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Effects of the original synthetic ligand of benzodiazepine receptors in long-term alcoholized mice

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Motivation and Aim: Currently, the role of the immune system in the pathogenesis of various mental disorders, in particular, chemical and behavioral addictions, is being actively studied. One of the molecular mechanisms underlying neuroimmune interactions, both in normal and pathological conditions, is the commonality of the receptor system of immunocytes and neurons with respect to such signaling molecules as cytokines and neurotransmitters. Of particular interest is the presence of gamma-aminobutyric acid (GABA) receptors type A, which are the main target of ethanol in the CNS, also on the surface of T-lymphocytes, which suggests the possibility of direct regulation of the immune response by inhibitory neurotransmitters. According to the modern paradigm, an essential pathogenetic mechanism of chronic alcoholism is neuroinflammation. Moreover, the cellular components of both non-adoptive and adoptive immunity are involved in the pathological process. Molecules of the GABA-receptor (GABA-R) complex, molecules of the Toll-like receptor family (TLRs), and cytokines play a key role in maintaining the pathological state. Ethanol consumption induces stable long-term activation of microglia predominantly in the hippocampus, mediated by TLRs [2]. Recently, an alternative pathway of TLR4 activation mediated by GABA-R has been identified, leading to a change in the balance of pro- and anti-inflammatory cytokine ligands on neurons, which is involved in the mechanisms of disturbance of higher nervous activity processes in alcoholism [1]. *ortho*-Fluorobenzonal is a synthetic ligand of the BDR-GABA-R complex, which, as we have previously established, have immunomodulatory properties mediated by lymphocytic GABA-R [3, 4]. The purpose of this work was to study the effect of the synthetic ligands of the GABA-R *ortho*-fluorobenzonal on the functional activity of the nervous and immune systems in experimental alcoholism.

Materials and Methods: Male (CBAxC57Bl/6)F1 mice in the state of alcohol dependence owing to 6-month 10 % ethanol exposure were undergoing intragastric administration of *ortho*-fluorobenzonal (100 mg/kg for 10 days). ortho-Fluorobenzonal was designed and synthesized in the Department of Biotechnology and Organic Chemistry, National Research Tomsk Polytechnic University. The formation of the alcohol dependence was evaluated by a single injection of naloxone (3 mg/kg, subcutaneously) followed by visual assessment of the signs of “withdrawal syndrome”. Animal's alcohol consumption, behavior and immune parameters before and after receiving the *ortho*-fluorobenzonal were estimated.

Results: Daily consumption of ethanol in mice with alcohol dependence decreased starting from 2 days of *ortho*-fluorobenzonal administration and stopping the

consumption of ethanol under free choice with water conditions on day 4, which indicated the alcohol motivation decrease. Chronic exposure to ethanol resulted also in significant decrease in animals behavioral activity in the Open Field Test. The pronounced changes in motor (horizontal locomotor) and exploratory (vertical locomotor) activities were registered after the 10 days substance administration: mice treated with ortho-fluorobenzonal showed increase in behavioral activity. Neuroimmune regulatory factors, including cytokines, contributed to changes in brain function and behavior associated with alcohol consumtion. In particular, interaction of the key regulatory cytokine IL-1β with GABAa-R might account for the locomotory-depressive effects of this cytokine. *ortho*-Fluorobenzonal treatment modulated the level of IL-1β, as well as other cytokines (IL-6, TNFα, IFN-γ, IL-10) in some brain regions (hippocampus, frontal cortex, striatum, hypothalamus) in mice with alcohol dependence, what might serve as one of the mechanisms of the demonstrated animal exploratory behavior stimulation. We have previously shown the *ortho*-fluorobenzonal ability of GABA-R – mediated modulation of spontaneous and mitogen-induced immune cells proliferative activity in mice with experimental alcohol dependence [3–5]. We also revealed the stimulation of the humoral and cellular immune response, estimated respectively by the relative number of antibody-producing spleen cells and the intensity of the delayed type hypersensitivity reaction. The immune response intensity in mice with experimental alcohol dependence after the *ortho*-fluorobenzonal treatment were comparable with that in the control group of equal age healthy animals.

Conclusion: The original synthetic ligand of benzodiazepine receptors *ortho*-fluorobenzonal, acting on molecular targets of ethanol in the nervous and immune systems, has a positive neuroimmunomodulatory effect, reduces alcohol motivation, so it has a promise in the treatment of alcoholism.

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