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# The effects of lymphocytes modulated *in vitro* by an original anticonvulsant on the brain functions in experimental alcoholism

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*Motivation and Aim*: A growing body of literature indicates that the immune system plays a critical role in the development and maintenance of alcohol use disorder. Long-term alcohol consumption reduces the effectiveness of the cell-mediated and humoral immune response to infection and vaccination, which may be associated with the conversion of the naive T-lymphocyte phenotype, with a reduction in the number of CD4 and CD8 subpopulations of lymphocytes and modulation of their functional activity [1, 2]. GABAA-receptors (GABAA-R) represent the main inhibitory neurotransmitter system in the brain and play a central role in mediating the effects of ethanol. Changes in GABAA-R activity, similar to the effects on neuronal cells, cause modulation of the immune cells functional activity [2]. We first demonstrated that original compound ortho-fluoro-benzonal, artificial GABAA-R ligand, has immunostimulating properties and is able to restored long-term alcoholized mice lymphocytes activity *in vitro* through GABAA receptors [3-5]. Based on the previous results the purpose of the present study was to evaluate the effects of peripherally injected ortho-fluoro-benzonal modulated lymphocytes in in long-term alcoholized recipient.

*Methods and Algorithms*: Male (CBAxC57Bl/6)F1 mice in the state of alcohol dependence owing to 6-month 10 % ethanol exposure were administered intravenously with syngeneic splenic lymphocytes pre-cultivated with *ortho*-fluorobenzonal (10 μg/ml). The compound was synthesized in the research laboratory of drug synthesis of the National Research Tomsk Polytechnic University in the process of searching for highly effective anticonvulsants. 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". Recipient's consumption of 10 % ethanol solution under conditions of free choice with water was recorded daily at 10:00 for 7 days, starting from the first day after lymphocytes transplantation. Exploratory behavior was assessed in the "Open field" test. The content of cytokines and BDNF in brain structures of syngeneic long-term alcoholized recipients was assessed by ELISA using specific test systems: BenderMed Systems (Austria) for the determination of IFN-γ and IL-6; R&D Systems Inc. (United States) for the determination of IL-1β, IL-10, TNF-α and BDNF in accordance with the manufacturer's instructions. Cells staining was perfomed with CFSE.

*Results*: Daily consumption of ethanol in mice with alcohol dependence decreased starting from 2 days after intravenous administration of syngeneic lymphocytes precultivated with ortho-fluorobenzonal, which proved the alcohol motivation decrease.

Chronic exposure to ethanol resulted also in significant decrease in animals' behavioral activity in the "Open Field" test, while the intravenous administration of syngeneic lymphocytes precultivated with ortho-fluorobenzonal restored the motor and exploratory activities almost up to the intact healthy animal's level. Depressive-like behavior in chronic alcoholism is mediated by neuroinflammation. Alcohol is thought to alter immune signaling and increase neuroinflammation via two primary mechanisms: indirectly by initiating systemic production of proinflammatory cytokines; and directly through actions in the brain, whereby alcohol and potentially alcohol-induced neural damage stimulate the release of inflammatory molecules In particular, interaction of the key regulatory cytokine IL-1β with GABAA-R might account for the locomotory-depressive effects of this cytokine [ 1]. In the present study we revealed that above mentioned stimulation of behavioral activity in the "Open Field" test was recorded against the background of decreased levels of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, IFN-γ in pathogenetically significant brain structures (frontal cortex, hippocampus and hypothalamus), which indicates a decrease in neuroinflammation. It is known that in chronic alcoholism neuroinflammation is associated with a decrease in the BDNF level, which causes in addition to neuroplasticity disorders the transition from occasional to compulsive alcohol consumption. We demonstrated a decrease BDNF level in the hippocampus of long-term alcoholized mice. After transplantation of lymphocytes modulated in vitro by the ortho-fluorobenzonal to syngeneic long-term alcoholized recipients an increase in the quantitative content of the hippocampal BDNF level was recorded, which indicates stimulation of neuroplasticity processes. There is also evidence that not only cytokines, but also immune cells can enter the brain and change the brain functions, including behavior, by direct contact with brain cells. The injected CFSE-labeled syngeneic lymphocytes modulated *in vitro* by ortho-fluorobenzonal 3 days after the systemic administration were recorded in the brain parenchyma of long-term alcoholized recipients. These lymphocytes were functionally active in the brain, which suggests, in particular, cell's direct influence on the brain functions.

*Conclusion*: Results demonstrated that transplantation of ortho-fluoro-benzonal-modulated lymphocytes caused positive psychoneuromodulating effect in long-term alcoholized recipients, manifested in editing behavioral patterns characteristic of alcoholism, reducing neuroinflammation and stimulating neuroplasticity.

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