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STUDY OF IMMUNOBIOLOGICAL PERFORMANCE ACTIVITIES IN CHRONIC POPTICIDE INTOXICATION WITH METSULFURON-METHYL

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Abstract

The purpose of the present study was to determine the effect of pesticide methylsulfuron-methyl (MSM) on the synthesis of interferon in human and animal cell culture in vitro experiments, as well as to study the effect of the domestic immunobiological preparations "Inducer of Liquid Bacterial Interferon" and "Antivirin-M" on the course of lethal viral infection in vivo, on a model of chronic MSM intoxication of mice.

Materials and methods. In laboratory mice with chronic MSM intoxication the interferon titer was determined by conventional methods with and without administration of drugs "Inducer of liquid bacterial interferon" and "Antivirin M". In the experiments, in mice with chronic MSM intoxication and infected by mice encephalomyocarditis virus (EMC) cultured in mouse fibroblast cells L_{929}, interferon and the cumulative mortality of animals were determined. The statistical processing of the results was carried out using the STATISTICA software package. Differences of values from the control at $P<0.05$ were considered as significant or authentic.

Results. Studies have shown that there is a direct correlation of immunosuppressive action of the pesticide to his administration: a high concentration of MSM leads to a stronger suppressive effect of the pesticide on the level of interferon synthesis in experimental animals. There are differences in the immunosuppressive effect of MSM on the activity of interferon synthesis in human and mice cells. Cells of murine fibroblasts showed somewhat greater resistance to toxic effects of MSM. The administration of "Liquid Bacterial Inducer of Interferon" and "Antivirin M" to mice with chronic MSM intoxication, not only helped to protect animals from the immunosuppressive action of the pesticide, but also stimulated the synthesis of interferon, and contributed to a twofold decrease in the cumulative mortality of animals from lethal viral infection on the background of chronic MSM intoxication.

Conclusion. The administration of drugs inducing the synthesis of interferon during chronic pesticide intoxication leads to the activation of antiviral resistance, and is also able to protect the body from the toxic effects of pesticides

Keywords: *pesticides, metsulfuron-metil, immunobiological preparations, interferon, interferon inductors, viruses.*

Introduction

At present, pesticides are widely used to intensify agricultural production in order to increase crop yields.

The content of pesticides and their transformation products in tissues and organs reflects the degree of risk to the human body (Hoppin JA et al, 2006; Benachour N et al, 2007; Tarazona JV et al, 2017). For example, the consumption of animal and vegetable food products with organophosphorus pesticide residues (even in acceptable residual amounts) can lead to adverse effects on the organism for inhibiting the activity of the cholinesterase enzyme, which is one of the key enzymes of blood and tissues, resulting in the accumulation of acetylcholine in synapses Peripheral nervous system. Prolonged intake of pesticides with food can lead

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to chronic poisoning of the body, which is closely related to the problems of nonspecific morbidity, carcinogenicity and allergic conditions (Thomas PT, 1995; Parks ChG et al, 2014; Lebov JF et al, 2015; Gangemi S, et al 2016). Endocrinological disorders caused by residual amounts of pesticides can be a consequence of a carcinogenic effect (Khamitova RYa, Imamov AA, 2006; Shevchenko MG, Sharina EG 2006; Panina NK, 2010).

Currently, methylsulfuron-methyl, as a pesticide, is widely used (EU Pesticides Database, 2017). The effect of this pesticide and its metabolites on animals and humans has been studied, but not enough (Botham PA, 1995, Lee YT et al, 1999; Samanta P et al, 2015; Zhao L et al, 2015; Bonner MR et al, 2017). At the same time, the issue of the effect of using immunobiological drugs for chronic poisoning with pesticides is not well understood (Arndt V et al, 1999; Elhalwagy MEA et al, 2015).

The purpose of the present work was to study the effect of methylsulfuron-methyl (MSM) on the production of interferon in human and animal cell culture in vitro experiments, as well as the effect of the domestic immunobiological preparations "Liquid Bacterial Inducer of Interferon" and "Antivirin-M" on the course of lethal viral infection in vivo, on a model of chronic MSM intoxication of mice.

MATERIAL AND METHODS

Metsulfuron-methyl (MSM) is a herbicide, derivative of sulfuron-urea (0.600 WG. 2012-02-21 Polgar agrochem., Ltd. Herbicide; Monitor WG). The required concentrations of MSM were used prepared ex tempore by dissolving dry granules in physiological saline.

Study protocol. To study the effect of MSM on the interferon production process, a model of chronic pesticide intoxication in laboratory mice was developed. To this end, 3 groups of laboratory mice were selected - only 60 white mice with 25-30 g weight:

The 1st group (control) received the placebo.

The 2nd group - injected the drug "Antivirin M" per os of 0.5 ml daily.

The 3rd group - injected the drug "Liquid Bacterial Inducer of Interferon"(LBII), 1.0 ml, subcutaneously, every other day.

In parallel, all mice of the three groups were administered MSM at a dose of 0.05 mg / ml within 15 days.

After 15 days, the mice of all groups were injected with the encephalomyocarditis virus (EMC) of mice, 1 LD_{50} (50% lethal doses of the virus), followed by observation of the animals.

Cell cultures. A human RD cell line was used, a transfected L_{929} murine fibroblast cell line, primary trypsinized mouse embryo fibroblasts (MEF) grown in plastic bottles of 25 cm^2 (Becman Dickinson) at a density of 104 cells/ml at 37° C for 24 hours in a humidified atmosphere containing 5% CO_2. To remove the cells, a solution of versene (0.02%) (PanEco) with trypsin (50 mg) (Samson-MED) was used. Cell cultures were stored in liquid nitrogen with the addition of cryoprotectant, 7.5% DMSO.

Nutrient media. Mediums 199 and Eagle's MEM ("SIGMA") (1: 1) with L-glutamine ("Sigma") and antibiotic gentamycin ("Gibco") enriched with growth components were used for culturing the cells: 10% calf embryonic ("LONZA", or bovine serum ("PanEco").

Viruses and inducers of interferon. The mouse encephalomyocarditis virus (EMC) was cultured in murine fibroblast L_{929} cells. The virus was used in experiments to determine interferon and determine the cumulative mortality of animals. EMC was used with infectious titre 4.5-5.5 lg $TCPD_{50}$ (50% of tissue cytopathogenic doses), and also adapted to RD cells.

The vaccine strain of the virus of Newcastle disease (VND), La Sota/46 (RF, Omsk) was used to induce α/β-interferon in cell culture. The virus was grown on chick embryos with an infectious titer of 5.5-6.0 lg (EID_{50}.). Viruses were stored in liquid nitrogen with addition of 7.5% DMSO cryoprotectant.

Immunobiological preparations. The domestic author's preparation "Liquid Bacterial Inducer of Interferon" (LBII) with a universal antiviral, immunomodulating and antitumor effect (State Registration of the Republic of Kazakhstan, AND 42-33-68-11 of 9.01.2012) was developed in the laboratory.

Antivirin M. The drug is an author's development received in the laboratory, has the property of stimulating interferon production (Aspetov DR.2001) Mice were administered per os a liquid solution prepared from a lyophilized dried series No 31/5.

Determination of interferon. Interferon was determined by a micro-method in 96-well plastic panels ("BD Falcon") with a monolayer of homologous cell cultures RD, FEM, L929 (Ershov FI, 1996).

Different dilutions of MSM were applied to the cell monolayer, in the culture medium (from 0.00002 to 0.005 mg/ml). The cells were incubated at 37 ° C for 24 hours, after which the MSM-containing medium was removed from the wells and 10^5 EID_{50} VND were injected as an interferon inducer.

After additional incubation of the cells at 37°C in a humid chamber containing 5% CO2, samples of the culture medium were obtained. After appropriate treatment, the samples were used to study the activity of interferon.

For the interferon titer, the greatest dilution of the interferon sample was taken, causing a 50% suppression of the cytopathogenic effect of the EMC test virus in homologous cell culture ($TCPD_{50}$).

The statistical analysis of the results was carried out using the STATISTICA software package. Differences of values from the control at $P<0.05$ were considered as significant or authentic

Bioethical considerations. The design and protocol of experimental studies were approved by the local bioethical committee of the Research center for Hygiene and Epidemiology named after Kh. Jumatov.

Results

The study of the effect of various concentrations of MSM on the synthesis of interferon in human cells RD. The results of interferon titration in RD cell samples show that treatment of the cell culture with various concentrations of MSM for 48 hours resulted in inhibition of the interferon synthesis process. Minor suppressive action of MSM was detected even when applying 0.00002 mg/well. Here, the activity of interferon was 890±58.2 U/ml, against a background of 930±74.6 U/ml in control samples. As the concentration of MSM increased in RD cells, the activity of interferonogenesis decreased. Interferon production in cells exposed to 0.005 mg/ml of MSM was 14±6.2 U/ml compared with a dose of 0.00002 mg/ml, which causes little immunosuppression.

The maximum concentration of MSM, which does not cause destructive effects on the cells, was 0.005 mg/ml. Similar results were obtained on mouse fibroblast cells (both transfected and primary-trypsinized), but we found differences in the immunosuppressive effect of MSM on the activity of interferon production in human and mouse cells. Cells of murine fibroblasts showed somewhat greater resistance to toxic effects of MSM. At a dose of 0.005 mg/ml, the level of interferon in mouse cells decreased by 15%, while under the influence of MSM on the culture of human cells at the same dose, the level of α/β-interferon production was reduced by 25%.

The study results showed that there is a direct correlation between the suppressive action of the pesticide and its dose: a higher concentration of MSM led to a stronger suppressive action of the pesticide on the level of interferon production.

Study of the effects of immunobiological preparations LBII and "Antivirin M" on the course of a viral infection in chronic MSM intoxication. To study the effect of MSM on the production of interferons, a model of chronic pesticide intoxication was developed in mice.

The results showed that in the control group of mice that did not receive LBII and Antivirin-M preparations, there was a twofold increase in the death rate as a result of the introduction of the lethal EMC virus. Thus, from the first day the loss of animals began, where on the 10^{th} day 19 animals died, while in the group of mice that received Antivirin-M, only 12 animals died in 10 days, and in the group that received LBII for the same period 10 animals died.

The observation showed a twofold decrease in the cumulative mortality of animals in the experimental groups to which the medications were administered, in comparison with the animals that did not receive the drugs (control).

Discussion

The question of the effect of pesticides on the interferon system remains poorly understood. In previous studies we found suppressive effect of low dose organophosphorus systemic herbicide glyphosate to interferon-producing function of transplanted human cell cultures (Aspet DR et al., 2012)

MSM also applies to new generation systemic herbicides, to which genetically modified crops are not susceptible. MSM is used in the processing of cereal crops for the destruction of weeds. In the available literature, no information found on the effect of MSM on synthesis of interferon in the body of humans and animals. Toxic effect of MSM is based on the inhibition of intracellular enzymes that ensure the growth and development of plants (Lokhin KB, 2007; Ning N et al, 2015).

At low application rates, the MSM is considered to be low-toxic to the environment. As the important part of the pathogenetic action of systemic herbicides is an enzymopatic effect, to overcome their toxic action, the use of drugs that enhance the activity of intracellular enzymes seems appropriate. As such means immunobiological preparations could serve. They increase the synthesis of interferon, which in turn stimulates the activity of the enzymes (2/5/-oligo-adenylate synthetase) involved in the intracellular biosynthetic processes. This problem points to the need to develop methods for overcoming the inhibitory effect of pesticides on the body.

Parenteral administration of the LBII or oral treatment of mice with the "Antivirin M" during their long-term injection of MSM pesticide not only protects the mice from the immunosuppressive action of the pesticide, but also restored the ability of the animals to the endogenous production of interferon, protecting them from damaging effects of MSM. Administration of drugs that induce the synthesis of interferon in the course of chronic pesticide intoxication is resulted in 50% reduction in cumulative mortality of animals from viral infection compared to controls that did not receive therapeutic drugs.

CONCLUSION

Thus, the data obtained in our studies show that there is a direct correlation between the immunosuppressive effect of a pesticide and its dose: a higher concentration of MSM led to a stronger suppressive effect of the pesticide on the level of interferon synthesis in experimental animals. There are some differences in immunosuppressive effect of MSM on the activity of interferon synthesis in human cells and mice. Cells of murine fibroblasts showed somewhat greater resistance to toxic effects of MSM than human.

Factors or medications stimulating interferon formation in cell culture, in humans and animals, and which contribute to the activation of antiviral resistance, are also able to protect the body from the toxic effects of pesticides, in particular, methsulfuron-methyl.

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