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ANTI-INFLAMMATORY ACTIVITY OF SYNAPTAMIDE IN TRAUMATIC BRAIN INJURY

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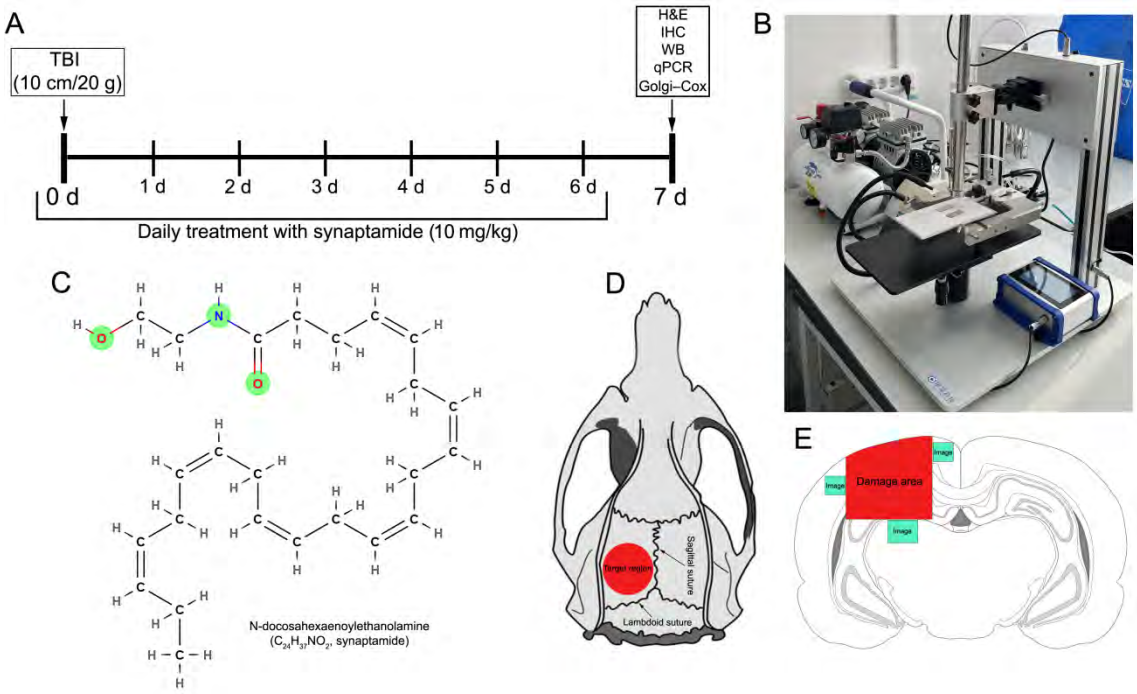
Abstract

Traumatic brain injuries (TBI) of varying severity are becoming more frequent all over the world. The process of neuroinflammation, in which macrophages and microglia are key players, underlies all types of brain damage [1]. The present study focuses on evaluating the anti-inflammatory potential of N-docosahexaenoylethanolamine (DHEA, synaptamide) in TBI.

All experimental procedures were performed on male C57BL/6 mice (3 months old, 26–28 g). Synaptamide (99,4 % purity) used in the present study was obtained from the digestive gland of *Berryteuthis magister* at the Pharmacology Laboratory of the NSCMB FEB RAS (see Figure, *C*). In the present study, the weight drop induced (WDI) cortical injury model (see Figure, *D*), previously developed by Feeney et al., 1981 [2], and was used to generate TBI. The model was generated using a specialized IMP-1020 impactor (Shanghai TOW Intelligent Technology, Shanghai, China) (see Figure, *B*). Synaptamide in a dose of 10 mg/kg and vehicle was injected subcutaneously in a volume of 100 µl, daily for 7 days from the day of surgery (see Figure, *A*). Material from for western blot, qPCR, histological and immunohistochemical studies was obtained after 7 days postoperatively. Images of the cortex and thalamus were acquired on a Zeiss Axio Imager microscope using an AxioCam 503 camera and AxioVision software (Zeiss, Muenster, Germany) (see Figure, *E*). Immunopositive staining was counted using ImageJ NIH (Bethesda, MD, USA). The present study examined the dynamics of changes in the activity of Iba-1- and CD68-positive microglia/macrophages, the level of production of pro-inflammatory cytokines (IL1β, IL6, TNFα) and pro-apoptotic proteins (Bad, Bax), the expression



(A) Experiment overall design; (B) Image of the IMP-1020 impactor; (C) Chemical structure of N-docosahexaenoylethanolamine (DHEA, synaptamide); (D) Anatomical mapping of the craniotomy and the target area for the rod force. The red dot indicates the site of force application. Institutional animal care committee approval was obtained for all experiments; (E) Green marks the ipsilateral thalamus and cortical regions that were subjected to immunohistochemistry examination. The area of the damage (marked in red)

of pro- and anti-inflammatory markers (CD68, CD206, arg-1). ATF3 transcription factor distribution and neuronal state in the thalamus and cerebral cortex of animals with sham injury, traumatic brain injury, and therapy are quantitatively assessed.

The obtained data showed that synaptamide: (1) has no effect on the total pool of microglia/macrophages; (2) inhibits the activity of pro-inflammatory microglia/macrophages and cytokines they produce; (3) increases the expression of CD206 but not arg-1; (4) has anti-apoptotic effect and (5) improves the morphological state of neurons. In conclusion, given that the pathological process seen following TBI involves excitotoxicity, apoptosis, and oxidative stress due to the development of uncontrolled neuroinflammation, the use of synaptamide as an immunomodulatory medication is fully justified.

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