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EXPRESSION OF CD68 IN THE RAT'S LIVER IN THE NORM AND AFTER 2 WEEKS CANNABIDIOL OIL APPLICATIONS

ЕКСПРЕСІЯ CD68 У ПЕЧІНЦІ ЩУРА В НОРМІ ТА ПІСЛЯ 2 ТИЖНІВ ЗАСТОСУВАННЯ ОЛІЇ КАНАБІДІОЛУ

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Introduction. Kupffer cells or stellate macrophages of the liver are the largest population of tissue-resident macrophages. Kupffer cells are in the sinusoids of the liver and are cells of the innate immune system that perform a protective function, regulate differentiation, cell proliferation or death, and take part in the remodeling of the intercellular matrix. Kupffer cells take part in the metabolism of lipids, proteins, come into direct contact with endotoxins, various bacteria, that is, stellate macrophages are the first immune cells of the liver, the function of which can change in various pathological processes and diseases [1].

To date, several scientific articles have been published demonstrating the potential therapeutic effects of cannabidiol, the non-psychoactive component of Cannabis sativa (marijuana), in various animal models of neuropsychiatric

disorders and in human clinical trials. In addition to therapeutic effects, the literature presents the results of experimental studies in which the authors express concern about the development of hepatotoxicity caused by cannabidiol [2; 3; 4]. Given that cannabidiol is now used in large quantities in cosmetics, nutritional supplements, and skin oils to treat various ailments, further experimental research is needed.

The **aim** of this study was to research of the expression of CD68 in the rat's liver in the norm and after 2 weeks cannabidiol oil applications.

Materials and methods. Experimental studies were performed on 24 sexually mature white male rats, weighing 180–230 g, aged 5–7 months at the beginning of the experiment. Experiments were conducted in compliance with moral and ethical norms in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 2010/63/EU, Law of Ukraine № 3447-IV "On protection of animals from cruel treatment". The experimental research protocols were approved by the bioethics committee of Danylo Halytsky Lviv National Medical University (protocol № 7 dated August 29, 2022). All animals were housed in the vivarium of Danylo Halytsky Lviv National Medical University. The main group consisted of 18 rats, which were given 5 drops of 10% cannabidiol oil, amounting to 3 mg, to the main feed once a day for 2 weeks. The control group consisted of 6 sexually mature white male rats, which were provided with water and food without any restrictions. Collection of biological material was carried out after euthanasia using diethyl ether. Liver samples were fixed in 10% buffered formalin. Then, according to the protocol, dehydration was carried out in alcohols of increasing concentration, embedded in paraffin according to the standard method. Histological sections with a thickness of 5±1 μm were made from paraffin blocks with liver tissue samples, which were applied to glass slides with a special adhesive coating. Deparaffined histological sections were stained according to the standard method with hematoxylin-eosin. In addition, immunohistochemical studies were performed to determine the expression of CD68 (clone KP1), a marker of macrophages – Kupffer cells and streptavidin peroxidase detection system with diaminobenzidine tetrachloride were used Visualization and microphotography were performed using a Leica DM 2500 light microscope (Leica Microsystems GmbH, Germany) with a Leica DFC450 C digital camera (Germany) and Leica Application Suit Version 3.8 software.

Results. The literature presents the results of experimental studies in which the authors express concern about the development of hepatotoxicity caused by cannabidiol [5]. We conducted an experimental study in which 5 drops (3 mg) of 10% cannabidiol oil were added to the main feed daily (1 time per day) for 2 weeks. After 2 weeks of the experiment, histological

and immunohistochemical studies of Kupffer cells in the sinusoidal capillaries of the liver were performed. In general, the analysis of the histological picture of the liver, as in our previous studies, showed that the structure of the liver lobe was not changed and did not differ from the control group [6]. The lining of the hepatic sinusoids is characteristic, it is formed by two different types of cells. Cells of one type – endothelial, are relatively thin and flattened. Other cells are much larger, star-shaped, located between endothelial cells and are clearly visualized. These are Kupffer cells. They were located both in the centrilobular areas of the liver lobes and in the periportal areas. Periportal Kupffer cells were, as a rule, larger and better visualized than centrilobular cells. An immunohistochemical study demonstrated the presence of a moderate number of Kupffer cells, and their hypertrophy in the periportal areas. According to the literature, the functions, and structures of Kupffer cells are specialized depending on their location. Periportal Kupffer cells are directly exposed to blood flow, as a rule, they are larger and have more lysosomal enzyme and phagocytic activity, while centrilobular Kupffer cells have little perfusion, create more superoxide radical [7; 8]. To date, the functional state of Kupffer cells in various pathological processes is described within the framework of the M1/M2 paradigm, where M1 are classically activated macrophages that have pro-inflammatory properties, and M2 are alternatively activated tolerogenic macrophages that contribute to the resolution of inflammation [9]. Kupffer cells play an important role in the pathogenesis of liver damage. For example, the presence of endotoxin induces strong M1 polarization of Kupffer cells. Many reactive oxygen species, pro-inflammatory cytokines and chemokines are produced by activated Kupffer cells, which leads to liver damage.

Conclusions. Thus, an experimental study demonstrated that after 2 weeks of using CBD as a dietary supplement, the localization, general histological characteristics of the liver, morphology of Kupffer cells, and immunohistochemical expression of CD68 were not different from the control group, and CBD had no toxic effect on the liver. Kupffer cells belong to the reticuloendothelial system, play an important role in physiology, homeostasis in the liver, providing an anti-inflammatory microenvironment during homeostasis, participate in the acute and chronic response of the liver to toxic compounds that may enter the body. Activation of Kupffer cells by the introduction of toxic agents directly or indirectly leads to the release of inflammatory mediators and reactive oxygen species [10]. In our opinion, additional experimental studies at other time intervals are needed to determine possible adverse effects on the liver, to analyze the phenotype of tissue macrophages, to assess risk factors, and to possibly determine optimal human drug use.

Bibliography:

1. Ju C, Tacke F. Hepatic macrophages in homeostasis and liver diseases: from pathogenesis to novel therapeutic strategies. Cell Mol Immunol. 2016; 13: 316–27. https://doi.org/10.1038/cmi.2015.104

2. Marx TK, Reddeman R, Clewell AE, Endres JR, Beres E, Vertesi A, Glavits R, Hirka G, Szakonyine IP. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. J. Toxicol. 2018; 8143582. DOI: 10.1155/2018/8143582.

3. Devinsky O, Cross, J.H., Wright, S. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. N. Eng. J. Med. 2017; 377:699–700. doi: 10.1056/NEJMoa1611618.

4. Thiele EA, Marsh ED, French JA, Mazurkiewicz-Beldzinska M, Benbadis SR, Joshi C, Lyons PD, Taylor A, Roberts C, Sommerville K. Cannabidiol in patients with seizures associated with Lennox-Gastaut syndrome (GWPCARE4): A randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2018; 391:1085–96. DOI: 10.1016/S0140-6736(18)30136-3.

5. Iffland K, Grotenhermen F. An update on safety and side effects of cannabidiol: a review of clinical data and relevant animal studies. Cannabis Cannabinoid Res. 2017; 2:139–54. DOI: 10.1089/can.2016.0034

6. Shevchuk, M.M. Macro– and microstructural liver arrangement in white rats in health. Bulletin of problems in biology and medicine. 2022; 3(166): 456-9 DOI:10.29254/2077-4214-2022-3-166-456-459

7. Nguyen-Lefebvre AT, Horuzsko A. Kupffer Cell Metabolism and Function. J Enzymol Metab. 2015;1(1):101. Epub 2015 Aug 14. PMID: 26937490; PMCID: PMC4771376.

8. Basit H, Tan ML, Webster DR. Histology, Kupffer Cell. 2022 Dec 30. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan –. PMID: 29630278.

9. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 2014; 6: 13. DOI: 10.12703/P6-13

10. Cubero FJ, Nieto N. Kupffer cells and alcoholic liver disease. Rev Esp Enferm Dig. 2006 Jun;98(6):460-72. English, Spanish. DOI: 10.4321/s1130-01082006000600007.