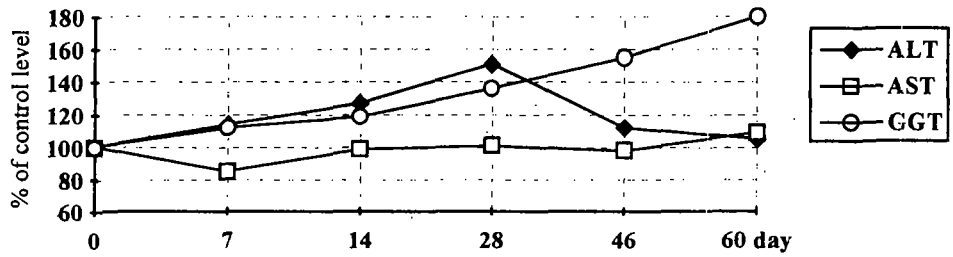
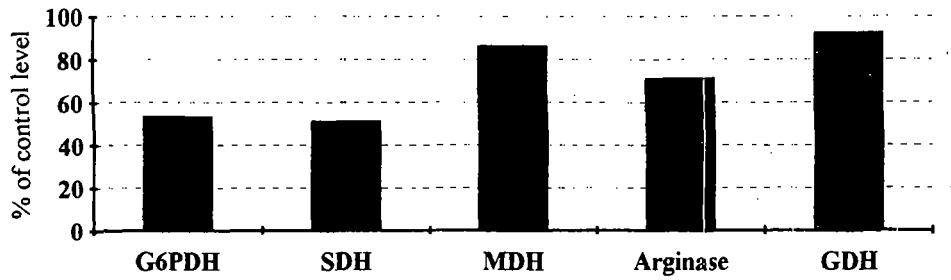


Fig.2. Enzyme activity in liver gomogenate in rats poisoned by herbicide 2,4-DA



At the same time, the activity of enzymes in liver homogenate was changed more significantly. G6PDH - the key enzyme of apothomic glucose cycle - had been reduced till 52,8% by the end of intoxication period. This may result in depression of both microsomal oxydation (and xenobiotics' detoxication) and biosynthetic reactions by the reason of cell NADPH pool depletion. To approve these suggestions the significant increase of hexenal-induced sleeping time in poisoned rats was found (1,6 to 1,7 times on days 7 and 14 of intoxication). Parallel inhibition of SDH (to 50,9%) and MDH enzyme activity (to 85,6% of control level) may cause the decrease of NADH pool and directly influence upon the cell energy metabolism[6]. The cytosol activity of arginase was also reduced to 70,7% of control level. Most of these enzymes are thyroid-dependent, and one of the reasons of its decrease may be the reduction of thyroid hormone level we previously determined[4]. So we can suggest the correction of liver metabolic disorders by stimulation of thyroid gland and/or administration of thyroid hormones is to be principally possible.

The GDH activity in LD_{50} poisoning was slightly decreased on day 28 (to 91,5%), though in 1/10 LD_{50} its level was much higher (166% of control). We suggest it as compensative elevation accompanying amino acid and xenobiotics metabolism induction in slight 2,4-DA intoxication, when higher doses of toxicant probably could cause some inhibition of GDH and/or its synthesis.

Fig.3. Changes of PNC properties in rats poisoned by herbicide 2,4-DA

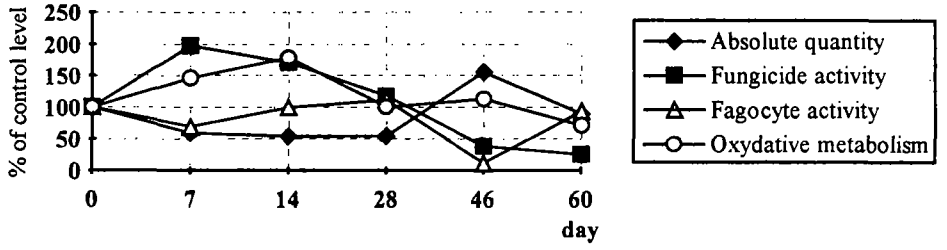
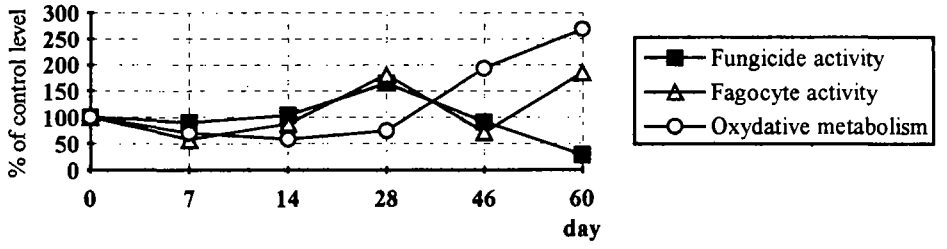


Fig.4. Changes of MNC prorerties in rats poisoned by herbicide 2,4-DA



On day 7 of intoxication a deep decrease of PNC quantity (to 59,4%) was found accompanied with the elevation of its fungicide activity (to 197% of control level) and

activation of oxygen-dependent reactions revealed by NBT-test[7,8]. Contralaterally, the MNC fungicide activity was not changed significantly and NBT-test levels were even decreased. Same tendention occured on day 14 and day 28 of experiment, but the fungicide activity of MNC (macrophages) to the end of intoxication period became elevated together with some inhibition of oxydative cell reactions by NBT-test.

On day 46 of experiment (18 days after toxicant cancel) the quantity of PNC was highly elevated from leucopenia to leucocytosis (up to 155,4% of control) but fungicide activity of these cells became low (37,6%), and its oxydative metabolism level together with fagocyte activity were falled down to 10 - 12% of control. The fungicide activity of MNC was also slightly changed near the control level, but some activation of oxydative MNC reactions in NBT-test was found. On day 60 of experiment (32 days from the end of intoxication) the quantity of PNC was returned to normal level, but its fungicide properties and NBT-test level stayed reduced. It seems to be important that the fungicide activity of MNC on day 60 was also decreased (28,6% of control level), and the oxydative metabolism (after the results of NBT-test) and fagocyte abilities of macrophages were contralaterally elevated.

Thus, the 28-day intoxication of rats by 2,4-DA at total dose LD_{50} causes deep leucopenia followed by leucocytosis on day 46 of observation. This leucocytosis was found to be accompanied with significant fall of fungicide and fagocyte activity and oxydative metabolism. These phenomena may be either a result of young leucocytes uptake to blood or cumulative influence of toxicant on phagocyte predcessors[7]. Returning PNC quantity to normal and staying of PNC and MNC oxydative reactions' deppression on day 60 is shown to indicate the progressive disfunction of phagocyte system.

The disorders of blood cell immune properties and changes of liver enzyme activity levels that were found out in rats in condition of experimental 2,4-DA intoxication testify the immuno- and hepatotoxic action of herbicide and the development of disregulation status as a whole. It seems to be a significant chain in pathogenesis of phenoxy herbicide toxic action and can be a basis for immunomodulation and possible administration of hepatoprotective drugs.

References

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