European Review for Medical and Pharmacological Sciences 2021; 25: 880-889

Analysis of neurotransmitters associated with neuropsychiatric status in workers following lead exposure

X.-J. CHEN[1,2], X. WANG[1], S.-J. MENG[4], L.-J. ZHANG[1], L.WU[1], F.-Y. CAO[1], Y.-S. ZHANG[1,3]

[1]School of Public Health, North China University of Science and Technology, Tangshan, China
[2]GongRen Hospital of Caofeidian District, Tangshan, China, [3]Experimental Animal Center, North China University of Science and Technology, Tangshan, China, [4]Hongci Hospital of Tangshan, Tangshan, China.

Abstract. – OBJECTIVE: To explore the correlation between neuropsychiatric status and blood neurotransmitter in lead workers, and to provide theoretical basis for the prevention and treatment of lead workers.

SUBJECTS AND METHODS: The study applied cross-sectional survey, 74 occupational lead exposed workers in a battery factory in a city of Hebei province were selected as the lead exposed group, and 62 workers (non-lead workers) were selected as the control group. The occupational health symptoms questionnaire and health examination and POMS (Profile of Mood State, POMS) emotional test questionnaire were applied to investigate the nearly emotional status of the studied objects, ICP-MS was used to determine the blood lead level of all subjects, HPLC (High performance liquid chromatography, HPLC) was applied to determine the concentration of neurotransmitter in peripheral blood of all studied subjects, and all results were applied the Pearson's correlation analysis.

RESULTS: The blood lead concentration of the lead workers group (163.23±40.77 ug/L) was significantly higher than that in the control group (43.62±14.50 ug/L), and the difference was statistically significant. From the analysis of neuropsychiatric status, the neurological symptoms in the lead workers group were higher than that in the control group, among which the symptoms of sleep disturbance, dizziness, fatigue, numbness of limbs and dampness and coldness of limbs were more obvious. Among the symptoms of digestive system, the incidence of abdominal pain, abdominal distension, constipation and nausea and vomiting were higher. According to the POMS emotion questionnaire, the scores of 5 negative emotions and 1 positive emotion in the lead exposure group were higher than that in the control group, and the difference was statistically significant. Related to the control group, the concentration of neurotransmitters such as DA, 5-HT, GABA, Gly, Trp and Glu were statistically decreased, $p<0.001$. There was a negative correlation between neurotransmitters in peripheral blood and blood lead levels in lead workers, among which 5-HT had the greatest correlation with lead levels (r=-0.569, $p<0.001$). 5-HT and Trp were significantly correlated with tension-anxiety (T), depression-depression (D), anger-hostility (A), Vigor-hyperactivity (V), fatigue-inertia (F), and confusion-confusion (C). 5-HT, Trp and GABA were significantly correlated with the survey symptoms, among which, the sleep disorder, constipation and fatigue had most significantly positive correlation with 5-HT or Trp, r-value was respectively 0.373, 0.233 and 0.563.

CONCLUSIONS: Lead exposure not only causes the alteration of neuropsychiatric behavior of lead workers, but also changes gastrointestinal symptoms. Serotonin may be involved as the main neurotransmitter synthesized in intestinal, and the synthesis and metabolism may be regulated by intestinal flora.

Key Words:
Lead exposure, POMS, Neurotransmitters, Neuropsychiatric status.

Introduction

Lead was a kind of environmental heavy metal neurotoxin, which entered the body through respiratory tract, digestive tract or skin contact, and caused toxic side effects on various systems and organs of the body[1,2]. Lead exposure could lead to neurobehavioral changes, but there were few reports on the relationship between lead exposure and depressive behavior. Depression-like behaviors include anxiety, irritability, irritability, depression, delayed speech, slow action and memory loss. Baker et al[3] showed that when the

Corresponding Author: Zhang Yanshu, Ph.D; e-mail: Yanshu_zhang@163.com

blood lead value of workers was between 400-800 g/L, their language, motor and sensory function were impaired; Escalona et al[4] show that lead exposure was related to the emotional state of workers[4]. Bouchard et al[5] studied the levels of blood lead, depression and panic disorder in American adolescents with the CIDI scale, the results showed that the incidence of depressive disorder and panic disorder was 2.32 and 4.94 times with blood lead level above 21.1 g/L higher than those with blood lead level of 2-7 g/L[5]. However, Parkinson et al[6] showed that there was no correlation between cumulative lead level and current lead level and neuropsychological behavior of lead exposed workers. Whether low-level occupational lead exposure was related to depressive behavior or not remained to be further explored, and the pathogenesis of lead exposure and depression is not clear.

The occurrence and development of depression and anxiety were closely related to the changes of neurotransmitters in the central nervous system[7-9]. Gamma aminobutyric acid (GABA) is an important neurotransmitter in adult brain, which played a role through its related receptors. Romeo et al[10] showed that the expression levels of GABA in brain and peripheral tissue of patients with major depressive disease (MDD) were decreased through meta-analysis. In animal experiments, the rats with depression-like behavior injected with GABA receptor antagonists showed antidepressant effect[11]. Dopamine (DA), a precursor of norepinephrine (NA), was synthesized in the substantia nigra and ventral tegmental area of the midbrain and projects to the striatum, cortex and other brain regions[12]. It widely existed in the central and peripheral tissues. DA played a role mainly through the activation of receptors. DA could regulate the frequent release of neurotransmitters and the excitability of neurons through the heterologous synapses of DR's family[13]. Dopamine and its receptor disorders were associated with a variety of neuropsychiatric diseases and involved in the occurrence of depression[14-15]. 5-hydroxytryptamine (5-HT) was a neurotransmitter distributed in the central nervous system and gastrointestinal tract, which is regulated by intestinal flora. About 95% of human 5-HT was synthesized by enterochromaffin cells (ECS) and intestinal neurons in the gut, while the remaining 5% was synthesized in central 5-HT neurons[16]. 5-HT in the blood could also pass through the blood-brain barrier and entered the central nervous system. The content of 5-HT in the brain was closely related to depression[17].

Therefore, the analysis of neurotransmitters related to neuropsychiatric status of lead exposed workers was of a positive significance for the prevention and treatment of lead exposed workers.

Subjects and Methods

Subjects

In this study, the method of cross-sectional survey was used to select the occupational lead exposed workers of a battery factory in a city of Hebei Province as the lead exposure group, and the workers in a non-occupational lead exposure enterprise with gender, age, weight and diet balance as the control group. In this study, 79 lead exposed workers and 65 non-lead exposed workers were tested. After excluding the incomplete test items, 136 subjects were included in the final analysis, including 74 lead exposed workers and 62 control workers. The inclusion criteria and exclusion criteria were as follows:

Inclusive criteria: the workers in the control group were not exposed to lead smoke, lead dust and other toxic and harmful exposed factors, and their occupational exposure factors did not cause related damage of nervous system.

Exclusion criteria: patients with neuropsychiatric system and other related diseases who could not complete the test independently due to hand movement disorders or visual and auditory disorders; patients with diseases that have an impact on neurobehavioral function test, such as daltonism, color weakness, color difference; people with diabetes mellitus or serious abnormal blood glucose; those who have excessive drinking behavior and taking sedative psychotropic drugs within 4 hours before the test; before the test Major life events occurred in the past year that affected the subjects.

The survey was approved with the Ethics Committee of North China University of Science and Technology, and informed consent was obtained by the subjects.

Methods

General Survey

Basic information

According to the designed requirements and designed scheme of employee health questionnaire, the basic information of occupational work-

ers was investigated by trained investigators in a one-to-one way. The survey contents including age, marital status, education level, type of work and change, smoking or not, diet, alcohol consumption, past disease history, and frequency of using computer.

Occupation

Look up the age, gender, time of occupational exposure and work posts of the front-line workers who have been exposed to lead for more than 3 years in the battery factory.

Health symptoms

The symptoms of nervous system (including headache, dizziness, feeling fatigue and weakness, sleep disorder, wet and cold hands and feet, numbness of limbs, stability of holding things, stabbing sensation of distal limbs, hand and foot twitch) and digestive system symptoms (nausea/vomiting, constipation, abdominal pain/bloating, anorexia) were measured by self-made questionnaire. In this study, the number of symptoms in each group was more than or equal to 3, which were regarded as the symptoms of nervous system and digestive system.

Emotional Status Survey

The subjects were asked to use the latest state of State Questionnaire (POMS) to test their emotion level in the recent week. POMS questionnaire is mainly an emotion test item, which is divided into six emotion subscales, namely, Tension anxiety (T), Depression (D), Anger hostility (A), Fatigue inertia (F), Vigor activity (V), and Confusion bewilderment (C).

Scoring method: according to the five levels of "almost no", "a little bit", "moderate", "quite a lot" and "extraordinary", the score of each item is assigned 0, 1, 2, 3 and 4 points. The original score of each subscale is accumulated. The higher the score, the more obvious the corresponding emotional state. T, D, A, F and C reflect negative emotions, and the higher was the score, the worse was the emotional state. V reflects positive emotions, and the higher was the score, the better the emotional state.

Determination of Blood Lead

Taking venous blood,1 ml whole blood was taken into Eppendorf (EP) tube with 50 ul heparin sodium, 200 ul concentrated nitric acid was added, and digested by microwave digestion instrument. The digested light yellow transparent liquid was transferred to 5 ml Eppendorf tube, and the volume was fixed to 4 ml with ultra pure water. The lead content in blood was determined by ICP-MS.

Determination of Blood Neurotransmitters in Lead Exposed Workers

Neurotransmitters with Aminoacid and monoamine were determined by HPLC and HPLC-ECD.

Statistical Analysis

All data were measured in the form of 'mean±SD'. SPSS 23.0 (IBM, Armonk, NY, USA) and Graph Pad Prism 5.0 were used for numerical statistics and mapping. Pearson's correlation analysis, *t*-test was used to the comparison between the two groups, $p<0.05$ showed a statistically significant difference.

Results

Basic Information of Research Subjects

A total of 74 lead exposed workers were investigated, the ratio of the male to the female was 0.85:1, the average age was (36.76±4.34) years, the average length of service was (7.67±3.52) years, the education level was mainly high school education or below, accounting for 89.2%; the control group was 62 people, the ratio of the male to the female was 1.38:1, the average age was (34.15±9.78) years, and the education level was also dominated by high school and secondary school, accounting for 54.1% (Table I).

Analysis of Blood Lead Level of Subjects

Through the analysis of blood lead concentration, the blood lead level of lead exposed workers was 163.23±40.77 g/L, which was significantly higher than that of the control group (43.62±14.50 g/L), and the difference was statistically significant, $^{**}p<0.001$, (Figure 1).

Effect of Lead Exposure on Mental Status of Workers

Analysis of neurological and digestive system symptoms in lead exposed workers

Through the analysis of the mental status of lead exposed workers, the incidence of nervous system symptoms was higher than that of the control group, among which, sleep disorders, dizziness, feeling fatigue, limbs' numbness and wet

Table I. Basic condition of age, working age, gender, education degree and living habits of subjects..

Subject	Column	Control N=62	Lead exposure N=74	*p*-value
Gender	male	36 (58.1)	34 (45.9)	0.159
	female	26 (41.9)	40 (54.1)	
Education degree				
	secondary school and below	22 (35.4)	26 (35.1)	0.168
	High school	34 (54.8)	40 (54.1)	
	College degree and above	6 (9.7)	8 (10.8)	
Smoke	yes	20 (32.3)	30 (40.5)	0.318
	no	42 (67.7)	44 (59.5)	
Alcohol	yes	28 (45.2)	24 (32.4)	0.128
	no	34 (54.8)	50 (67.6)	
Working age		7.85 (3.2-12.5)	7.67 (4.15-11.19)	0.454
Age		34.15 (24.37-43.93)	36.76 (32.42-41.1)	0.283

cold of hands and feet were more evident; in the symptoms of digestive system, the incidence of abdominal pain, abdominal distension, constipation and nausea and vomiting were higher, as shown in Table II.

Analysis of neurobehavioral function of subjects exposed to occupational lead

The POMS emotional questionnaire of lead exposed workers showed that the scores of five negative emotions for "tension-anxiety", "depression-depression", "anger-hostility", "fatigue-inertia", and "confusion-confusion" of lead exposed workers were higher than those of the control group, and the score of positive emotions was lower than those of the control group, and the differences were statistically significant. These suggested that lead exposure could affect neuropsychiatric behavior, as shown in Table III.

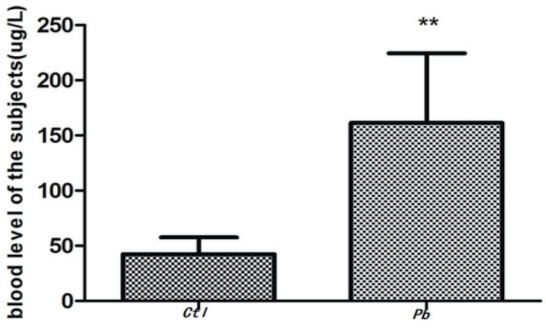

Figure 1. Content of blood lead in the subjects (mean±SD).

Effects of lead exposure on peripheral blood neurotransmitters in workers

Compared with the control group, the neurotransmitters DA, 5-HT, GABA, Gly, Trp and Glu in the lead exposed group were significantly lower than those in the control group (p<0.001, Table IV).

Correlation Between Neurotransmitters And Neuropsychiatric Status

Correlation between neurotransmitters and blood lead in lead exposed workers

There was a negative correlation between the levels of neurotransmitters and blood lead in lead exposed workers, and the correlation between 5-HT and lead level was the highest (r =-0.569, p<0.001).

Correlation analysis of neurotransmitters and emotional status scores

From the correlation analysis of neurotransmitters and emotional status, it is found from Table V that 5-HT and Trp are significantly correlated with tension-anxiety (T), depression-depression (D), anger-hostility (A), Vigor-hyperactivity (V), fatigue inertia (F) and confusion-confusion (C), p<0.001.

Correlation analysis of neurotransmitters and mental status

Table VI showed that 5-HT, Trp and GABA were significantly correlated with the investigated symptoms (p < 0.001), among which sleep disor-

Table II. Effect on nervous system and digestive system of subjects exposed to occupational lead (mean±SD).

		Control (n=62)		Pb (n=74)			
Subject	Symptom	n	Incidence (%)	n	Incidence (%)	χ^2	p
Neurological symptoms	Sleep disorders	4	6.5	18	24.3	7.947	0.005
	Languor	16	25.8	40	54.1	11.113	<0.001
	Dizzy	0	0	28	37.8	29.542	<0.001
	Headache	0	0	18	24.3	17.382	<0.001
	Brotherhood of raw	0	0	36	48.6	41.021	<0.001
	Limb numbness	0	0	30	40.5	32.249	<0.001
	Tingling in the distal extremities	0	0	16	21.6	15.193	<0.001
Digestive symptoms	Abdominal pain or bloating	0	0	18	24.3	17.382	<0.001
	Constipation	2	3.2	26	35.1	21.010	<0.001
	Nausea or vomiting	0	0	20	27.0	19.646	<0.001
	Anorexia	0	0	8	10.8	7.122	0.008

ders, constipation, and sensory fatigue were most significantly correlated with 5-HT or Trp, with R values of 0.373, 0.233 and 0.563, respectively.

Discussion

The healthy hazards of lead to occupational population were mainly chronic lead poison of lead dust or lead smoke and their compounds. Studies showed that there was no "safe" level of adverse effects of lead on nervous system function. At present, due to the improvement of production process and technology and the strengthening of protective measures, there were few cases of acute lead poisoning in occupational population. Clinical diagnosis of occupational chronic lead poisoning was based on the data of laboratory examination, employment history and clinical symptoms such as urine lead≥120 g/L, blood lead≥600 g/L[18], the main clinical symptoms were the comprehensive symptoms of nervous system, digestive system and hematopoietic system. Our results showed that the blood lead concentration of occupational lead- exposed workers was 163.23±40.77 g/L, which was significantly higher than that of the control group (43.62±14.50 g/L), which could lead to neurological toxicity, including the minor cases of neurological behavioral function and the severe cases of sustained damage to the central nervous system. Some studies also showed that lead exposure affected the executive ability, short-term memory ability and psychological emotion of lead exposed workers, POMS emotional test questionnaire results show that blood zinc protoporphyrin level was correlated with anxiety and depression in lead exposed workers[19,20].In this study, POMS emotional questionnaire was used to investigate, and the results showed that the emotional state and neuropsychiatric aspect of lead exposed workers were changed, mainly manifested as the increase of anxiety, depression, hostility, fatigue, confusion and other negative emotions. The incidence of digestive system symptoms in lead exposed workers was 31.1 times higher than that in the control group, including nausea and vomit-

Table III. Effects on neurobehavioral function of subjects exposed to occupational lead (mean±SD).

Neurobehavior	Index	Control (n=62)	Lead exposure (n=74)	t	p
mental status	Tension-anxiety (T)	3.81±2.98	10.22±5.63	8.475	<0.001
	Depression (D)	3.32±3.93	13.92±10.49	8.040	<0.001
	anger-hostility (A)	3.52±3.49	12.05±8.03	8.261	<0.001
	Vigor activity (V)	22.68±2.73	17.16±5.98	15.27	<0.001
	Fatigue inertia (F)	3.06±2.78	9.05±5.44	8.150	<0.001
	Confusion- Confusion (C)	6.00±2.55	10.65±3.89	8.360	<0.001

Table IV. Effect on neurotransmitter of subject exposed to occupational lead (mean±SD).

Neurotransmitter	Control (ug/L) (n=62)	Lead exposure (ug/L) (n=74)	t	p
DA	4573.16±694.78	4066.04±763.42	4.018	<0.001
5-HT	265.38±41.31	215.06±29.87	8.225	<0.001
GABA	2543.70±320.22	2127.25±483.09	5. 802	<0.001
Gly	2516.92±497.52	2164.88±578.62	3.363	<0.001
Trp	2686.35±484.73	1844.74±449.45	10.49	<0.001
Glu	1696.25±461.68	1199.40±401.97	6.708	<0.001

Table V. Correction of neurotransmitter and emotional status.

		T	D	A	V	F	C
DA	r	-0.127	-0.127	-0.069	-0.065	0.010	-0.201**
	p	0.071	0.071	0.227	0.454	0.010	0.089
5-HT	r	-0.267**	-0.202**	-0.210**	0.218**	-0.241**	-0.256**
	p	0.001	0.009	0.007	0.005	0.002	0.001
GABA	r	-0.036	-0.096	-0.078	0.276**	-0.122	-0.010
	p	0.339	0.132	0.184	0.001	0.079	0.452
Gly	r	-0.174*	-0.132	-0.126	0.127	-0.159*	-0.056
	p	0.021	0.063	0.073	0.071	0.032	0.260
Trp	r	-0.382**	-0.411**	-0.443**	0.202**	-0.355**	-0.373**
	p	0.000	0.000	0.000	0.009	0.000	0.000
GLu	r	-0.199*	-0.230**	-0.253**	0.170*	-0.138	-0.123
	p	0.010	0.003	0.001	0.024	0.054	0.076

ing, loss of appetite, abdominal pain, abdominal distension, constipation and metallic taste in the mouth. The incidence of constipation was 10.96 times higher than that in the normal group. It suggested that the neurobehavioral changes of lead exposed workers were often accompanied by gastrointestinal symptoms, which may be related to the low level of serotonin in intestinal neurons of lead exposed workers.

Gao et al[21] showed that lead exposure disrupted the normal development of genetic diversity and flora composition of intestinal microorganisms, the synthesis and metabolism pathway of metabolites, induced oxidative stress, and activated the defense and anti-virus mechanism of intestinal flora. Lead exposure increased the levels of amino acids, including glycine, glutamic acid, threonine, serine and proline, but decreased the level of Alanine. Human intestine registered hundreds of millions of microorganisms, about 1000 kinds of bacteria, known as the "second organ tube" of the human body. Intestinal flora affected the function and behavior of the brain through the "gut-brain axis", and participated in movement and secretion[22].Imbalance of intestinal flora could lead to irritable bowel syndrome (IBS),inflammatory bowel disease (IBD) and hepatic encephalopathy (HE) and other intestinal brain diseases[23-25].This kind of disease not only showed evident intestinal symptoms such as defecation difficulty, abdominal pain, abdominal distension, dyspepsia and intestinal inflammation, but also showed neuropsychiatric symptoms such as anxiety, depression and cognitive impairment[26].Animal experiments had shown that the lack of 5-HT in intestinal neurons of mice could lead to constipation[27].The lack of 5-HT in the intestine reduced the peristalsis ability of intestine and slowed down the transmission speed of intestine. The results showed that there was a significant correlation between blood lead concentration and neurotransmitters in lead exposed workers, among which 5-HT in peripheral blood was significantly lower than that in control group (215.06±29.87 *vs.* 265.38±41.31). Moreover, through the correlation analysis between 5-HT and the related symptoms (including gastrointestinal symptoms), we found that 5-HT was not only significantly correlated with neurobehavioral changes induced by lead exposure, but also negatively correlated with gastrointestinal symptoms. These suggested that 5-HT was involved in the regulation of intestinal flora-gut-

brain axis in enterocerebral diseases. Studies had shown that intestinal flora or its metabolites stimulated enterochromaffin cells and monocytes to release 5-HT, and produce inflammatory factors such as IL-6, IL10,TNF-α and IFN-γ, which mediated the imbalance of intestinal flora-intestinal mucosal immune system, and realized the bidirectional regulation of gut-brain axis through the connection of vagal afferent nerve and synapse. The interaction of microorganism, intestine and brain affected the change of intestinal flora[28,29]. Studies reported that taking amoxicillin for 7 days would lead to the imbalance of intestinal flora in healthy people, manifested with the increase in the number of Clostridium, Bacteroides and enterobacteria, while taking compound probiotics (*Lactobacillus* and *Bifidobacterium*) for 3 weeks could restore the intestinal flora composition to the normal structure before taking antibiotics[30]. In an experimental rat model of water avoidance stress, the combination of *Lactobacillus Swiss* and *Lactobacillus rhamnosus* prevented the overproliferation of stress-induced intestinal mucosal bacteria and the translocation of the bacteria from the intestinal epithelium to the mesenteric lymph nodes[31]. In addition, the combined use of *Lactobacillus helveticus* and *Bifidobacterium longum* could reduce the anxiety and depression behavior of rats[32]. Probiotics could not only adjust intestinal flora, improved intestinal symptoms, but also regulated function and behavior of the brain. These suggested that probiotics supplementation provided a new intervention for the prevention and treatment of occupational lead exposure workers.

As a neurotransmitter widely distributed in the central nervous system and gastrointestinal tract, the synthesis, metabolism and physiological function of 5-HT were regulated by intestinal flora.

Table VI. Correction of neurotransmitter and investigated symptom.

		Investigated symptom					
Neurotransmitter		Memory decline	Sleep disorders	Tingling in the distal extremities	Limb numbness	Brotherhood of raw	Sweat on hands and feet
DA	r	-0.191*	-0.073	0.262**	0.036	0.161	0.286
	p	0.026	0.401	0.002	0.678	0.061	0.001
5-HT	r	0.212	0.373**	0.193*	0.335**	0.418**	0.264
	p	0.013	0.000	0.024	0.000	0.000	0.002
GABA	r	0.373**	-0.146	0.335**	0.633*	0.461**	0.248
	p	0.000	0.089	0.000	0.000	0.006	0.003
Gly	r	0.193*	0.335**	0.136	0.466**	0.402*	0.246*
	p	0.024	0.000	0.115	0.000	0.000	0.004
Trp	r	0.233**	0.369**	0.153	0.519**	0.436**	0.431
	p	0.006	0.000	0.076	0.000	0.000	0.000
Glu	r	0.315**	0.130	0.042	0.254**	0.293**	0.171*
	p	0.000	0.133	0.628	0.003	0.001	0.046

		Investigated symptom							
Neurotransmitter		Headache	Dizzy	Languor	Constipation	Hate to vomit	Abdominal pain	Abdominal distension	Anorexia
DA	r	0.30[2**]	0.316**	0.100	0.026	0.121	0.287	0.034	0.280
	p	0.000	0.000	0.248	0.760	0.160	0.001	0.697	0.001
5-HT	r	0.229**	0.233	0.563**	0.233*	0.345**	0.315	0.193*	0.257*
	p	0.007	0.006	0.000	0.006	0.000	0.000	0.024	0.002
GABA	r	0.300**	0.369**	0.444	0.171*	0.156	0.182	0.087	0.399*
	p	0.006	0.000	0.000	0.007	0.000	0.002	0.002	0.024
Gly	r	0.127	0.153	0.344*	0.040	0.106	0.396	0.008	0.297*
	p	0.141	0.076	0.000	0.645	0.219	0.000	0.923	0.00
Trp	r	0.660**	0.266	0.387	0.370**	0.405**	0.230	0.266*	0.182*
	p	0.000	0.002	0.000	0.000	0.000	0.007	0.002	0.034
Glu	r	0.239**	0.289	0.304**	0.353**	0.113	0.104	0.181*	0.143
	p	0.005	0.001	0.000	0.000	0.189	0.229	0.035	0.098

**showed Significant correlation

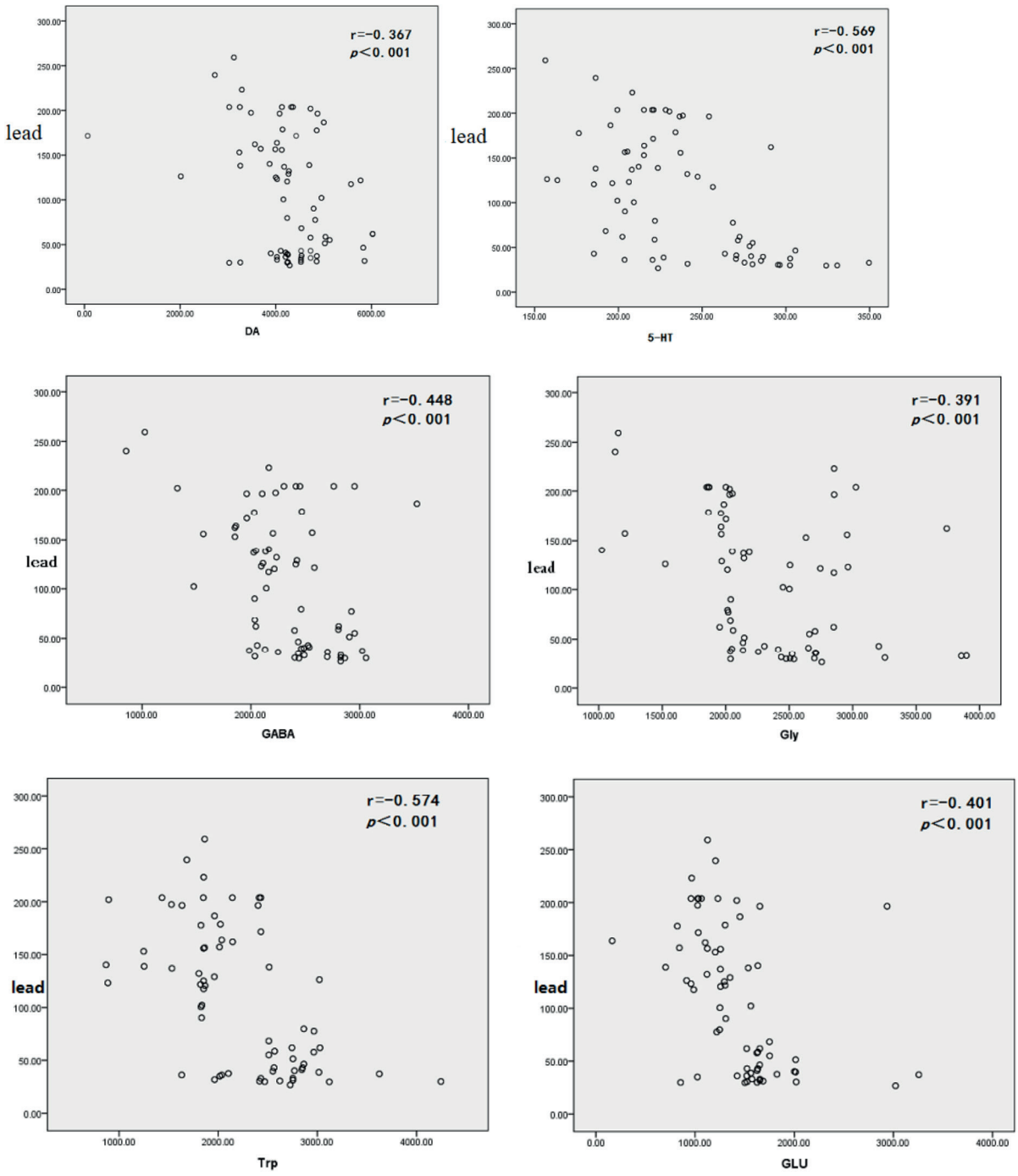

Figure 2. Relative analysis of neurotransmitter and lead.

About 95% of 5-HT was synthesized in the gut through enterochromaffin cells (ECs) and enteric neurons, while the remaining 5% was synthesized in central 5-HT neurons. 5-HT Synthesized and released in the intestine could activate the subtype receptors distributed in intestinal epithelium, intestinal neurons and immune cells, and regulated intestinal motility, secretory reflex and immune response. 5-HT distributed in the striatum of the brain chose the corresponding receptors and participated in neuropsychiatric behavior. Studies had shown that depression was closely related

to 5-HT. Increasing the concentration of 5-HT in the brain or reducing the reuptake of 5-HT by platelets could play an antidepressant role and improved the depression-like mood[33].Although the pathogenesis of depression was still unclear, the monoamine neurotransmitter hypothesis was still dominant. The abnormal decrease of serotonin in the brain leaded to the occurrence of depression. Dopamine was an important neurotransmitter in the brain, a precursor of NA, synthesized in substantia nigra and ventral tegmental area of midbrain and projected to related brain areas of the frontal cortex and the striatum regulated multiple signal pathways in the brain through activating dopamine receptor[34,35]. Prisco et al[36] had reported that striatal dopamine content had a delayed effect on depressive behavior. The results showed that the level of DA in peripheral blood was negatively correlated with the score of negative emotion, but positively correlated with the score of positive emotion. Prisco et al[37] reported that DA could regulate the release frequency of glutamate (Glu), gamma aminobutyric acid (GABA) and cholinergic terminals through heterologous synapses of dopamine receptor (DR) family, change the projection mode of neurons and regulate the excitability of neurons. Therefore, it was necessary to explore the interaction between various neurotransmitters to improve the treatment system of depression. In this study, we analyzed the correlation between 5-HT, DA, Trp, GABA, Glu and Gly with all the investigated symptoms, including emotional symptoms, and found that 5-HT, Trp and GABA were significantly correlated with the investigated symptoms. Among them, sleep disorders, constipation, and sensory fatigue were most significantly correlated with 5-HT or Trp (r=0.373, 0.233 and 0.563, respectively).

Conclusions

In summary, lead exposure not only led to changes of neuropsychiatric behavior, but also changed gastrointestinal symptoms. Serotonin, as the main neurotransmitter of intestinal synthesis, may participate in it, and its synthesis and metabolism may be regulated by intestinal flora. We hoped that the analysis could provide a reference for researchers to explore the mechanism of intestinal flora regulating 5-HT in neuropsychiatric changes induced by lead exposure in animal models.

References

1) Sampson TR, Mazmanian SK. Control of brain development, function, and behavior by the microbiome. Cell Host Microbe 2015; 17: 565-576.
2) Noureddini M, Verdi J, Mortazavi-Tabatabaei SA, Sharif S, Azimi A, Keyhanvar P, Shoae-Hassani A. Human endometrial stem cell neurogenesis in response to NGF and bFGF. Cell Biol Int 2012; 36: 961-966.
3) Baker EL, White RF, Pothier LJ, Berkey CS, Dinse GE, Travers PH, Harley JP, Feldman RG. Occupational lead neurotoxicity: improvement in behavioural effects after reduction of exposure. Br J Ind Med 1985; 42: 507-516.
4) Escalona E, Yanes L, Feo O, Maizlish N. Neurobehavioral evaluation of Venezuelan workers exposed to organic solvent mixtures. Am J Ind Med 1995; 27: 15-27.
5) Bouchard MF, Bellinger DC, Weuve J, Matthews-Bellinger J, Gilman SE, Wright RO, Schwartz J, Weisskopf MG. Blood lead levels and major depressive disorder, panic disorder, and generalized anxiety disorder in US young adults. Arch Gen Psychiatry 2009; 66: 1313-1319.
6) Parkinson DK, Ryan C, Bromet EJ, Connell MM. A psychiatric epidemiologic study of occupational lead exposure. Am J Epidemiol 1986; 123: 261-269.
7) Kala SV, Jadhav AL. Region-specific alterations in dopamine and serotonin metabolism in brains of rats exposed to low levels of lead. Neurotoxicology 1995; 16: 297-308.
8) Minnema DJ, Greenland RD, Michaelson IA. Effect of in vitro inorganic lead on dopamine release from superfused rat striatal synaptosomes. Toxicol Appl Pharmacol; 84: 400-411.
9) Wirbisky SE, Weber GJ, Lee JW, Cannon JR, Freeman JL. Novel dose-dependent alterations in excitatory GABA during embryonic development associated with lead (Pb) neurotoxicity. Toxicol Lett 2014; 229: 1-8.
10) Romeo B, Choucha W, Fossati P, Rotge JY. Meta-analysis of short- and mid-term efficacy of ketamine in unipolar and bipolar depression. Psychiatry Res 2015; 230: 682-688.
11) Jacobson LH, Vlachou S, Slattery DA, Li X, Cryan JF. The Gamma-Aminobutyric Acid B Receptor in Depression and Reward. Biol Psychiatry 2018; 83: 963-976.
12) Baik JH. Dopamine signaling in reward-related behaviors. Front Neural Circuits 2013; 7: 152.
13) Berlanga ML, Price DL, Phung BS, Giuly R, Terada M, Yamada N, Cyr M, Caron MG, Laakso A, Martone ME, Ellisman MH. Multiscale imaging character-ization of dopamine transporter knockout mice reveals regional altera-tions in spine density of medium spiny neurons. Brain Res 2011; 1390: 41-49.
14) Jonas RK, Montojo CA, Bearden CE. The 22q11. 2 deletion syndrome as a window into complex

neuropsychiatric disorders over the lifespan. Biol Psychiatry 2014; 75: 351-360.

15) Seppi K, Weintraub D, Coelho M, Perez-Lloret S, Fox SH, Katzenschlager R, Hametner EM, Poewe W, Rascol O, Goetz CG, Sampaio C. The Movement Disorder Society Evidence-Based Medicine Review Update: Treatments for the non-motor symptoms of Parkinson's disease. Mov Disord 2011; Suppl 3: S42-80.

16) Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015; 161: 264-276. Erratum in: Cell 2015; 163: 258.

17) Sharon G, Garg N, Debelius J, Knight R, Dorrestein PC, Mazmanian SK. Specialized metabolites from the microbiome in health and disease. Cell Metab 2014; 20: 719-730.

18) ACGIH. 2015 TLVs® and BEIs. Cincinnati, OH: American Conference of Governmental Industrial Hygienists; 2015.

19) Wu J, Basha MR, Brock B, Cox DP, Cardozo-Pelaez F, McPherson CA, Harry J, Rice DC, Maloney B, Chen D, Lahiri DK, Zawia NH. Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD. J Neurosci 2008; 28: 3-9.

20) White LD, Cory-Slechta DA, Gilbert ME, Tiffany-Castiglioni E, Zawia NH, Virgolini M, Rossi-George A, Lasley SM, Qian YC, Basha MR. New and evolving concepts in the neurotoxicology of lead. Toxicol Appl Pharmacol 2007; 225: 1-27.

21) Gao B, Chi L, Mahbub R, Bian X, Tu P, Ru H, Lu K. Multi-Omics Reveals that Lead Exposure Disturbs Gut Microbiome Development, Key Metabolites, and Metabolic Pathways. Chem Res Toxicol 2017; 30: 996-1005.

22) Zmora N, Zeevi D, Korem T, Segal E, Elinav E. Taking it Personally: Personalized Utilization ofthe Human Microbiome in Health and Disease. Cell Host Microbe 2016; 19: 12-20.

23) Plaza-Díaz J, Ruiz-Ojeda FJ, Vilchez-Padial LM, Gil A. Evidence of the Anti-Inflammatory Effects of Probiotics and Synbiotics in Intestinal Chronic Diseases. Nutrients 2017; 9: 555.

24) Brzozowski B, Mazur-Bialy A, Pajdo R, Kwiecien S, Bilski J, Zwolinska-Wcislo M, Mach T, Brzozowski T. Mechanisms by which Stress Affects the Experimental and Clinical Inflammatory Bowel Disease (IBD): Role of Brain-Gut Axis. Curr Neuropharmacol 2016; 14: 892-900.

25) Casén C, Vebø HC, Sekelja M, Hegge FT, Karlsson MK, Ciemniejewska E, Dzankovic S, Frøyland C, Nestestog R, Engstrand L, Munkholm P, Nielsen OH, Rogler G, Simrén M, Öhman L, Vatn MH, Rudi K. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment Pharmacol Ther. 2015; 42(1): 71–83. doi:10.1111/apt.13236

26) Bhattarai Y, Schmidt BA, Linden DR, Larson ED, Grover M, Beyder A, Farrugia G, Kashyap PC. Human-derived gut microbiota modulates colonic secretion in mice by regulating 5-HT3 receptor expression via acetate production. Am J Physiol Gastrointest Liver Physiol 2017; 313: G80-G87.

27) Nagakura Y, Kontoh A, Tokita K, Tomoi M, Shimomura K, Kadowaki M. Combined blockade of 5-HT3- and 5-HT4-serotonin receptors inhibits colonic functions in conscious rats and mice. J Pharmacol Exp Ther 1997; 281: 284-290.

28) Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 2006; 124: 1269-1282.

29) Brierley SM, Linden DR. Neuroplasticity and dysfunction after gastrointestinal inflammation. Nat Rev Gastroenterol Hepatol 2014; 11: 611-627.

30) Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, Patel B, Mazzola MA, Liu S, Glanz BL, Cook S, Tankou S, Stuart F, Melo K, Nejad P, Smith K, Topçuolu BD, Holden J, Kivisäkk P, Chitnis T, De Jager PL, Quintana FJ, Gerber GK, Bry L, Weiner HL. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun 2016; 7: 12015.

31) Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil 2011; 23: 255-264.

32) Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of Lac-tobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci USA 2011; 108: 16050-16055.

33) Mao YK, Kasper DL, Wang B, Forsythe P, Bienenstock J, Kunze WA. Bacteroides fragilis polysaccharide A is necessary and sufficient for acute activation of intestinal sensory neurons. Nat Commun 2013; 4: 1465.

34) Saijo T, Takano A, Suhara T, Arakawa R, Okumura M, Ichimiya T, Ito H, Okubo Y. Electroconvulsive therapy decreases dopamine D2 receptor binding in the anterior cingulate in patients with depression: a controlled study using positron emission tomography with radioligand [11C]FLB 457. J Clin Psychiatry 2010; 71: 793-799

35) Prisco S, Pagannone S, Esposito E. Serotonin-dopamine interaction in the rat ventraltegmental area: an electrophysiological study in vivo. J Pharmacol Exp Ther 1994; 271:83-90.

36) Prisco S, Esposito E. Differential effects of acute and chronic fluoxetine administration on the spontaneous activity of dopaminergic neurones in the ventral tegmental area. Br J Pharmacol 1995; 116: 1923-1931.