The Role of MARK ERK1/2 and p38 in Regulation of Functions of Neural Stem Cells and Neuroglia under Conditions of β-Amyloid-Induced Neurodegeneration

G. N. Zyuz'kov, L. A. Miroshnichenko, A. V. Chaikovsky, and L. Yu. Kotlovskaya

Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 173, No. 4, pp. 431-435, April, 2022

Original article submitted January 28, 2022

The role of ERK1/2 and p38 in the realization of the growth potential of neural stem cells and secretion of neurotrophic growth factors by glial cells was studied using in vitro model of β-amylloid-induced neurodegeneration. It was shown that amyloid-β fragment 25-35 significantly inhibits the cell cycle progression of neural stem cells against the background of stimulation of their differentiation and reduced production of growth factors by neuroglia. The inhibitory role of ERK1/2 and p38 in relation to the proliferative activity of neural stem cells and the secretory activity of glial elements was revealed. ERK1/2 and p38 inhibitors increased proliferation of progenitor cells of the nervous tissue and reduced the intensity of their specialization, as well as stimulated production of growth factors by neuroglial cells under conditions of simulated β-amylloid-induced neurodegeneration.

Key Words: Alzheimer’s disease; neural stem cells; neuroglia; intracellular signal transduction; mitogen-activated protein kinases

Alzheimer’s disease (AD) is the most common form of dementia in the elderly. In recent years, a dramatic increase in the incidence of AD and a significant decrease of the debut age are observed [2,8]. AD is characterized by a progressive impairment of cognitive functions and the loss of practical skills and self-care abilities, which ultimately leads to death due to causes unrelated to the underlying disease (infections, food aspiration, etc.). The development of these disorders occurs against the background of decompensation of adaptive mechanisms in various compartments of the nervous tissue [7,15]. The synthesis of neurotoxic β-amylloid peptides and the formation of neurofibrillary tangles are accompanied by a significant reorganization of the CNS and the formation of a qualitatively new pathogenic pattern of activity of individual brain structures [1,5].

Currently used drug therapy is based on the impact on the three main putative causes of AD: cholinergic, amyloid, and tau-protein hypotheses [6,8]. However, the drugs developed within the framework of these concepts are little effective [3], which indicates the relative failure of the cholinergic, amyloid, and tau-hypotheses, at least as the trigger mechanisms for the formation of the pathology. However, the neurotoxic effect of β-amylloid (Aβ) and its critically important pathogenetic role in AD course are beyond doubt [9].

The death of neurons and destruction of intercellular contacts (synapses) under the influence of Aβ are observed against the background of the loss of the ability for balanced neuro-, neurito-, and synaptogenesis in the nervous tissue [2]. Therefore, stimulation of neurogenesis coordination with pharmacological agents, regulators of regeneration-competent cells of various classes (including neural stem cells (NSC) and neuroglial elements), it a promising approach in the search for AD therapy options [11-15]. This approach
The studies were carried out on C57BL/6 mice (n=30, age 2-2.5 months, body weight 20-22 g), the 1st category (conventional mice) obtained from the Department of Pharmacology and Regenerative Medicine. The study was conducted in compliance with the principles of humane treatment of experimental animals and were approved by the Local Ethical Committee.

Amyloid β Fragment 25-35 (Calbiochem) was used to simulate Aβ-induced neurodegeneration in vitro. This neurotoxic agent in a concentration of 1 mM was incubated for 7 days at 37°C, 5% CO₂, and 100% humidity for protein aggregation. Then, Aβ was introduced in vitro into the culture medium to a final concentration of 20 μM.

Using cultural methods, we studied the direct effect of MAPK inhibitors ERK1/2 (PD98059) and p38 (SB202190) (Calbiochem) on the realization of the growth potential of NSC and the secretion of growth factors by glial cells in vitro. The working concentration of inhibitors was determined in preliminary experiments and was in vitro 100 and 10 μM, respectively. Cell cultures without inhibitors of signaling molecules served as controls.

NSC were studied by culturing unfractionated cells of the subventricular zone of the cerebral hemispheres. To this end, cells at a concentration of 10⁵/ml were incubated in MACS Neuro Medium (Miltenyi Biotec) for 5 days in a CO₂ incubator at 37°C, 5% CO₂, and 100% air humidity. After incubation, the content of clonogenic cells, in particular NSC (CFU-N, neurospheres containing >100 cells) and committed neural precursors (CIFU-N, neurospheres consisting of 30-100 cells) were counted, their mitotic activity and intensity of specialization of CFU were evaluated. The proliferative activity of progenitor cells was assessed by the hydroxyurea cell suicide method. The intensity of specialization processes (differentiation/maturation) of progenitor elements was determined by calculating the CIFU/CFU ratio (differentiation index) [14,15].

The secretory function of glial cells, i.e. total production of growth factors stimulating CFU-N (colony-stimulating activity) was assessed by the effect of conditioned media of 2-day cell cultures of the subventricular region of the cerebral hemispheres (containing Aβ and inhibitors of ERK1/2 or p38) on the level neurosphere formation in the test-system, a culture of intact cells of the subventricular zone (concentration 10⁵/ml) in MACS Neuro Medium [12,15].

The results were processed by the method of variation statistics using the Statistica 6.0 software (StatSoft, Inc.) using the nonparametric Mann—Whitney U test (mean value of the indicator, the significance of differences in indicators between groups at p<0.05).
The revealed phenomena correspond to the published data on the disruption of the mitotic activity of the ancestral cells of the nervous tissue under the influence of Aβ [2,6]. At the same time, the results obtained indicate a significant uncoupling of the processes of NSC proliferation and differentiation under conditions of Aβ-induced neurodegeneration. At the same time, it is known that accelerated maturation of progenitor elements can be accompanied by de novo development of functionally defective mature cells [5,9]. The detected reaction of neuroglia under the action of Aβ should also be considered ambiguous. The revealed decrease in the colony-stimulating activity of supernatants obtained by culturing neuroglia in the presence of a toxic agent could be associated with both a decrease in the secretion of growth factors and an increase in the production of inhibitors of NSC proliferation by glial cells. These substances include a wide range of proinflammatory cytokines produced primarily by microglial cells [6,12,15]. At the same time, it is believed that pronounced inflammatory re-

action in the nervous tissue in AD has a pathogenic significance [6].

The study of the involvement of MAPK-dependent signal transduction pathways in the realization of the growth potential of neural progenitors revealed a number of interesting phenomena. The introduction of ERK1/2 and p38 inhibitors into the culture medium in both cases (in the presence and without Aβ) led to an increase in the level of colony formation and the rate of CFU-N division (Fig. 1, a, c). The number of CFU-N and their mitotically active forms under conditions of neurodegeneration modeling reached 138.6 and 187.1% with ERK1/2 blockade and 188.7 and 243.5% with p38 inactivation from the corresponding control levels (Fig. 1). Moreover, the values of these indicators under the influence of ERK1/2 and p38 inhibitors were achieved under conditions of modeling Aβ-induced neurodegeneration, values similar to those in the medium without Aβ. That is, the pharmacological agents used completely leveled the negative effect of Aβ on the progression of the NSC cell cycle. In

---

**Fig. 1.** Number of CFU-N (a), CFU-N (b), proportion of S-phase CFU-N (c), and intensity of CFU-N differentiation (d) in the cell culture of the subventricular zone of the brain of intact C57BL/6 mice incubated without βA (intact culture) and with βA after addition of inhibitors of ERK1/2 and p38. *p*<0.05 in comparison with *intact culture, *culture without inhibitors.*

- No inhibitors
- ERK1/2 inhibitor
- p38 inhibitor

Arb. units

%
addition, inhibitors of ERK1/2 and p38 in the presence of Aβ reduced the differentiation index of progenitor cells to that of intact cells (Fig. 1, d). Disruption of signal transmission through protein kinases was also accompanied by a significant increase in the colo-ny-stimulating activity of conditioned media of nerve cells during their cultivation both in the presence of Aβ and without it (Fig. 2). Under conditions of modeled neurodegeneration with blockade of ERK1/2 and p38, this parameter increased to 855.6 and 411.1% of the control level (medium with Aβ without inhibitors of signaling molecules), respectively.

In general, the results of studies indicate a pro-nounced discoordination of the functioning of NSC and a violation of the implementation of the humoral neurotrophic function (aimed at stimulating the pro-gression of the cell cycle [11,12]) by neuroglial cells under the influence of Aβ. The revealed changes (de-synchronization of the functions of regeneration-com-petent cells) can cause the formation of mature cells of the nervous tissue, which have certain functional “defects” [5,9], which are under the influence of phosphorylated tau-proteins and disorders of the cholinergic system in AD [8,9] in situ will only be more pronounced and aggravated. At the same time, it was shown that ERK1/2 and p38-dependent signaling play an important role in the development of the discov-ered mechanisms of disadaptation [9,10,12]. The pos-sibility of conjugation of the processes of proliferation and differentiation of NSC, as well as activation of the compensatory response of neuroglia in Aβ-induced neurodegeneration using selective inhibitors of ERK1/2 and p38, was demonstrated for the first time.

The results indicate the expediency of finding a solution to the problem of neurogenesis disorders in AD in the framework of the “Strategy of Target-ed Pharmacological Regulation of Intracellular Signal Transduction in Regeneration-Competent Cells” [10-15]. At the same time, the prospect of developing approaches to stimulate coordinated (full-fledged) neuroregeneration based on ERK1/2 and p38 inhibitors is obvious.

The study was supported by the Russian Science Foundation (grant No. 22-25-00069; https://rsfc.ru/project/22-25-00069/).

REFERENCES

1. Coronel R, Lachgar M, Bernabeu-Zornoza A, Palmer C, Dominguez-Alvaro M, Revilla A, Ocaña I, Fernández A, Martínez-Serrano A, Cano E, Liste I. Neuronal and glial differentiation of human neural stem cells is regulated by amyloid precursor protein (APP) levels. Mol. Neurobiol. 2019;56(2):1248-1261. doi: 10.1007/s12035-018-1167-9
2. Han F, Bi J, Qiao L, Arancio O. Stem cell therapy for Alzheimer's disease. Adv. Exp. Med. Biol. 2020;1266:39-55. doi: 10.1007/978-981-15-4370-8_4
3. Kaeser G, Chun J. Brain cell somatic gene recombination and its phylogenetic foundations. J. Biol. Chem. 2020;295(36):12 786-12 795. doi: 10.1074/jbc.REV120.009192
4. Lee MH, Siddoway B, Kaeser GE, Segota I, Rivera R, Romanow WJ, Liu CS, Park C, Kennedy G, Long T, Chun J. Somatic APP gene recombination in Alzheimer's disease and normal neurons. Nature. 2018;563:639-645. doi: 10.1038/s41586-018-0718-6
5. Lu J, Li Y, Molinari C, Garaci E, Merlo D, Pei G. Amyloid-β oligomers-induced mitochondrial DNA repair impairment contributes to altered human neural stem cell differentiation. Curr. Alzheimer Res. 2019;6(10):934-949. doi: 10.2174/1567205016666191023104036
6. Ozben T, Ozben S. Neuro-inflammation and anti-in-flammatory treatment options for Alzheimer’s disease. Clin. Biochem. 2019;72:87-89. doi: 10.1016/j.clinbiochem.2019.04.001
7. Udut VV, Naumov SA, Evtushenko DN, Udut EV, Naumov SS, Zyuz’kov GN. A case of xenon inhalation therapy for respiratory failure and neuropsychiatric disorders associated with COVID-19. EXCLI J. 2021;20:1517-1525. doi: 10.17179/excli2021-4316
8. Vaz M, Silvestre S. Alzheimer’s disease: Recent treat-ment strategies. Eur. J. Pharmacol. 2020;887:173554. doi: 10.1016/j.ejphar.2020.173554
9. Wang Z, Chen Y, Li X, Sultana P, Yin M, Wang Z. Amyloid-β1-42 dynamically regulates the migration of neural stem/progenitor cells via MAPK-ERK pathway. Chem. Biol. Interact. 2019;298:96-103. doi: 10.1016/j.cbi.2018.11.001
10. Zyuz’kov GN. Targeted regulation of intracellular signal transduction in regeneration-competent cells: a new di-rection for therapy in regenerative medicine. Biointerface Res. Appl. Chem. 2021;11(4):12 238-12 251. doi: 10.33263/briarc11.1223812251
11. Zyuz’kov GN, Miroshnichenko LA, Polykova TY, Simani-na EV, Stavrova LA. Targeting cAMP-pathway in rege-
neration-competent cells of nervous tissue: potential to create a novel drug for treatment of ethanol-induced neurodegeneration. Cent. Nerv. Syst. Agents Med. Chem. 2021;21(3):172-180. doi: 10.2174/1871524921666210907102

12. Zyuz’kov GN, Miroshnichenko LA, Polyakova TY, Zhdanov VV, Simanina EV, Stavrova LA, Churin AA, Fomina TI. Role of MAPK ERK1/2 and p38 in the regulation of secretory functions of different populations of neuroglia in ethanol-induced neurodegeneration. Bull. Exp. Biol. Med. 2021;171(6):699-703. doi: 10.1007/s10517-021-05298-x

13. Zyuz’kov GN, Miroshnichenko LA, Polyakova TY, Zhdanov VV, Simanina EV, Stavrova LA, Danilets MG. Specific features of intracellular signal transduction in the regulation of functions of neural stem cells and committed neuronal progenitors. Bull. Exp. Biol. Med. 2021;170(4):522-527. doi: 10.1007/s10517-021-05100-y

14. Zyuz’kov GN, Miroshnichenko LA, Simanina EV, Stavrova LA, Polykova TY. Intracellular signaling molecules of nerve tissue progenitors as pharmacological targets for treatment of ethanol-induced neurodegeneration. J. Basic Clin. Physiol. Pharmacol. 2021. doi: 10.1515/jbcpp-2020-0317

15. Zyuz’kov GN, Stavrova LA, Miroshnichenko LA, Polyakova TYu, Simanina EV. Prospects for the use of NF-κB inhibitors to stimulate the functions of regeneration-competent cells of nerve tissue and neuroregeneration in ethanol-induced neurodegeneration. Biointerface Res. Appl. Chem. 2021;11(1):8065-8074. doi: 10.33263/BRIAC111.80658074