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EFFECTS OF PROGENITOR CELL CONDITIONED MEDIA ON THE AMOUNT OF BRAIN CORTEX NEURONS IN A RAT MODEL OF TRAUMATIC BRAIN INJURY

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Aim. The purpose of the study was to examine beneficial effect of conditioned media (CM) of progenitor cells of different origin (neurogenic progenitor cells, or NPCs, and adipose-derived mononuclear cells, or AMCs) as a source of mesenchymal multipotent stromal cells (MMSCs) on brain cortex neurons in rats with traumatic brain injury (TBI).

Methods. TBI was reproduced in outbred sexually mature male rats by developing the model of free-falling load (50 g) with damage to the left hemisphere of the brain. The rats were injected 3 times with an interval of every other day intraperitoneally with NPCs CM and AMCs CM (dose 0.8 mg by total protein) that were obtained from cell cultures of fetal rat brain and adult rat adipose tissue. On the 5th day after TBI, the morphologic study of brain tissue was performed.

Results. The number of neurons in the cortex of rats on the 5th day after TBI in damaged hemisphere as well as in contralateral hemisphere compared to control group decreased by half. Three i.p. injections of NPCs CM or AMCs CM increased the number of neurons in the cortex in both hemispheres in rats of corresponding groups compared to the rats with TBI without additional treatment.

Conclusion. Obtained results indicate that CM obtained from NPCs and AMCs have noticeable neuroprotective effect on the damaged neurons and might be considered as an additional mode to treatment of TBI.

Key words: neurogenic progenitor cells, mesenchymal multipotent stromal cells, traumatic brain injury, conditioned medium, neuron viability.

The pathogenetic mechanism of traumatic brain injury (TBI) involves structural changes in nervous tissue as a result of primary mechanical damage as well as a cascade of secondary effects, which causes increased death of neurons and the emergence of functional deficits considered as neurological and functional consequences of trauma [1]. Significant number of ongoing studies has shown that stem cells and their metabolic products, such as anti-inflammatory and growth factors have potential in treatment of brain injury [2, 3]. While these findings, albeit tentatively, seem to confirming beneficial effects and usefulness of stem cells derived treatments, the question of their exact impact remains up for discussion [4].

The purpose of the study was to determine and register curative effect of conditioned media (CM) of neurogenic progenitor cells (NPCs) and adipose-derived mononuclear cells (AMCs) as a source of mesenchymal multipotent stromal cells (MMSCs) on brain cortex neurons in rats with TBI.

Materials and Methods

All procedures with experimental animals used in the work were carried out in compliance with legal norms and requirements of the Law of Ukraine no. 3447 IV "On protection of animals from cruel treatment", "European Convention for the protection of vertebrate animals used for research and other scientific purposes" (Strasbourg, 1986), principles of bioethics and biosafety standards. The study was approved by the Ethics and Bioethics Committee of the SI "INS NAMS" (protocol No. 26 of May 11, 2018).

The study was performed with outbred sexually mature male Wistar rats divided into 4 experimental groups (n = 6 in each group): 1) TBI; 2) TBI + NPCs CM; 3) TBI + AMCs CM; 4) control (intact rats). TBI was reproduced by developing the model of a free-falling load (50 g) with damage to the left hemisphere of the brain. The rats from groups 2 and 3 were injected three times i.p. with an interval of every other day NPCs CM or AMCs CM (dose 0.8 mg by total protein). NPCs CM or AMCs CM were obtained from cell cultures of fetal (E14) rat brain and adult rat adipose tissue [5]. On the 5th day after TBI, the brains were subsequently removed, fixed in 10% formalin solution, set in paraffin, cut (5–7 μm serial sections) and stained with hematoxylin-eosin and thionine. Microscopic examination and photo registration of tissue slices (×100) were performed with the use of Nikon Eclipse E200 microscope with ImageView software. Comparative histopathological and quantitative analysis of the samples was performed using QuPath and ImageJ software. Data are presented as M (25%; 75%), where M is the median, (25%; 75%) – the quartile interval between 25th and 75th percentiles.

Results and Discussion

On the 5th day after TBI the general number of neurons in the cortex of rats decreased almost two-fold. This decrease was observed in the damaged hemisphere and in the contralateral one (Fig). The medians were correspondingly 65 (59;76) and 72 (58;86) compared to 129 (125;147) in control (p = 0,000005, Mann-Whitney U-test).

Three i.p. injection of progenitor cells CM significantly increased the number of neurons in cortex of rats on 5th day after TBI compared to this value in rats with TBI with no additional treatment: the medians were

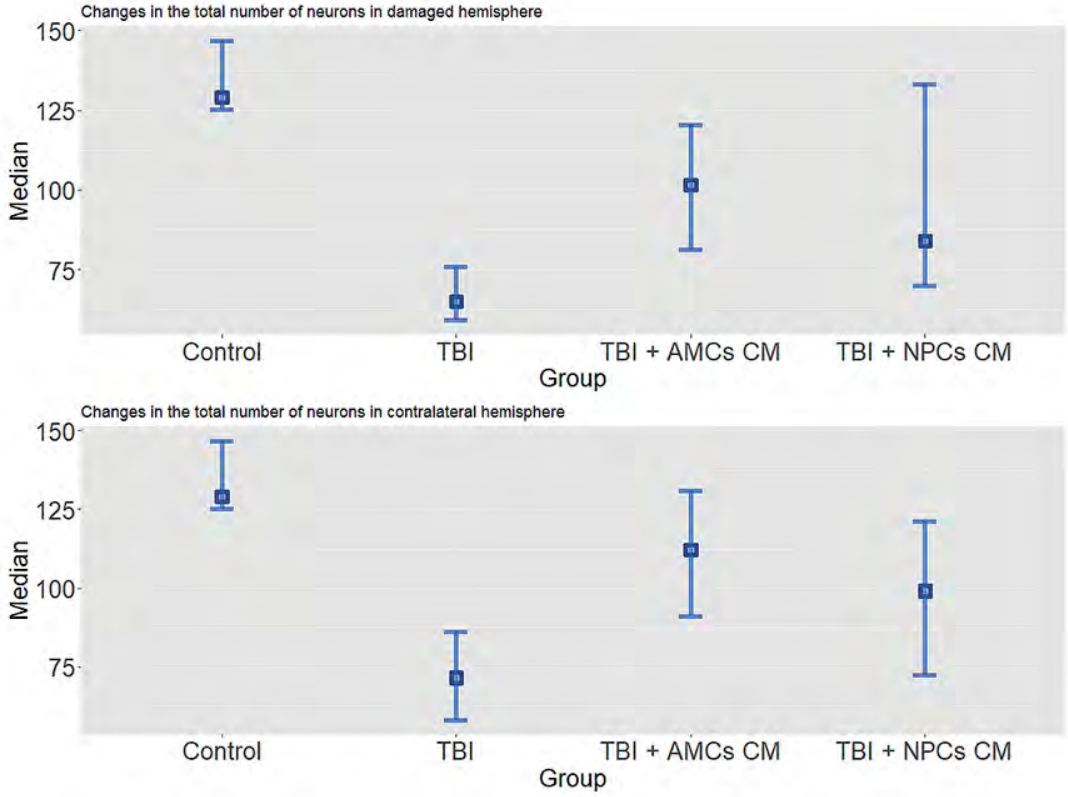

Fig. **The effect of conditioned media obtained from neurogenic progenitor cells and adipose-derived mononuclear cells on the total number of neurons in mice brain cortex (test area 2.55 mm^2),** $P \leq 0.05$

correspondingly 84 (70;133) and 99 (72;121) in damaged and contralateral hemisphere after NPCs CM impact ($P = 0{,}002$, $P = 0{,}02$, Mann-Whitney U-test) as well as 102 (81;120) and 112 (91;131) correspondingly after AMCs CM influence ($P = 0{,}000002$, $P = 0{,}00004$, Mann-Whitney U-test).

The increase of the neurons number in the brain cortex of rats treated with NPCs CM and AMCs CM after TBI comparing to untreated ones suggests the general neuroprotective effect of CM obtained from these cells on nervous tissue. The increase in total number of neurons in damaged as well as unaffected hemispheres could indicate anti-apoptotic and life-sustaining effect of the media. While comparing the action of CM of different origin slightly higher potential of AMCs CM should be noted. At the same time, the regenerative impact of CM of both origins should be further investigated.

Generally, obtained data confirm the concept regarding the paracrine mechanism of influence of the used progenitor cells due to their secretome [2, 3, 5]. In particular, such biologically active molecules — known components of the NPCs and MMSCs secretome as NGF, BDNF, CNTF, GDNF [2, 6], that prevent death and enhance the regeneration of target cell populations — can contribute to the neuroprotective effects of NPCs CM and AMCs CM.

Conclusions

The study demonstrates that conditioned media obtained from neurogenic progenitor cells and adipose-derived mononuclear cells (as a source of mesenchymal multipotent stromal cells) have potential as a therapy for damaged nervous tissue. They also might be considered as an additional treatment mode of traumatic brain injury.

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