INFLUENCE OF CHRONIC INTOXICATION WITH THE ORGANOPHOSPHORUS PESTICIDE ANTIO ON THE ACTIVITY OF RAT LYMPHOCYTE ACETYLCHOLINESTERASE

Khaydarova Dilorom Safoevna
Samarkand State Medical University, Department of Pathophysiology
https://doi.org/10.5281/zenodo.7875743

Abstract. *Chronic poisoning of rats with the organophosphate pesticide antio at doses of 3.5 mg/kg/day and 17.5 mg/kg/day (1/100 and 1/20 LD50, respectively) in the experiment revealed the presence of acetylcholinesterase activity in their lymphocytes. The results of the experiment showed that the activity of acetylcholinesterase is not the same in different subpopulations of lymphoid cells. The revealed dose-dependent inhibition of acetylcholinesterase in rat lymphocytes during chronic administration of the organophosphorus pesticide antio suggests that there may be a previously unknown mechanism of the immunosuppressive effect of organophosphorus compounds, implemented through the interference of these drugs in the processes of neurotransmitter regulation of the functions of lymphoid cells.*

Keywords: *organophosphate pesticides, chronic poisoning, rat lymphocytes, acetylcholinesterase activity, acetylcholinesterase inhibition.*

Introduction. The widespread use of pesticides in agriculture has led to the problem of the harmful effects of these substances on human health. Among the mechanisms that cause a decrease in resistance and immunosuppression in humans upon contact with pesticides, great importance is attached to the toxic effect of these drugs on internal organs, metabolic disorders and the mechanisms of neurohumoral regulation of the body's defense reactions [1–6, 8]. It is known that human lymphocytes contain on their membrane not only receptors for acetylcholine, but also the enzyme acetylcholinesterase (AChE) [3]. These data give grounds to consider acetylcholine, released by nerve endings into the microenvironment of lymphoid cells, as a natural factor in the regulation of their activity, and AChE of lymphocytes as a factor limiting the immunomodulatory effect of the neurotransmitter. As a result, the question arose about the possible connection of the immunosuppressive effect of pesticides with their acetylcholinesterase activity and the violation of the physiological mechanisms of cholinergic regulation of the functions of cells of the body's immune system.

The aim of this work was to study the effect of chronic administration of the widely used organophosphorus pesticide antio on the AChE activity of rat lymphocytes in primary and secondary lymphoid organs.

Material and Methods. The experiments were carried out on male Wistar rats weighing 180-240 g. Animals for 2 months daily received an oral solution of the pesticide Antio at doses of 3.5 mg/kg/day and 17.5 mg/kg/day (1/ 100 and 1/20 LD50, respectively). Control animals simultaneously received an equal volume of solvent. Antio in its original form does not have an anticholinesterase effect, however, in the body it easily undergoes oxidative desulfurization and, turning into a P-O derivative, becomes a strong inhibitor of AChE [6]

At the end of the inoculation period, the animals were sacrificed, and the thymus and spleen were isolated. Suspensions of lymphocytes were obtained using conventional methods (gentle crushing of the organ, filtration through a nylon filter, centrifugation in a density gradient of ficcol-verografin with a specific gravity of 1.090, washing the suspension in cold buffered phosphate saline with pH 7.2). The content of lymphocytes in the suspension was counted. Then, suspensions of lymphocytes from experimental and control animals were homogenized in Potter homogenizers. In a lymphocyte homogenate, the activity of AChE was determined separately using acetylcholine as a substrate [8, 9] and nonspecific esterases (NSE), including carboxylestrase, for which p-nitrophenylacetate served as a substrate [7]. The data were processed statistically using Student's t-test.

Results. Chronic intoxication of rats with organophosphorus pesticide antio at doses of 1/100 and 1/20 LD50 did not affect the general condition and behavior of the animals; the experimental rats gained weight in the same way as the control animals. All rats taken in the experiment survived until the end of the experiment. When determining AChE in lymphocyte homogenates, a significant difference was found in the activity of this enzyme in thymus and spleen cells: the activity of AChE in splenocytes was 15–20 times higher than the activity of AChE in thymocytes. When NSE of lymphocytes was detected, not so significant differences were obtained between organs: the activity of NSE of splenocytes was, on average, 4 times higher than the activity of these enzymes in thymocytes (Table).

Table

Drug and dose	Thymocytes		Splenocytes	
	AChE	NSE	AChE	NSE
Control	1,30±0,30 (n=10)	15,5±4,1 (n=10)	29,7±5,3 (n=11)	67.3±8,5 (n=11)
Antio 3.5 mg/kg/day	0,90± 0,30 (n=9)	10,8±2,8 (n=9)	15,2±2,2* (n=9)	44,4±9,1 (n=9)
Antio 17.5 mg/kg/day	0,74±0,18 (n=9)	10,6±1,3 (n=9)	11,9±1,6* (n=9)	48,3±4,9 (n=9)

Note: * - statistically significant ($p<0.05$) difference from control; n is the number of animals studied

Discussion. The reasons for the difference in the AChE activity of thymus and spleen lymphocytes are currently unknown. The higher activity of the enzyme in homogenates of spleen cells, apparently, has little to do with the admixture of erythrocytes, since calculations have shown that such an admixture in the suspensions used can provide an increase in AChE activity by a maximum of 1.5%. Most likely, the revealed difference is due to the fact that the primary and secondary organs of immunogenesis, which are the thymus and spleen, contain different populations of lymphocytes. It is known that thymocytes are 95% immature T-lymphocytes, and splenocytes are a mixture of mature T- and B-lymphocytes [2].In this regard, it can be assumed that the high activity of AChE in splenic cell homogenates is due to the presence of B-lymphocytes in the suspension. On the other hand, T-lymphocytes, unlike thymocytes, are mature

immunocompetent cells; in the spleen, they come into contact with the antigen and are activated. Therefore, it cannot be ruled out that both maturation and activation of T cells can be accompanied by an increase in AChE activity on the membrane of these cells. The need to take into account this possibility is indicated by the data that the maturation or activation of T-lymphocytes by mitogens or antigens leads to an increase in the number of m-cholinergic receptors on their membrane [2, 3]. Thus, the available data suggest that higher splenocyte AChE activity may be associated with the presence of B-lymphocytes, mature T-lymphocytes, or activated T-lymphocytes in the spleen, which indicates the existence of differences in AChE activity in different subpopulations of lymphocytes. This issue requires targeted research.

Under the influence of antio, there is a decrease in the activity of AChE in splenocytes; for thymocytes, only a tendency to a decrease in the activity of this enzyme is noted (table). It should be noted that higher doses of the drug cause a more pronounced inhibition of AChE in spleen lymphocytes. As for the NSE, intoxication with a pesticide slightly reduced the activity of these enzymes (not statistically significant).

Conclusion. Thus, the experiments performed made it possible to reveal the presence of acetylcholinesterase activity in rat lymphocytes and show that AChE activity in different subpopulations of lymphoid cells differs significantly. The observed dose-dependent inhibition of AChE in rat lymphocytes after chronic administration of the organophosphorus pesticide Antio suggests the existence of a previously unknown mechanism of the immunosuppressive effect of pesticides, realized through the interference of these drugs in the processes of neurotransmitter regulation of the functions of lymphoid cells.

REFERENCES

1. Anuchina A.V. Toksicheskoe deystvie pesticidov na organism cheloveka i jivotnih (The toxic effect of pesticides on the human body and animals) // Mejdunarodniy studencheskiy nauchniy vestnik (International Student Scientific Bulletin). - 2019. - № 1. (in Russia)
2. Gerunov T.V., Redkin Yu.V., Gerunova L.K. Immunotoksichnost pesticidov: rol v patologii jivotnih i cheloveka (Immunotoxicity of pesticides: role in the pathology of animals and humans) // Uspehi sovremennoy biologii (Advances in modern biology). - 2011. - T. 131, № 5. - P. 474-482. (in Russia)
3. Zabrodskiy P.F. Immunotoksikologiya fosfororganicheskih soedineniy (Immunotoxicology of organophosphorus compounds). – Saratov. Izdatelstvo “Saratovskiy istochnik” (Saratov. Publishing house "Saratov source"). - 2016. - 289 p. (in Russia)
4. Kaloyanova-Simeonova F. Pesticidi. Toksicheskoe deystvie i profilaktika (Pesticides. Toxic effect and prevention). – Moskva: Medicina (Moscow: Medicine), 1980. - 304 p. (in Russia)
5. Xaydarova D. S. Pestitsidlar bilan surunkali zaharlanishda kortikosteron kontsentratsiyasi va spetsifik immunitet ko’rsatkichlarining o’zgarishi //Science and Education. – 2023. – T. 4. – №. 2. – P. 562-566. (in Uzbek)
6. Rozengart V.I., Sherstobitov O.E. Izbiratelnaya toksichnost fosfororganicheskih insektoakaricidov. Sravnitelno-biohimicheskie aspekti (Selective toxicity of organophosphate insectoacaricides. Comparative biochemical aspects). - L.: Nauka 1978. - 173 p. (in Russia)
7. Telyabaev Z. Svoystva holinesterazi i karboksilesterazi gangliev saranchi (Properties of cholinesterase and carboxylesterase of locust ganglia) // Biochemistry, 1979. – V. 44. – P. 2083. (in Russia)

8. Ellman, G. L., Courtney, K. D., Anders, V. & Featherstone, R. M. A new and rapid colorometric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **7**, 88–95 (1961).
9. Singh, K.D., Labala, R.K., Devi, T.B. *et al.* Biochemical efficacy, molecular docking and inhibitory effect of 2, 3-dimethylmaleic anhydride on insect acetylcholinesterase. *Sci Rep* **7,** 12483 (2017)
10. Wang X., Anadón A., Wu Q. et al. Mechanism of Neonicotinoid Toxicity: Impact on Oxidative Stress and Metabolism // Annu. Rev. Pharmacol. Toxicol. – 2018. – V. 58. – P. 471–507.