ORIGINAL ARTICLE

CHANGES IN THE STATE OF VEGETATIVE NERVOUS SYSTEM OF ALBINO RATS AT THE BACKGROUND OF CHRONIC ROUNDUP POISONING

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ABSTRACT

The aim: The aim of this research was the study of low dose roundup, a well-known herbicide, chronic poisoning on the state of the vegetative nervous system in albino rats.

Materials and methods: The state of vegetative nervous system was assessed by the method of variation pulsometry The two-week chronic roundup poisoning at a dose of 40 mcg/kg having been simulated on 30 albino rats.

Results: The chronic roundup poisoning was accompanied by impaired state of vegetative nervous system that revealed itself in the growing indices of variation pulsometry: tension index – 1.6 times (P<0.001), index of regulatory system activity – 1.52 times (P<0.001), vegetative balance index – 2.36 times (P<0.001), rhythm vegetative index – 1.39 times (P<0.001). Moderate regulatory system stress, requiring extra functional reserves to provide adaptation to environment, was observed. Such condition occurs in the process of adaptation to adverse environmental factors with impairing adaptive self-regulation mechanisms.

Conclusions: Internal two-week use of the roundup on albino rats in a dose of 40 mcg/kg is accompanied by functional disorders of vegetative nervous system, which reveal themselves in the growing values of variation pulsometry. The results obtained were indicative of prevailing vegetative system sympathetic division as compared with parasympathetic one, as well as of disordered regulation of vegetative nervous system tone.

KEY WORDS: chronic roundup poisoning, variation pulsometry, vegetative nervous system disorders

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INTRODUCTION

Statistically, glyphosate, an agent used for fighting weeds, is the most popular herbicide worldwide. About 800 thousand tons are produced and thereafter embedded into the soil annually [1]. The WHO has repeatedly issued warnings about the herbicide accumulating in body tissues, thus causing disorders in the functional state of the nervous system, heart, liver, and kidneys. Glyphosate increases the risk of tumours, infertility, and embryonic disorders in humans and animals manyfold [2]. First developed by Monsanto (USA) in 1974, glyphosate is an analogue of glycine amino acid, capable of blocking EPSP synthase enzyme activity that is accompanied by inhibition of aromatic amino acid synthesis and, consequently, protein and chlorophyll synthesis, ultimately resulting in the death of plants. This agent is an acting base of the roundup (R), a well-known herbicide. In time, weeds develop resistance to R, so necessitating repeated use of the herbicide. As regards the frequency of use, R was 17th in the world rating in 1987 and gradually rose to ranking 1st in 2001. The annual requirement for R is about 500 thousand tons, and sales in 2011 reached $5.6 billion [1].

Herbicide manufacturers constantly stress the need for the agricultural use owing to the assumptive risk of $1.4 billion losses for the EU in case the herbicides are prohibited. Of all EU countries, France is the most categorical and definite. According to the president Emmanuel Macron statement, within the next three years the country is going to prohibit the use of glyphosate in the fieldwork.

The growing use of R world-wide taken into consideration, the issue of researching low dose chronic poisoning pathogenesis is taking on great importance. Pathogenesis is often accompanied by the disorders of the vegetative nervous system [3], which aggravate pathological state by making its treatment more complicated. So far, no research report on the effect of acute or chronic glyphosate and R poisoning is available.

THE AIM

The aim of this research is the study of low dose chronic R poisoning on the state of the vegetative nervous system in albino rats.

MATERIALS AND METHODS

30 pubertal albino male rats (180-220 b.w.) were used. Strict adherence to the regulations of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) was provided [4]. The rats were kept

in Fengshi plastic cages, 5 in each, on the litter of wooden chips. Light conditions: 12 hours – light, 12 hours – dark. Temperature regime – 19-25 ° C. Relative humidity – 50-70%. Temperature and humidity indices were taken daily. Ventilation was controlled with the anemometer and by measuring carbon dioxide and ammonia air content. Ventilation regime, providing 15-room volume per hour, carbon dioxide concentration up to 0.15 volume percent, and ammonia – up to 0.001 mg/l, was set. The rats were fed twice a day, water available ad libitum.

The animals were blindly randomized into 2 groups (n=15): 1 – intact animals; 2 – those which were given R for two weeks in a dose of 40 mcg/kg. The reasons for the dose were as follows: maximum allowable R concentration in the water is 1 mg/l [5]; rat's daily need for water – 40 ml/kg [6]. Under this condition, the rats take 40 mcg/kg of R with water daily. That is the dose we have chosen for the research. The state of vegetative nervous system was assessed by the method of variation pulsometry (VP) [7]. For this, a computer program (PULS), capable of recording time lags between ECG QRS spikes, was used. The program analyses the values automatically, presenting results in the form of conventional histogram. The program operates with a peripheral device, connected to the port (LPT1). The device consists of a timer, generating impulses (1 msec., 1000 Hz), a unit that normalizes input signal from the sensor to the standard logical level of 50 msec. an electrocardiograph serving as signal transmitter. Measurement accuracy for time intervals is not less than 5%.

Standards for the measurements and physiological interpretation of vegetovascular regulation were used [8]. The Neurosoft "Vita Rhythm" computer system, electrocardiographic complex "МКА 01" and rheographic device "РПІКА 2-01" with the cardiographic canal "МЕДАСС" were used. Attached to the computer, all the mentioned complexes provide formation of dynamic series of cardiointervals with digitization frequency of electrocardiographic signal up to 1000 Hz and over. Measurement accuracy for RR-intervals was ± 1ms.

Strict adherence to the regulations of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the directive 2010/63/EU of the European parliament and of the council on the protection of animals was provided.

The digital data obtained were processed by variation statistical method using Mann–Whitney test and single-factor dispersion analysis (ANOVA). Mean arithmetic value (M), mean arithmetic errors (m), variation coefficients, and mean quadratic deviations were calculated. Changes were regarded as reliable at $p \leq 0.001$. The significance level in the tables was specified for reliable results only. MicrosoftExcel XP (USA) and StatsoftSTATISTICA program was used for calculations.

RESULTS

Today, VP is among the most informative and non-invasive methods of studying the state of vegetative nervous system, of regulatory mechanisms general activity, as well as of the correlation between sympathetic and parasympathetic divisions of vegetative nervous system [9]. When analysing VP, fluctuations of cardiac intervals size are regarded as an effect of multicircuit, hierarchy and multilevel system for physiological functions control. Dynamic series of cardiac intervals are supplied digitally. At that, changes of the cardiac rhythm are regarded in relation to the adaptation response of integral organism, as a manifestation of different phases of general adaptation syndrome.

As a component of multiparametric interaction, the heart reacts to any change in homeostasis. When performing VP on the basis of cardiac intervals succession, the state of heart control system can be judged by the variations of their length. To calculate VP indices, a computer program in use constructs a variation curve of cardiac intervals distribution – histogram, – and establishes its basic characteristics: M (mode), MA (mode amplitude), and MxDMn (variation range). VP primarily makes possible to assess the functional state of sinus node, the latter being regarded both as an indicator of cardiac muscle automatism and as an index of vegetative nervous system state on the whole.

Four most informative variation pulsometry indices were determined: index of regulatory systems activity (IRSA), index of vegetative balance (IVB), vegetative rhythm index (VRI), and tension index (TI) [10].

Chronic R poisoning caused reliable changes of most VP indices (Table I).

Chronic R poisoning caused heart rate increase by 34 beats per minute in all rats, as well as reliable 1.52 times increase in IRSA ($P<0.001$). Meanwhile, the tension index at the background of chronic R poisoning was 1.60 times the value of intact animals ($P<0.001$), whereas the index of vegetative balance was 2.36 times the value of intact rats ($P<0.001$). The use of the roundup resulted in 1.39 times increase of the vegetative rhythm index ($P<0.001$).

IRSA values are expressed in points – from 1 to 10. Our finding (IRSA increase to 3.23 points) indicates, in accordance with recommendations of the clinical use of the method, the state of moderate regulatory systems tension and a need for additional functional reserves for adaptation to the environment. Such states (IRSA=3-4) occur in the process of adaptation to adverse occur ecological factors [8].

Tension index makes possible to differentiate the degree of regulatory systems tension and to evaluate systemic adaptation capabilities. It is calculated in points by a special algorithm including statistical indicators, histogram indices, and findings of cardiointervals spectral analysis. Index of regulatory systems tension reflects the degree of centralization in cardiac rhythm regulation and characterizes the activity of sympathetic regulation mechanisms and functional state of the central control circuit. Activation of the central control circuit is accompanied by enhancement of the sympathetic regulation under mental or physical loads and by decreased variability of cardiac intervals length as well as in the increased amount of single-type intervals, the histograms narrowing and increasing in height.

Normally, the tension index is within 80-150 standard

Table I. Effect of roundup on the VP indices in the rats with chronic R poisoning.

Group	Heart rate, beats per minute	Tension index, standard units	Index of vegetative balance, standard units	Vegetative rhythm index, standard units	IRSA, standard units
Intact rats	299.7±6.2	110.1 ±4.6	0.99±0.05	0.53 ±0.03	2.12±0.07
Roundup, 40 mcg/kg for 2 weeks	333.5±7.8	176.3,4±7.4*	2.34±0.62*	0.74±0.10*	3.23 ±0.09*

Note: *-reliable index changes (P <0.001) regarding intact animals.

units, being extremely sensitive to the enhanced tone of sympathetic nervous system. A slight load (physical or emotional) results in 1.5-2 times increase in the tension index, whereas major loads lead to 5-7 times increase. In case of essential tension of regulatory systems, the tension index rises to 600 standard units. We observed 1.60 times increase in the tension index. Such changes are considered to be the proof of impaired balance between anaerobic and aerobic ways of energy supply to the organism [7].

The VRI obtained is also indicative of prevailing tone of the sympathetic division over parasympathetic one, as well as of the disorders in the regulation system of the tone, imbalanced ratio of aerobic and anaerobic ways of metabolism, imperfect circulation regulation, and regulation failure of energy metabolism aerobic mechanisms.

DISCUSSION

In summary, it can be argued that the used simulation of low dose chronic R poisoning causes regulatory vegetative systems disorder, revealing itself first of all in the moderate activation of sympathetic division. Disbalance between the tones of sympathetic and parasympathetic divisions of vegetative nervous system occurs. Obviously, the used model of chronic R poisoning exposes an organism to a certain stress. According to current sanitary standards, the use of R in a dose of 40 mcg/kg should have been harmless and non-toxic. However, it is not like that. Our findings indicate adverse effect of two-week R administration in a dose of 40 mcg/kg on the vegetative nervous system.

Our findings are essential in view of increasing annual use of R in the agriculture that results in the growing concentrations of the agent in water and foodstuff. Excessive amount of the compound, which is found in food, calls forth the risk of R's adverse effect on the human health. According to the European Organization for Food Safety, 0.025-5 mg/kg of the roundup have been found in various foodstuffs. Recent research are indicative of even higher R content in the food and fodder derived from GM plants owing to the intensive use of herbicides in the cultivation. Since 1995, when the fields had been first sown with GM soya, 30 g more of R/acre of crops were used each successive year. Eventually, in 2016 the use of R per sown unit area increased 2.5-fold [1].

The roundup capacity of causing oxidative stress even in small concentrations has been revealed by previous researchers of R toxicity mechanisms in the experiments on both cellular cultures and animals. This is due to its capacity of binding series of ions that leads to the impairment of mitochondrial function, disordered process of oxidative phosphorylation, and formation of numerous active oxygen forms. The most typical are the impairments of nervous tissue, brain, kidneys, and liver function [11]. A whole number of epidemiologic studies has revealed close correlation between R use and increased frequency of autism and senile dementia [12]. Previous studies have shown that R effect on the organism is largely due to its neurotoxicity. The roundup has been found to increase Ca (2+) inflow due to the activation of of NMDA-receptors and voltage-dependent Ca (2+) channels, leading to the oxidative stress and death of nerve cells [13]. The mechanisms underlying R neurotoxicity include CaMKII and ERK activation. Besides, R increases H-glutamate release into synaptic gap alongside with decreasing GSH content, increasing lipid peroxidation, excitotoxicity, and oxidative damage to tissues, primarily the nerve one [14]. Since the activity of vegetative nervous system is inseparably connected with the activity of lower brain sections, it can be assumed that the above-mentioned neurotoxicity mechanisms underlie the toxic effect of the roundup on the autonomic nervous system.

CONCLUSIONS

Internal two-week use of the roundup on albino rats in a dose of 40 mcg/kg is accompanied by functional disorders of vegetative nervous system, which reveal themselves in the growing values of variation pulsometry: tension index – 1.6-fold (P<0.001), index of regulatory systems activity – 1.52-fold (P<0.001), index of vegetative balance – 2.36-fold (P<0.001), and vegetative rhythm index – 1.39-fold (P<0.001).

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Conflict of interest:
The Authors declare no conflict of interest.

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