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N.I. Hnida¹, N.H. Lotysh¹, D.V. Usenko¹, I.M. Hnidoi², V.N. Kukushkin² Psychological testing in children with elevated blood lead level: the effect of spirulina

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The search for effective and safe remedies to reduce the negative effects of lead on a child's cognitive sphere is an urgent problem.

Aim — to analyze changes in psychological testing indicators in children with elevated blood lead level and the effect of spirulina on them.

Materials and methods. A total of 33 children aged 7 to 15 years were examined. The children were divided into 2 groups depending on the lead level in their blood: up to and including 49 µg/l (control group) and from 50 µg/l or more (main group). Statistical processing of the results was carried out by the method of variation series using the Student's t-test, and analysis of variance was carried out using the F-criterion.

Results. In children with elevated blood lead level, there was a significant increase in cognitive impairment (memory and attention) compared to control scores. According to WHO recommendations, nutritional intervention was selected to correct the identified changes. For this purpose, children of the main group received a course of spirulina according to the following scheme: 0.5 g twice a day for 10 days, then 1 g twice a day for 20 days. As a result, the level of lead in the blood of children in this group decreased: (62.64±5.73) µg/l, compared to the initial value (75.55±1.99) µg/l. Cognitive indicators also improved: the number of correctly reproduced images significantly increased and the time to switch attention decreased, compared to the indicators before taking microalgal drugs.

Conclusions. Spirulina has a neuroprotective effect in children with chronic lead intoxication. The analysis of variance confirmed this conclusion: the score on the number of correctly reproduced images according to criterion F was 4.58. The effect on the time of switching attention according to criterion F was 8.58.

The research was carried out in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the Local Ethics Committee of the participating institution. The informed consent of the patient was obtained for conducting the studies.

No conflict of interests was declared by the authors.

Keywords: lead poisoning, children, cognitive disorders, psychological testing, spirulina.

Психотестування в дітей із підвищеним рівнем свинцю у крові: вплив спіруліни

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Пошук ефективних і безпечних засобів для зменшення наслідків негативного впливу свинцю на когнітивну сферу дитини є актуальною проблемою.

Мета — проаналізувати зміни показників психотестування в дітей із підвищеним рівнем свинцю у крові і вплив на них препарату спіруліни.

Матеріали і методи. Обстежено 33 дитини віком від 7 до 15 років. Дітей було поділено на 2 групи залежно від рівня свинцю в їхній крові: до 49 мкг/л включно (контрольна група) і від 50 мкг/л і більше (основна група). Статистичну обробку результатів проводили методом варіаційних рядів з використанням t-критерію Стьюдента, а дисперсійний аналіз — за F-критерієм.

Результати. У дітей із підвищеним рівнем свинцю у крові встановлено достеменно збільшення когнітивних розладів (пам'яті та уваги) порівняно з показниками контролю. Згідно з рекомендаціями ВООЗ, для корекції виявлених змін вибрано нутритивне втручання. З цією метою діти основної групи отримали курс спіруліни за схемою: по 0,5 г 2 рази на день протягом 10 діб, надалі по 1 г 2 рази на день протягом 20 діб. Унаслідок цього рівень свинцю у крові дітей цієї групи знизився: (62,64±5,73) мкг/л, порівняно з початковим значенням (75,55±1,99) мкг/л. Покращились показники когнітивної сфери: достеменно збільшилась кількість правильно відтворених образів та зменшився час переключення уваги, порівняно з показниками до прийому препарату з мікроводоростей.

Висновки. Спіруліна має нейропротективну дію під час її застосування в дітей із хронічною свинцевою інтоксикацією. Дисперсійний аналіз підтвердив цей висновок: оцінка за критерієм F дії на кількість правильно відтворених образів становила 4,58, p<0,05. Вплив на час переключення уваги за критерієм F був 8,58.

Дослідження виконано відповідно до принципів Гельсінської Декларації. Протокол дослідження ухвалено Локальним етичним комітетом установи. На проведення досліджень отримано інформовану згоду батьків, дітей.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: отруєння свинцем, діти, когнітивні розлади, психотестування, спіруліна.

Introduction

Armed conflicts and military activities are associated with environmental pollution caused by metals, particularly lead [10]. In Ukraine, this problem has become especially urgent in the last two years. It is known that lead is included in the list of 10 most dangerous chemicals for children by the World Health Organization

(WHO) [9]. Even in low doses, this heavy metal can affect children's brain development and lead to cognitive impairment [3,7]. Therefore, it is necessary to find effective remedies to reduce the consequences of such exposure. Numerous *in vivo* studies have confirmed the potential of spirulina for brain health [11]. Spirulina, a filamentous microalga, is used all over the world as a nutraceutical dietary supplement. Recent studies have focused

on examining its chelating activity and antioxidant properties, especially as a candidate for protection against neurotoxicity caused by heavy metals [8].

The **aim** of the study – to investigate changes in psychological testing indicators when spirulina was used in children with elevated blood lead level.

Materials and methods of the study

A total of 33 children from a random sample aged 7 to 15 years were examined. According to the lead level in the blood, the children were divided into 2 groups: the Group 1 (control) – up to 49 µg/l, n=22; the Group 2 (main) – from 50 to 99 µg/l, n=11. According to the peculiarities of the course of pregnancy and childbirth in the mother, the age of the children, their sex, diseases that the children suffered from, education and social status of their parents, the groups were balanced. The indicators at the beginning and end of the observation were compared in each group.

Psychological testing was carried out using several techniques recommended by the Ukrainian Scientific and Methodological Center for Practical Psychology and Social Work [5], as previously described [3].

4 groups of tests were used: to study memory (short-term and long-term); to study attention; to study the peculiarities of the thinking process; and to study personality traits. Short-term memory studies were carried out by recognizing shapes and memorizing numbers and images. The assessment of the level of number recognition was carried out by deviation from the optimal value (1 unit) and expressed in points: 0 points – no deviation; 1 point – deviation up to 0.5 units; 2 points – deviation from 0.51 to 1.00; 3 points – deviation from 1.01 or more. Long-term memory was assessed by memorizing (from 3–5 attempts) 10 words and reproducing them after 1 hour. In examining attention, a proofreading test (Bourdon test), Schulte tables, and a red-and-black table were used. The Attention Switching Index (ASI) in the Bourdon test was calculated using the formula:

$$ASI = 100 - R_{er.} / R_{tot.} \times 100,$$

where $R_{er.}$ – the number of erroneously processed rows; $R_{tot.}$ – the total number of rows in this task.

The results were expressed as a percentage. Attention switching time was defined as the difference between the time spent working with the red-black table and the total time working with

the first and second Schulte tables. Children after the age of 9 were also tested to correct 10 errors in the text. To study thinking in children after 10 years of age, a test for finding simple analogies was used. The study of personality traits was carried out in children after 10 years of age using the Eysenck Personality Questionnaire.

The determination of lead concentration was carried out in heparinized venous blood by atomic absorption spectrometry with electrothermal atomization (ET AAS).

Statistical processing of the results was carried out by the method of variation series using the Student's t-test, and analysis of variance was carried out using the F-criterion. Differences at $p < 0.05$ were considered significant.

The research was carried out in accordance with the principles of the Declaration of Helsinki. The research protocol was approved by the Local Ethics Committee of an participating institution. The informed consent of the patient was obtained for conducting the studies.

The inclusion of children in the study was carried out with the informed consent of the children's parents after providing detailed information about the procedure and objectives of the work.

Results of the study and discussion

The results of the study are presented in Table 1. Analysis of the personality test (Eysenck's Personality Questionnaire) did not reveal any significant differences between the groups of children on the scales of introversion, extroversion and neuroticism. Therefore, its results were not included in Table 1.

As observations showed, the indicators of psychological testing in children depended on the level of lead accumulation in their body. Thus, in children of the Group 2 (main), who had a higher level of lead in the blood than children of the Group 1 (control), the coefficient of deviation from the optimal level of figure recognition was also higher. The Bourdon Proofreading Test revealed a slower switching attention in children of the Group 2 than in children of the Group 1. The children of the Group 2 made more mistakes when working with the red-and-black table, in contrast to the children of the Group 1 (control). The time of switching attention in the children of the Group 2 was longer than in the Group 1. The results obtained are consistent with the literature and our previous data [3,7].

To correct the detected changes, a nutritional intervention was chosen, according to WHO

Table 1

Indicators of psychological testing of children of different groups at the beginning of observation

Indicators	Group 1	Group 2
Deviations from the optimal level of recognition of figures, points	0.73±0.20	1.46±0.31*
Number of correctly rendered numbers	3.73±0.38	3.64±0.34
Number of properly rendered images	9.14±0.61	8.00±0.73
Number of memorized words reproduced after 1 hour	8.82±0.22	7.82±0.66
Attention span according to the Bourdon test, c.u.	126.41±21.30	103.02±26.54
ASI, according to the Bourdon test, %	83.14±3.92	67.50±4.87*
Average time to work with Schulte tables, s	60.07±5.46	59.69±8.84
Working time with the red and black table, s	221.52±13.06	255.24±24.17
Number of mistakes made when working with the red-and-black table	1.04±0.17	2.91±0.61*
Attention switching time, s	98.95±9.72	147.26±14.06*
Number of uncorrected bugs in the bug fix test	2.64±0.02 (n=22)	3.70±0.45 (n=10)
Number of errors in finding analogies	2.53±0.84 (n=17)	2.70±0.79 (n=10)

Note: * — the difference is significant ($p < 0.05$) with the indicators of the Group 1.

Table 2

Indicators of psychological testing of children of different groups at the end of observation

Indicators	Group 1	Group 2	Criterion F in Group 2
Deviations from the optimal level of recognition of figures, points	0.82±0.27	1.09±0.39	0.53
Number of correctly rendered numbers	3.95±0.35	4.36±0.36	2.15
Number of properly rendered images	9.54±0.46	10.64±0.89**	4.58**
Number of memorized words reproduced after 1 hour	8.54±0.31	8.91±0.28	2.32
Attention span according to the Bourdon test, c.u.	108.13±15.26	84.34±12.28	0.41
ASI according to the Bourdon test, %	78.84±4.64	69.02±6.71	0.03
Average time to work with Schulte tables, s	58.70±5.67	62.29±11.05	0.03
Working time with the red and black table, s	229.40±13.94	216.47±23.52	1.33
Number of mistakes made when working with the red-and-black table	1.14±0.21	1.91±0.25	2.30
Attention switching time, s	113.65±8.54	97.97±9.36**	8.58**
Number of uncorrected bugs in the bug fix test	2.23±0.44 (n=22)	3.00±0.47 (n=10)	1.16
Number of errors in finding analogies	2.71±1.09 (n=17)	1.60±0.54 (n=10)	1.32

Note: ** — the difference is significant ($p < 0.05$) with the indicators at the beginning of the observation.

recommendations [9]. For this purpose, children of the Group 2 received a course of spirulina according to the following scheme: 0.5 g twice a day for 10 days, then 1 g twice a day for 20 days.

After the use of spirulina, the lead level in the blood of children of the Group 2 decreased: (62.64±5.73) µg/l, $p < 0.05$, compared to the initial value (75.55±1.99) µg/l. This coincides with modern data on the sorption properties of microalgae [1,8].

In parallel with the decrease in the blood of children of the Group 2 (main), the level of lead has improved some indicators of psychological testing (Table 2).

As we can see, the children of the Group 2 had an increase in the number of properly reproduced images. In the same group of children, there was also a decrease in the time to switch attention. In the Group 1 (control), no significant changes

were found between the indicators at the beginning and at the end of the observation.

Positive changes in the indicators of psychological testing of children treated with spirulina can be explained primarily by the elimination of the causal factor — a decrease in lead load, as evidenced by a significant decrease in the lead level in the blood of the examined children.

According to the literature, one of the mechanisms of the negative effect of lead on the cognitive sphere is the oxidative stress caused by this heavy metal in brain cells [11]. By reducing lead levels, spirulina helps to slow down the process of excessive oxidation. This is confirmed by scientific sources and the previously obtained results [4,11]. It is due to this property that microalgae have a neuroprotective effect [11]. This effect is also facilitated by spirulina's ability to enhance antioxidant protection in the body, which further reduces the

manifestations of oxidative stress, according to the literature [2,6,11] and our preliminary results [4]. This, in turn, enhances the neuroprotective effect of spirulina.

Conclusions

The obtained results indicate an associative relationship between the lead accumulation in the child's body and indicators of psychological testing.

In children with elevated blood lead levels, a certain increase in cognitive impairment (memory and attention) has been established.

The use of spirulina has a positive effect on such indicators of the cognitive sphere as the number of properly reproduced images (which reflects the improvement of memory in the child) and the time of switching attention (which reflects the improvement in the mobility of the processes of the nervous system).

The neuroprotective effect of spirulina when used in children with lead intoxication is based on the elimination of the causative factor (lead accumulation) and, probably, the improvement of pro-oxidant-antioxidant homeostasis in brain cells.

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