INTERLEUKIN-1β AND TNF-α ARE ELEVATED IN THE AMYGDALA OF ADULT RATS PRENATALLY EXPOSED TO ETHANOL

V.S. Kokhan[1]*, P.K. Anokhin[1], T.V. Proskuryakova[1], V.A. Shokhonova[1], R.A. Ageldinov[2], I.Yu. Shamakina[1]

[1]National Scientific Center for Narcology —
Branch of the V.P. Serbsky National Medical Research Centre for Psychiatry and Narcology,
3 Malyj Mogil'cevskij lane, Moscow, 119002 Russia; *e-mail: viktor_kohan@hotmail.com
[2]Scientific Center of Biomedical Technologies of the Federal Medical and Biological Agency of Russia,
settl. Svetlye gory, Moscow Region, 143442 Russia

Affective disorders, including anxiety and depression, developed in adult offspring of the mothers who consumed alcohol during pregnancy could be associated with an imbalance in neuroimmune factors in the amygdala (*corpus amygdaloideum*) resulted in impaired emotional stimulus processing. The aim of this study was to compare the content of cytokines TNF-α, IL-1α, IL-1β, IL-10, and IL-17 in the amygdala of adult female rats exposed to alcohol *in utero* and control rats. Cytokine levels were evaluated using a multiplex immunoassay system; mRNA expression was investigated using a real-time reverse transcription-polymerase chain reaction (RT-qPCR) assay. Prenatal alcohol exposure led to the increase in the content of TNF-α and IL-1β without significant changes in the mRNA expression level. Our data suggest that ethanol exposure to the fetus during pregnancy can result in long-term alterations in the content of the key neuroinflammatory factors in the amygdala, which in turn can be a risk factor for affective disorders in the adulthood.

Key words: prenatal alcohol exposure; affective disorders; amygdala; cytokines; TNF-α; IL-1β

DOI: 10.18097/PBMC20236905300

INTRODUCTION

Morphological, physiological, affective, behavioral, and cognitive disorders developed in offspring due to alcohol consumption by a pregnant woman are summarized by the term "fetal alcohol spectrum disorder" (FASD). The severity of these disorders depends on the duration and frequency of alcohol consumption and characteristics of the maternal organism, particularly, the activity of ethanol-metabolizing enzymes. Fetal alcohol syndrome (FAS), the most severe form of FASD, is diagnosed already in the early postnatal period and manifests as a specific pathology of the facial region, slow growth, dysfunction of the central and peripheral nervous system [1, 2]. However, FAS is a relatively rare pathology; pathological conditions that are not associated with craniocephalic dysmorphia and growth retardation, but manifest themselves in the formation of maladaptive behavior and cognitive dysfunction at later stages of postnatal development, are more common [3]. These disorders are known as "neurodevelopmental disorder associated with prenatal alcohol exposure" (ND-PAE) [4]. Typically, ND-PAE does not appear at an early age, and symptoms can only appear during primary school education, with the main problems being difficulties in adaptation, low resistance to stress, impulsivity, and difficulties in social interaction [5]. Experimental models of prenatal alcoholization in rodents reasonably well reflect the clinical picture of FASD in humans [6]. Among the identified changes in animals in adolescence and adulthood, the most common affective disorders include increased anxiety and depressive-like behavior [7].

Considering the role of neuroimmune mechanisms in the formation of the central nervous system during critical periods of development [8], researchers have hypothesized the participation of microglial cells and the neuroinflammatory factors they express in the mechanisms of the central nervous system dysfunction caused by prenatal exposure to alcohol [9, 10]. The effect of alcohol on microglial functions and the development of the central nervous system in the prenatal and early postnatal period have been studied in sufficient detail [10]. It has been suggested that the effects of prenatal alcoholization on microglial cells are not transient and may underlie CNS dysfunction in the adulthood [11]. It is important to note that most experimental studies have focused on analyzing changes of microglial functioning in the hippocampus, prefrontal cortex, and cerebellum in rats and mice in various models of FASD [12, 13]. At the same time, taking into consideration the key role of the amygdala in processing of emotionally significant stimuli and the development of adequate behavioral strategies [14, 15] as well as the involvement of neuroinflammation factors in this brain structure in the formation of affective pathology [16, 17], it is reasonable to suggest that the influence of prenatal alcoholism on the affective state in the adulthood may be mediated by changes in a cytokine/chemokine balance in the amygdala.

In this study, we have evaluated the content of the cytokines TNF-α, IL-1α, IL-1β, IL-10, and IL-17 in the amygdala of juvenile prenatally alcoholized and control female rats. The choice of these cytokines was determined by their established physiological role in the mechanisms of synaptic transmission and neuroplasticity in the adult brain [18–21].

MATERIALS AND METHODS

Animals

Outbred Wistar rats obtained from the Stolbovaya laboratory animal nursery of the Scientific Center for Biomedical Technologies were used in experiments. Animals were kept at 22±2°C, humidity 50–70% under natural light conditions. Animals of all groups had free access to standard pelleted food (GOST R 5258-92) and drinking water.

A Model of Prenatal Alcohol Exposure

To obtain offspring, two 3 months old female rats were placed in a cage with a 3 months old male rat for three days (the total number of rats included 5 males and 10 females). The first day of pregnancy was defined as the day of detection of sperm in the vaginal smear of females. Females mating with one male were randomly divided into two groups. We used the model of forced (one-bottle) exposure to ethanol that we described earlier [22]: females in the experimental group drank a 10% ethanol solution as the only source of liquid throughout pregnancy. Control females consumed only water. After the birth of offspring, all females were placed in individual cages with free access to water for the period of feeding. Thus, the offspring were alcoholized in the prenatal period, similar to the first two trimesters of pregnancy in women. On day 30 of life, the rat pups were separated from their mothers, separated by gender, and placed 6 pups per cage, where they had free access to water and food.

To conduct the experiment, 7 prenatally alcoholized (PA group) and 7 control females were randomly selected from different litters.

At 60 days of age rats were euthanized by decapitation. This age corresponds to the average age of first contact with alcohol, when transferred to humans. The amygdala was isolated according to the Paxinos and Watson rat brain atlas [23]. The dissection area corresponded to the following coordinates: *AP* -1.8÷-3.3 mm, *intraraural* 7.20÷5.70 mm, *lateral to midline* 4÷5 mm, *DV* 8÷9 mm. The resulting tissue was immediately frozen in liquid nitrogen and stored at -70°C until analysis.

Protein Isolation and the Multiplex Immunofluorescence Assay

Samples were homogenized by means of glass beads using a MagNA Lyser 230B homogenizer (Roche, Switzerland) and a buffer solution containing 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM PMSF, 0.05% Tween-20 and 1% (v/v) protease inhibitor cocktail II (ab201116, Abcam, USA). The resulting homogenates were centrifuged for 15 min at 12,000 *g* and 3°C, and the supernatant was collected for further analysis. Protein content was determined by the Bradford method using a commercially available Quick Start Bradford Protein Assay kit (Bio-Rad, USA). For analysis, if necessary, samples were diluted to a target protein concentration of 200–900 μg/ml.

Multiplex immunofluorescence analysis using microspheres (xMAP technology) was carried out using commercially available kits for the determination of cytokines in rat brain tissue (Cloud-Clone Corp., China) according to the manufacturer's instructions. The analysis was performed using a Bio-Plex MAGPIX Multiplex Reader with a Bio-Plex Pro Wash Station (Bio-Rad). The concentration of cytokines in the test samples was determined automatically using standard calibration dilutions, the computer programs Bio-Plex Manager Software v.6.1 (for equipment management and initial processing of results) and Bio-Plex Data Pro Software v.1.2 (for final processing of results) (Bio-Rad). The content of target proteins was normalized to the total protein in the sample.

RNA Extraction and Real-Time Quantitative PCR (RT-qPCR)

Total RNA was isolated using the standard guanidine isothiocyanate method using phenol-chloroform extraction (PureZOL RNA Isolation Reagent, Bio-Rad). The resulting RNA was treated with DNase (ThermoScientific, USA) according to the manufacturer's instructions. cDNA synthesis was carried out using the Mint revertase kit (Evrogen, Russia) according to the manufacturer's recommendations. The isolated cDNA was used as a template for quantitative real-time PCR; the sequences of the primers used (DNA-synthesis, Russia) are given in Table 1. Amplification

Table 1. Oligonucleotide primer sequences for RT-qPCR

Gene	Oligonucleotide primer sequence, 5′→3′	
	Forward	Reverse
TNF-α	AAATGGGCTCCCTCTCATCAGGTTC	TCTGCTTGGTGGTTTGCTACGAC
IL-1β	CACCTCTCAAGCAGAGCACAG	GGGTTCCATGGTGAAGTCAAC
β-actin	CACTGCCGCATCCTCTTCCT	AACCGCTCATTGCCGATAGTG

was carried out in 25 μl of the mixture using the commercially available qPCRmix-HS SYBR kit (Evrogen) on a CFX96 Real-Time System C1000 Thermal Cycler amplifier (Bio-Rad). The β-actin gene was used as a reference. To validate the specificity of amplification, melting curves were analyzed. Quantitative assessment of mRNA expression was carried out using the $2^{-\Delta\Delta Ct}$ method [24].

Statistical Data Processing

Statistical data processing was carried out using Statistica v.12 software. The normality of data distribution in the sample was checked using the Shapiro-Wilk test. Since all obtained datasets obeyed the Gaussian distribution law (p>0.05; values not shown), further data analysis was carried out using Student's t-test for independent samples. Data were presented as the arithmetic mean ± standard deviation.

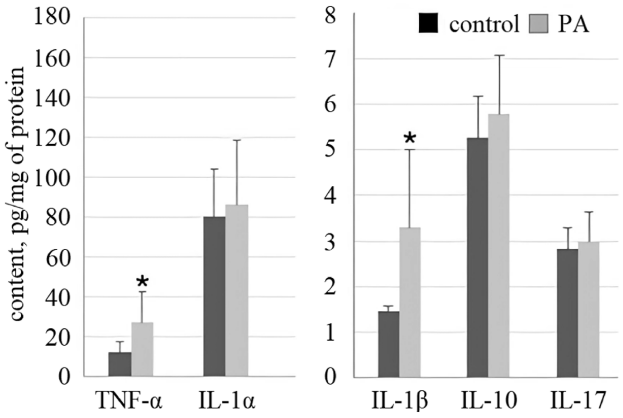

Figure 1. Content of cytokines TNF-α, IL-1α, IL-1β, IL-10, and IL-17 in the amygdala of adult prenatally alcohol exposed (PA, n=7) and control (n=7) female rats. * – p<0.05 (Student's t-test for independent samples).

RESULTS AND DISCUSSION

Prenatal ethanol exposure led to an increase in the levels of TNF-α and IL-1β in the amygdala, by 119.3% (p=0.03) and 125.2% (p=0.01), respectively, as compared to the control (Fig. 1). The content of IL-1α, IL-10, and IL-17 in the experimental and control groups differed insignificantly.

The expression level of *TNF-α* and *IL-1β* mRNA in the amygdala of rats from the PA group and control animals did not differ significantly (Fig. 2).

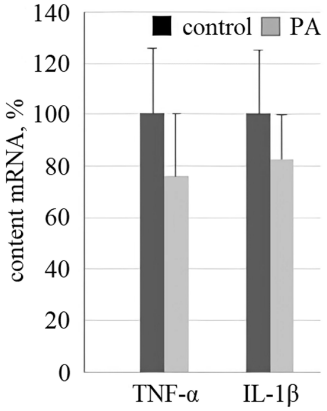

Figure 2. mRNA levels of cytokines TNF-α and IL-1β in the amygdala of adult prenatally alcohol exposed (PA, n=7) and control (n=7) female rats.

It should be noted that we studied the level of cytokines in the amygdala of rats in the absence of experimental factors with a proinflammatory effect after the birth of rats. Under these conditions, the level of proinflammatory cytokines in the brain is quite low [25]. Our experiment showed that the content of the analyzed cytokines in the amygdala varied in the range from 1.46 pg/mg to 86 pg/mg of protein; slightly higher values for the content of some cytokines could be associated with more efficient extraction from tissues due to the presence the surfactant Tween-20 in the isolation buffer. At the same time, the content of TNF-α corresponds to the literature data [25].

Based on the fact that protein levels can be regulated epigenetically, we have investigated the expression levels of cytokines that showed significantly altered concentrations in the amygdala. Due to the absence of significant differences, we consider several possible scenarios that would explain the obtained results. This discrepancy may be explained by the time point of the analysis, when the transcription level was normalized before the protein content. Such discrepancies are often found in studies [26]. Taking into consideration an important role of posttranscriptional regulation of cytokine expression in the brain [27], another possibility explaining the discrepancy may be regulation at the posttranscriptional level. It has been shown that adenine/uracil rich regulatory elements (AREs) in the 3′-untranslated region (3′-UTR) of mRNA play an important role in the mechanisms of regulation of cytokine translation [27]. AREs bind to a number of proteins that either promote mRNA degradation in response to various intra- and extracellular signals or regulate translation. This mechanism of translation regulation is best studied for TNF-α [27]. The 3′-UTR of TNF-α mRNA contains a conserved ARE of 34 nucleotides. The AGO2 and FXR1 proteins have been shown to bind to TNF-α mRNA and activate translation. In turn, in humans, binding of these proteins to AREs is regulated by miR369-3 microRNA thus confirming the role of microRNAs in the translation regulation [28]. Thus, it can be assumed that the effect of prenatal alcohol exposure on the level of TNF-α in the amygdala may be associated with changes at the posttranscriptional level, including the interaction of AREs, RNA-binding proteins, and microRNAs. In addition, *de novo* synthesis of TNF-α can be initiated by activation of the ATP ionotropic P2X7 receptor, mitogen-activated kinases (p38MAP),

extracellular signal-regulated kinases (ERK), c-Jun-N-terminal kinase (JNK) [29]. The mechanisms of the influence of prenatal alcoholization on post-transcriptional regulation of cytokine expression require further research.

It is generally accepted that the cytokines TNF-α, IL-1α, IL-1β, and IL-17 are pro-inflammatory, while IL-10 is anti-inflammatory; however, certain experimental evidence exists that this division is conditional [30]. For example, under various conditions, TNF-α can be both a pro- and anti-inflammatory cytokine. TNF-α exists in transmembrane (tmTNF) and soluble form (sTNF) forms. TNF-α-converting enzyme (TACE) cleaves tmTNF to form sTNF, which has proinflammatory effects. Currently, two TNF-α receptors have been described: TNFR1 and TNFR2. The transmembrane form interacts with both TNFR1 and TNFR2, while the soluble form can only bind to the type 1 receptor. Activation of TNFR1 is related to pathological processes and promotes cell death, while activation of TNFR2 leads to tissue repair and cell survival [31]. Thus, TNF-α has a direct effect on the functioning and survival of neurons in the brain; it regulates production and secretion of neurotransmitters, and affects blood-brain barrier permeability [31, 32]. It has been shown that the level of sTNF is elevated in the blood of patients with severe depression and bipolar disorder [30]. Interestingly, antidepressants, mood stabilizers, and antipsychotic drugs exhibit anti-inflammatory properties; particularly, lithium and valproate reduce production of TNF-α *in vitro* [33]. At the same time, a number of studies indicate the direct role of the increased TNF-α content in the amygdala in the genesis of the anxiety state [34]. Injection of TNF-α neutralizing antibodies (infliximab) into the basolateral amygdala (BLA) had an anxiolytic effect in a mouse model of inflammatory pain. *In vitro* experiments have shown that TNF-α facilitates glutamate AMPA-dependent neurotransmission and suppresses $GABA_A$-dependent synaptic transmission in the BLA [34]. Other cytokines, IL-1 (α and β), regulate GABAergic transmission in the amygdala [18].

The proinflammatory cytokines IL-1α and IL-1β bind to their receptor IL-1R1, leading to the activation of a number of intracellular signaling pathways (NF-κB, JNK, and p38MAPK) [35]. Researchers have shown that interleukin-1 receptor antagonist protein (*IL1RN*) knockout mice have a low alcohol preference [36]. This suggests that IL-1 plays an important role in the mechanisms mediating alcohol preference under free choice conditions. The level of IL-1β increased in the blood of patients with alcoholism and chronically alcoholized animals [37–39]. In addition, administration of IL-1β into the brain ventricles of chronically alcoholized rats increased the state of anxiety during alcohol withdrawal [40]. IL-1 regulates the basal level of activity of GABA neurons in the central amygdala and makes a significant contribution to the effects of alcohol in this brain region [41]. Thus, it can be assumed that an increase in the level of TNF-α and IL-1β in the brain amygdala in prenatally alcoholized animals may be one of the reasons for the affective disorders described in the literature and the predisposition to the formation of alcohol dependence.

We found no changes in the amygdala IL-10 content of prenatally alcoholized animals. This cytokine is considered as anti-inflammatory, immunosuppressive, and it promotes survival of neurons and glial cells [42]. No change has been found the IL-17 content, which is currently considered to be one of the main triggers of synaptic dysfunction and impairments of cognitive processes in some CNS pathologies [43, 44]. During intermittent prenatal alcoholization of rats, an increased level of IL-10 expression and an increased content of IL-17 were found in the brain of embryos; in the postnatal period these changes were absent [45, 46]. Thus, several scenarios are likely to explain the lack of significant changes in IL-10 and IL-17. First, embryonic changes may not have persisted into the postnatal period; secondly, the model of prenatal alcoholization used in this study, which assumes alcohol consumption throughout the entire period of pregnancy, compared with the interval scheme, could have a specific effect on the cytokine system of the brain of the offspring.

CONCLUSION

The data obtained in this work suggest that the increase in the level of TNF-α and IL-1β in the amygdala of the brain of prenatally alcoholized female rats may be one of the risk factors for the development of affective pathology in adulthood. Understanding the functional role of cytokines in the pathogenesis of FASD may be culminated the development of therapeutics for treatment of neuropathologies associated with the prenatal alcohol exposure.

FUNDING

The work was carried out within the framework of the State Assignment.

COMPLIANCE WITH ETHICAL STANDARDS

All experiments with rats were performed in accordance with the “Principles of Good Laboratory Practice” (approved by the Ministry of Health of the Russian Federation, order No. 199n dated April 1, 2016). Experiments with animals also complied with the requirements of Directive 2010/63/EU of September 22, 2010 of the European Parliament and of the Council of the European Union on the protection of animals used for scientific

purposes. The experimental protocol for working with animals was approved by the Ethics Committee of the V.P. Serbsky National Medical Research Center on Psychiatry and Addiction, (protocol No. 3 of March 03, 2022).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. *Voutilainen T., Rysä J., Keski-Nisula L., Kärkkäinen O.* (2022) Self-reported alcohol consumption of pregnant women and their partners correlates both before and during pregnancy: A cohort study with 21,472 singleton pregnancies. Alcohol. Clin. Exp. Res., **46**(5), 797-808. DOI: 10.1111/acer.14806
2. *Popova S., Lange S., Probst C., Gmel G., Rehm J.* (2017) Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: A systematic review and meta-analysis. Lancet Glob. Heal., **5**(3), e290-e299. DOI: 10.1016/S2214-109X(17)30021-9
3. *Sanders J.L., Netelenbos N., Dei S.O.* (2020) Construct and factorial validity of neurobehavioral disorder associated with prenatal alcohol exposure (ND-PAE). BMC Psychol., **8**, 53. DOI: 10.1186/s40359-020-00405-5
4. *Brown J.M., Bland R., Jonsson E., Greenshaw A.J.* (2019) The standardization of diagnostic criteria for fetal alcohol spectrum disorder (FASD): Implications for research, clinical practice and population health. Can. J. Psychiatry, **64**(3), 169-176. DOI: 10.1177/0706743718777398
5. *Hagan J.F. Jr., Balachova T., Bertrand J., Chasnoff I., Dang E., Fernandez-Baca D., Kable J., Kosofsky B., Senturias Y.N., Singh N., Sloane M., Weitzman C., Zubler J.* (2016) Neurobehavioral disorder associated with prenatal alcohol exposure. Pediatrics, **138**(4), e20151553. DOI: 10.1542/peds.2015-1553
6. *Razumkina E., Anokhin P., Proskuryakova T.V., Shamakina I.* (2019) Experimental approaches to the study of behavioral impairments associated with prenatal exposure to alcohol. Neurosci. Behav. Physiol., **49**, 894-902. DOI: 10.1007/s11055-019-00816-x
7. *Wang A.L., Micov V.B., Kwarteng F., Wang R., Hausknecht K.A., Oubraim S., Haj-Dahmane S., Shen R.Y.* (2023) Prenatal ethanol exposure leads to persistent anxiety-like behavior during adulthood indicated by reduced horizontal and vertical exploratory behaviors. Front. Neurosci., **17**, 1163575. DOI: 10.3389/fnins.2023.1163575
8. *Cowan M., Petri W.A. Jr.* (2018) Microglia: Immune regulators of neurodevelopment. Front. Immunol., **9**, 2576. DOI: 10.3389/fimmu.2018.02576
9. *Wilhelm C.J., Guizzetti M.* (2015) Fetal alcohol spectrum disorders: An overview from the glia perspective. Front. Integr. Neurosci., **9**, 65. DOI: 10.3389/fnint.2015.00065
10. *Wong E.L., Stowell R.D., Majewska A.K.* (2017) What the spectrum of microglial functions can teach us about fetal alcohol spectrum disorder. Front. Synaptic. Neurosci., **9**, 11. DOI: 10.3389/fnsyn.2017.00011
11. *Kane C.J., Drew P.D.* (2016) Inflammatory responses to alcohol in the CNS: Nuclear receptors as potential therapeutics for alcohol-induced neuropathologies. J. Leukoc. Biol., **100**, 951-959. DOI: 10.1189/jlb.3mr0416-171r
12. *Drew P.D., Johnson J.W., Douglas J.C., Phelan K.D., Kane C.J.* (2015) Pioglitazone blocks ethanol induction of microglial activation and immune responses in the hippocampus, cerebellum and cerebral cortex in a mouse model of fetal alcohol spectrum disorders. Alcohol. Clin. Exp. Res., **39**, 445-454. DOI: 10.1111/acer.12639
13. *Airapetov M.I., Eresko S.O., Bychkov E.R., Lebedev A.A., Shabanov P.D.* (2021) Prenatal exposure to alcohol alters TLR4 signaling in the prefrontal cortex in rats. Biomeditsinskaya Khimiya, **67**(6), 500-506. DOI: 10.18097/PBMC20216706500
14. *O'Neill P.K., Gore F., Salzman C.D.* (2018) Basolateral amygdala circuitry in positive and negative valence. Curr. Opin. Neurobiol., **49**, 175-183. DOI: 10.1016/j.conb.2018.02.012
15. *Sah P.* (2017) Fear, anxiety, and the amygdala. Neuron, **96**, 1-2. DOI: 10.1016/j.neuron.2017.09.013
16. *Hu P., Lu Y., Pan B.X., Zhang W.H.* (2022) New insights into the pivotal role of the amygdala in inflammation-related depression and anxiety disorder. Int. J. Mol. Sci., **23**(19), 11076. DOI:10.3390/ijms231911076
17. *Zhang W.H., Zhang J.Y., Holmes A., Pan B.X.* (2021) Amygdala circuit substrates for stress adaptation and adversity. Biol. Psychiatry, **89**, 847-856. DOI: 10.1016/j.biopsych.2020.12.026
18. *Bajo M., Varodayan F.P., Madamba S.G., Robert A.J., Casal L.M., Oleata C.S., Siggins G.R., Roberto M.* (2015) IL-1 interacts with ethanol effects on GABAergic transmission in the mouse central amygdala. Front. Pharmacol., **6**, 49. DOI: 10.3389/fphar.2015.00049
19. *Hiles S.A., Baker A.L., de Malmanche T., Attia J.* (2012) A meta-analysis of differences in IL-6 and IL-10 between people with and without depression: Exploring the causes of heterogeneity. Brain Behav. Immun., **26**, 1180-1188. DOI: 10.1016/j.bbi.2012.06.001
20. *Brigas H.C., Ribeiro M., Coelho J.E., Gomes R., Gomez-Murcia V., Carvalho K., Faivre E., Costa-Pereira S., Darrigues J., de Almeida A.A., Buée L., Dunot J., Marie H., Pousinha P.A., Blum D., Silva-Santos B., Lopes L.V., Ribot J.C.* (2021) IL-17 triggers the onset of cognitive and synaptic deficits in early stages of Alzheimer's disease. Cell Rep., **36**(9), 109574. DOI: 10.1016/j.celrep.2021.109574
21. *Simon D.W., Raphael I., Johnson K.M., Dixon C.E., Vagni V., Janesko-Feldman K., Kochanek P.M., Bayir H., Clark R.S.B., McGeachy M.J.* (2022) Endogenous interleukin-17a contributes to normal spatial memory retention but does not affect early behavioral or neuropathological outcomes after experimental traumatic brain injury. Neurotrauma Rep., **3**(1), 340-351. DOI: 10.1089/neur.2022.0017
22. *Kokhan V.S., Chaprov K., Ninkina N.N., Anokhin P.K., Pakhlova E.P., Sarycheva N.Y., Shamakina I.Y.* (2022) Sex-related differences in voluntary alcohol intake and mRNA coding for synucleins in the brain of adult rats prenatally exposed to alcohol. Biomedicines, **10**(9), 2163. DOI: 10.3390/biomedicines10092163
23. *Paxinos G., Watson C.* (1998) The Rat Brain in Stereotaxic Coordinates, 4th edn. New York, NY: Academic Press, 237 p.
24. *Schmittgen T.D., Livak K.J.* (2008) Analyzing real-time PCR data by the comparative CT method. Nat. Protoc., **3**(6), 1101-1108. DOI: 10.1038/nprot.2008.73
25. *Zahr N.M., Luong R., Sullivan E.V., Pfefferbaum A.* (2010) Measurement of serum, liver, and brain cytokine induction, thiamine levels, and hepatopathology in rats exposed to a 4-day alcohol binge protocol. Alcohol. Clin. Exp. Res., **34**(11), 1858-1870. DOI: 10.1111/j.1530-0277.2010.01274.x

26. *Kokhan V.S., Mariasina S., Pikalov V.A., Abaimov D.A., Somasundaram S.G., Kirkland C.E., Aliev G.* (2023) Neurokinin-1 receptor antagonist reverses functional CNS alteration caused by combined γ-rays and carbon nuclei irradiation. CNS Neurol. Disord. Drug Targets, **21**(3), 278-289. DOI: 10.2174/1871527320666210122092330
27. *Palanisamy V., Jakymiw A., van Tubergen E.A., d'Silva N.J., Kirkwood K.L.* (2012) Control of cytokine mRNA expression by RNA-binding proteins and microRNAs. J. Dent. Res., **91**(7), 651-658. DOI: 10.1177/0022034512437372
28. *Scalavino V., Piccinno E., Valentini A.M., Mastronardi M., Armentano R., Giannelli G., Serino G.* (2022) A novel mechanism of immunoproteasome regulation via miR-369-3p in intestinal inflammatory response. Int. J. Mol. Sci., **23**(22), 13771. DOI: 10.3390/ijms232213771
29. *Raffaele S., Lombardi M., Verderio C., Fumagalli M.* (2020) TNF production and release from microglia via extracellular vesicles: Impact on brain functions. Cells, **9**(10), 2145. DOI: 10.3390/cells9102145
30. *Probert L.* (2015) TNF and its receptors in the CNS: The essential, the desirable and the deleterious effects. Neuroscience, **302**, 2-22. DOI: 10.1016/j.neuroscience.2015.06.038
31. *Uzzan S., Azab A.N.* (2021) Anti-TNF-α compounds as a treatment for depression. Molecules. **26**(8), 2368. DOI: 10.3390/molecules26082368
32. *Alvarez Cooper I. Beecher K., Chehrehasa F., Belmer A., Bartlett S.E.* (2020) Tumour necrosis factor in neuroplasticity, neurogenesis and alcohol use disorder. Brain Plasticity, **6**(1), 47-66. DOI: 10.3233/BPL-190095
33. *Leu S.J., Yang Y.Y., Liu H.C., Cheng C.Y., Wu Y.C., Huang M.C., Lee Y.L., Chen C.C., Shen W.W., Liu K.J.* (2017) Valproic acid and lithium meditate anti-inflammatory effects by differentially modulating dendritic cell differentiation and function. J. Cell. Physiol., **232**, 1176-1186. DOI: 10.1002/jcp.25604
34. *Chen J., Song Y., Yang J., Zhang Y., Zhao P., Zhu X.J., Su H.C.* (2013) The contribution of TNF-α in the amygdala to anxiety in mice with persistent inflammatory pain. Neurosci. Lett., **541**, 275-280. DOI: 10.1016/j.neulet.2013.02.005
35. *Garlanda C., Dinarello C.A., Mantovani A.* (2013) The interleukin-1 family: Back to the future. Immunity, **39**(6), 1003-1018. DOI: 10.1016/j.immuni.2013.11.010
36. *Blednov Y.A., Ponomarev I., Geil C., Bergeson S., Koob G.F., Harris R.A.* (2012) Neuroimmune regulation of alcohol consumption: behavioral validation of genes obtained from genomic studies. Addict. Biol., **17**(1), 108-120. DOI: 10.1111/j.1369-1600.2010.00284.x
37. *Vallés S.L., Blanco A.M., Pascual M., Guerri C.* (2004) Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. Brain Pathol., **14**(4), 365-371. DOI: 10.1111/j.1750-3639.2004.tb00079.x
38. *Qin L., He J., Hanes R.N., Pluzarev O., Hong J.S., Crews F.T.* (2008) Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. J. Neuroinflammation, **5**, 10. DOI: 10.1186/1742-2094-5-10
39. *Lippai D., Bala S., Petrasek J., Csak T., Levin I., Kurt-Jones E.A., Szabo G.* (2013) Alcohol-induced IL-1β in the brain is mediated by NLRP3/ASC inflammasome activation that amplifies neuroinflammation. J. Leukoc. Biol., **94**(1), 171-182. DOI:10.1189/jlb.1212659
40. *Breese G.R., Knapp D.J., Overstreet D.H., Navarro M., Wills T.A., Angel R.A.* (2008) Repeated lipopolysaccharide (LPS) or cytokine treatments sensitize ethanol withdrawal-induced anxiety-like behavior. Neuropsychopharmacology, **33**(4), 867-876. DOI: 10.1038/sj.npp.1301468
41. *Bajo M., Patel R.R., Hedges D.M., Varodayan F.P., Vlkolinsky R., Davis T.D., Burkart M.D., Blednov Y.A., Roberto M.* (2019) Role of MyD88 in IL-1β and ethanol modulation of GABAergic transmission in the central amygdala. Brain Sci., **9**(12), 361. DOI: 10.3390/brainsci9120361
42. *Burmeister A.R., Marriott I.* (2018) The interleukin-10 family of cytokines and their role in the CNS. Front. Cell Neurosci., **12**, 458. DOI: 10.3389/fncel.2018.00458
43. *Chen J., Liu X., Zhong Y.* (2020) Interleukin-17a: The key cytokine in neurodegenerative diseases. Front. Aging Neurosci., **12**, 566922. DOI: 10.3389/fnagi.2020.566922
44. *Frank K., Abeynaike S., Nikzad R., Patel R.R., Roberts A.J., Roberto M., Paust S.* (2020) Alcohol dependence promotes systemic IFN-γ and IL-17 responses in mice. PLoS One, **15**(12), e0239246. DOI:10.1371/journal.pone.0239246
45. *Terasaki L.S., Schwarz J.M.* (2016) Effects of moderate prenatal alcohol exposure during early gestation in rats on inflammation across the maternal-fetal-immune interface and later-life immune function in the offspring. J. Neuroimmune Pharmacol., **11**(4), 680-692. DOI: 10.1007/s11481-016-9691-8.
46. *Pascual M., Montesinos J., Montagud-Romero S., Forteza J., Rodriguez-Arias M., Minarro J., Guerri C.* (2017) TLR4 response mediates ethanol-induced neurodevelopment alterations in a model of fetal alcohol spectrum disorders. J. Neuroinflammation, **14**(1), 145. DOI: 10.1186/s12974-017-0918-2

Received: 24. 07. 2023.
Revised: 08. 08. 2023.
Accepted: 28. 08. 2023.

ИНТЕРЛЕЙКИН-1β И TNF-α ПОВЫШЕНЫ В МИНДАЛИНЕ ПРЕНАТАЛЬНО АЛКОГОЛИЗИРОВАННЫХ ВЗРОСЛЫХ КРЫС

В.С. Кохан[1]*, П.К. Анохин[1], Т.В. Проскурякова[1], В.А. Шохонова[1], Р.А. Агельдинов[2], И.Ю. Шамакина[1]

[1]Национальный научный центр наркологии — филиал Национального медицинского исследовательского центра психиатрии и наркологии им. В.П. Сербского, 119002, Москва, Малый Могильцевский пер., 3; *эл. почта: viktor_kohan@hotmail.com
[2]Научный центр биомедицинских технологий, 143442, пос. Светлые горы, Московская область

Аффективная патология (тревога, депрессия) у взрослого потомства матерей, употреблявших алкоголь во время беременности, может быть связана с изменением баланса нейроиммунных факторов в миндалине мозга (*corpus amygdaloideum*) и, как следствие, нарушением её функций в процессинге эмоционально значимых стимулов. Целью исследования было изучение содержания цитокинов TNF-α, IL-1α, IL-1β, IL-10 и IL-17 в миндалине мозга взрослых пренатально алкоголизированных и контрольных самок крыс. Для количественной оценки содержания целевых цитокинов был использован мультиплексный иммунофлуоресцентный анализ. Уровень экспрессии мРНК анализировали методом ПЦР в режиме реального времени после обратной транскрипции. Увеличение содержания провоспалительных цитокинов TNF-α и IL-1β в миндалине взрослых пренатально алкоголизированных животных не было связано с изменением уровня экспрессии мРНК. Наши данные свидетельствуют о том, что воздействие этанола на плод во время беременности может приводить к долгосрочным изменениям содержания ключевых провоспалительных цитокинов в миндалине, которые, в свою очередь, выступают фактором риска аффективных патологий в позднем возрасте.

Полный текст статьи на русском языке доступен на сайте журнала (http://pbmc.ibmc.msk.ru).

Ключевые слова: пренатальная алкоголизация; аффективные нарушения; миндалина; цитокины; TNF-α; IL-1β

Финансирование. Работа выполнена в рамках государственного задания.

Поступила в редакцию: 24.07.2023; после доработки: 08.08.2023; принята к печати: 28.08.2023.