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New Insights into Alzheimer's Drug Discovery Targeting NF-κB

Gleb Nikolaevich Zyuz'kov [1,*], Larisa Arkad`evna Miroshnichenko [1], Larisa Yur`evna Kotlovskaya [1], Alexander Vasil`evich Chayikovskyi [1]

[1] Laboratory of Pathological Physiology and Experimental Therapy, Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russia, 3 Lenin Prospect, Tomsk, 634028, Russia; nii@pharmso.ru (G.N.Z..);
* Correspondence: zgn@pharmso.ru (G.N.Z.);

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Abstract: The lack of effective treatments for Alzheimer's disease (AD) makes it highly relevant to develop fundamentally new drugs for its therapy. Promising is the creation of approaches to stimulate neurogenesis. The search for targets among intracellular signaling molecules is promising as part of implementing this direction. Much interest is focused on NF-κB. The aim of the work was to study the effect of the NF-κB inhibitor on CNS dysfunction, the realization of the growth potential of nervous tissue progenitors (neural stem cells (NSCs) and neuronal-committed progenitors (NCPs)) and the secretion of neurotrophic factors by neuroglial cells (astrocytes, oligodendrocytes, microglial cells) in aged mice as a model of Alzheimer's disease (16-month-old C57BL/6 male mice). Significant changes in maladaptive exploratory behavior and disturbances in conditioned reflex activity with signs of senile dementia were revealed in aged mice. The course administration of the NF-κB inhibitor led to a significant correction of the decline in age-related memory and exploratory behavior. This developed against the background of the increase in the content of NSCs and NCPs in the subventricular zone of the cerebral hemispheres. Simultaneously, the mitotic activity of progenitors increased. Moreover, in the NSCs, the change in this parameter was significantly more pronounced than in the NCPs. Also, there was a significant decrease in the NSC intensity specialization. In addition, a decrease in the production of neurotrophic growth factors by astrocytes was revealed with the increase in their secretion by oligodendrocytes and microglial cells under the influence of the NF-κB inhibitor. The results open new prospects for discovering drugs for Alzheimer's disease based on NF-κB inhibitors.

Keywords: Alzheimer's disease; neuroregeneration; neurogenesis; exploratory behavior; old mice; neural stem cells; intracellular signal transduction; NF-κB.

1. Introduction

Alzheimer's disease (AD) is a steadily progressive neurodegenerative disease most common in countries with high life expectancy [1, 2]. The currently used drug approaches to AD therapy, based on the protection and/or modulation of the functions of mature cells of the nervous tissue preserved under pathological conditions, are not able not only to restore the CNS functions lost in AD but also to prevent the progression of the disease [1-3].

Despite the unknown etiology of this disease [4, 5], many aspects of its pathogenesis have been studied. For example, there is no doubt about the neurotoxicity of pathogenic fragments of β-amyloid (Aβ) on nerve cells [6, 7], the pathogenic role of tau proteins [8],

somatic mutations [9, 10], cholinergic disorders [11], etc. At the same time, it has been experimentally proven that the dysfunction of neurons and their death under conditions of the formation of a pathological biochemical continuum in the nervous tissue occurs against the background of the loss of its plasticity and the ability to neurogenesis [4, 5, 7].

Previously, we have shown that impaired neurogenesis in AD occurs due to the disadaptation of the system of cellular renewal of the nervous tissue [5, 7, 9]. Under the conditions of the development of this disease, there is a desynchronization of the activity of neural stem cells (NSCs) and neuronal-committed progenitors (NCPs). Moreover, the appearance of this phenomenon is accompanied by the dissociation of proliferation and differentiation of NSCs against the background of impaired secretory function of neuroglial cells [7, 9].

Proceeding from this, the development of pharmacological methods of AD therapy based on the principle of regulating the functions of regeneration-competent cells (RCCs) of the nervous tissue and coordinating the functions of their individual representatives (NSCs, committed precursors, neuroglial cells) looks promising [5, 7, 9]. The implementation of this approach looks promising within the framework of the "Strategy of targeted regulation of intracellular signal transduction in RCCs" [12-17]. In this case, the effectiveness of the impact is determined by the unique role of several intracellular signaling molecules in the regulation of the functions of certain progenitors of the nervous tissue and/or cells that regulate their functions (primarily, neuroglial cells) [12, 13, 18].

Based on previous in vitro experiments, we hypothesized that NF-κB-signaling in RCCs acts as one of the mechanisms of disadaptation of the nervous tissue cell renewal system in AD [7, 13, 19]. At the same time, under in vitro conditions, the ability of NF-κB inhibitors to synchronize the activity of NSCs and NCPs was revealed. In addition, it was found that the blockade of nuclear transcription factors is accompanied by an increase in the proliferative activity of NSC and its association with their differentiation [13]. Moreover, such targeted regulation of intracellular signal transduction, as it turned out, can significantly correct Aβ-induced disturbances in neurotrophin secretion by certain types of neuroglial cells [7]. There is also strong data on the prospect of using NF-κB inhibitors as drugs for treating AD due to their anti-inflammatory properties [20].

This work aimed to study the effect of the NF-κB inhibitor on disorders of the CNS activity in aged C57BL/6 mice as a model of Alzheimer's disease and on the functioning of neural tissue progenitors and neuroglial cells of various types in them.

2. Materials and Methods

2.1. Chemicals and drugs.

Amyloid β-Protein Fragment 25-35 (Sigma-Aldrich, CIIIA); NF-κB inhibitor JSH-23 (J4455, Sigma-Aldrich, USA); MACS Neuro Medium; anti-PSA-NCAM MicroBeads; anti-ACSA-2 MicroBead Kit; Anti-O4 MicroBeads; Anti-CD11b (Microglia) MicroBeads (all manufactured by Miltenyi Biotec, Germany); hydroxyurea (Calbiochem, USA); dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA); Primaria Cell Culture Plate (size 96 well) (Corning, USA).

2.2. Animals and experimental design.

Experimental work complied with the principles of humane experimental technique (EU Directive 2010/63/EU for animal experiments). The study was approved by the Institute`s local Ethics Committee (Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences) (protocol GRIPh & RM-2022-01/12). The studies were carried out on male C57BL/6 mice aged 16 months (n=68). The aged C57BL/6 mice are considered to be quite adequate models of endogenous Aβ plaque formation and AD [21-23]. Moreover, this model is devoid of many disadvantages typical for various transgenic mouse models AD [23, 24].

When assessing the CNS activity disorders in aged mice, 2-month-old male C57BL/6 mice (n=14) were used as controls. The mice were obtained from the Department of Experimental Biological Models of the Tomsk National Research Medical Center. The experiments were carried out in the autumn-winter period. Animals were sacrificed by decapitation under deep anesthesia in a CO_2 chamber.

The percentage survival of male C57BL/6 mice at the age of 16 months was 87.5% (4 out of 32 mice died). At 16 months, mice were randomly selected into the experimental and control groups. The NF-κB inhibitor was injected subcutaneously daily for 7 days at a 1 mg/kg dose. The control 2-month-old and old mice received subcutaneously in a similar mode in an equivalent volume of the solvent (0.2 ml of 0.5% DMSO).

On days 1, 3, 7, 14, and 21 after the start of administration of the NF-κB inhibitor, the exploratory behavior of mice was studied. On day 1, the conditioned passive avoidance reflex was developed. On days 3, 7, and 21, the reproducibility of the conditioned passive avoidance response (CPAR) was tested (n=14 in the experimental and control groups).

On days 3, 7, and 14, the content of NSCs and NCPs in the subventricular zone of the cerebral hemispheres (SVZ), their proliferative activity, and the intensity of specialization (differentiation/maturation) were studied. During these periods, the production of neurotrophic growth factors by neuroglial cells (astrocytes, oligodendrocytes, microglial cells) was also determined (n=6 in the experimental and control groups (aged mice)).

2.3. Testing exploratory behavior.

The exploratory behavior was studied in the open field test [14]. Testing exploratory behavior in an open field is one of the most frequently used methodological techniques to judge the CNS's functional state. The “open field” experimental setup was a 40×40×20 cm chamber with a square floor and white walls. Its floor, divided into 16 squares, had a round hole 3 cm in diameter in each of them. From above, the chamber was illuminated by a 100 W lamp located at a height of 1 m from the floor. The mouse was placed in one of its corners, and the behavior was recorded for 3 min. Total, horizontal, and vertical locomotor activity, hole-board exploration, self-grooming, and defecation were assessed. The coefficient of asymmetry of the locomotor activity was also calculated (the ratio of horizontal activity to the total number of actions). The indicators of the first minute and subsequent 2-3 minutes were studied separately.

2.4. Testing conditional reflex activity.

To study the effect of a pharmacological substance on the mnestic function of the brain, we used the CPAR test [14]. The test demonstrates the reproduction of the acquired skill of passive avoidance by mice of electric pain stimulation (with a current of 0.45 mA) that occurs

when they enter the dark compartment of a special chamber. The method is based on suppressing the innate unconditioned reflex preference for dark space, which is available in rodents. To do this, they had to be in the light compartment of the camera. The reflex was considered to be developed if, during all 3 minutes of observation, the animal never visited the dark compartment. The quality of the reflex was estimated by the proportion of animals that developed this reflex.

2.5. Progenitor cells study.

The cellular material for the study was taken from the subventricular area of the cerebral hemispheres (SVZ). The content of NSCs and NCPs was determined by the number of CFU in the unfractionated and $CD56^+$ cell culture, respectively. $CD56^+$ ($PSA\text{-}NCAM^+$) cells were obtained by positive selection using an immunomagnetic separator MIniMACS Cell Separator (Miltenyi Biotec, Germany) [13, 18]. The cells at a concentration of 10^5 / ml were incubated in MACS Neuro Medium for 5 days in a CO_2 incubator Thermo Scientific 8000 DH (Thermo Fisher Scientific Inc., USA) при 37^0C, 5% CO2 и 100% влажность воздуха (under standard conditions). After incubation, the content of clonogenic cells, their mitotic activity, and the intensity of specialization were calculated. The number of NSCs and NCPs was determined by the colony-forming capacity of the respective cell cultures (CFU, neurospheres containing more than 100 cells). The proliferative activity of CFUs was assessed by the cell suicide method using hydroxyurea (at a concentration of 1 μM) inhibiting DNA synthesis in the S phase of the cell cycle. The intensity of specialization (differentiation/maturation) of progenitors was determined by the ratio of the corresponding cluster-forming units (ClFU, neurospheres of 30 - 100 cells) to CFU (specialization index).

2.6. Neuroglial cells study.

Using anti-ACSA-2 MicroBead, Anti-O4 MicroBeads, and Anti-CD11b MicroBeads and the immunomagnetic separator MIniMACS Cell Separator, the following fractions were isolated from the cells of the SVZ of experimental animals by positive selection: astrocytes, oligodendrocytes, and microglial cells, respectively. The isolated cells were incubated in MACS Neuro Medium in a Thermo Scientific 8000 DH CO_2 incubator at a concentration of 2×106/mL for 2 days to obtain supernatants (under standard conditions). To determine their secretory activity (production of neurotrophins, a combination of growth factors active against CFU), the effect of conditioned media on the level of neurosphere formation (neurospheric stimulating activity, NSA) in the test system was studied [7, 17].

2.7. Statistical analysis.

The results were analyzed by one-way ANOVA followed by Dunnett's test, Wilcoxon test for dependent samples, and Mann-Whitney test for independent samples. The data are expressed: in tables as arithmetic means and standard error of the mean (M±SEM); in the figures as arithmetic means—significance level $p < 0.05$ [25].

3. Results and Discussion

3.1. Neuropsychiatric disorders in aged mice.

In aged mice, a significant decrease in locomotor activity was observed on all days of the experiment (Table 1). At the same time, a decrease in total, horizontal, and vertical locomotor activity, as well as hole-board exploration, was recorded in the first (1 min) and in the second (2-3 min) monitoring periods. However, the most pronounced were the changes in vertical locomotor activity and hole-board exploration in the open field test. For example, the decrease in vertical locomotor activity in aged mice on the 14th and 21st days of observation reached 6.1% and 3.4% (in 1 min) and 16.7% and 8.3% (in 2-3 min) from similar parameters in 2-month-old mice, respectively. This was the reason for a very significant increase in the coefficient of asymmetry of the locomotor activity in aged mice on all days of the experiment. While this was noted against the background of an increase in the number of self-grooming. The revealed shifts in the behavior of age-related animals indicated not only the presence of locomotor problems (associated with age-related degenerative changes in the musculoskeletal system [26]). They showed the development of pronounced disturbances in exploratory behavior and cognitive functions of the CNS [21, 22, 27].

In addition, in aged mice, there was a sharp drop in the level of CPAR reproduction on the 3rd, 7th, 14th, and 21st days (up to 18.2% of the level of the same indicator in 2-month-old mice on the 21st day) (Table 2).

Thus, the data of studies [21, 26, 27] on the development of senile dementia, maladaptive exploratory behavior, and other disorders of cognitive activity in aged C57BL/6 mice characteristic of AD were confirmed.

Table 1. Parameters of exploratory behavior in 2-month-old male C57BL/6 mice (1); aged male C57BL/6 mice (2); aged male C57BL/6 mice treated with the NF-κB inhibitor (3), arb. units (M±SEM).

Groups	Horizontal locomotor activity	Vertical locomotor activity	Hole-board exploration	Self-grooming	Defecation	Total locomotor activity	Asymmetry coefficient
Day 1							
Frist period (minute 1)							
1	17.40 ± 0.92	10.80 ± 0.79	5.33 ± 0.30	0.87 ± 0.22	1.07 ± 0.30	35.47 ± 1.94	0.49 ± 0.01
2	8.54 ± 1.09*	2.62 ± 0.54*	2.54 ± 0.48*	0.92 ± 0.50	0.38 ± 0.14	15.00 ± 1.60*	0.57 ± 0.03*
3	6.14 ± 1.57*	1.79 ± 0.59*	1.43 ± 0.64*	0.43 ± 0.20	0.57 ± 0.25	10.36 ± 2.22*	0.60 ± 0.07
Second period (minutes 2-3)							
1	36.93 ± 1.61	12.53 ± 0.84	15.47 ± 1.43	0.53 ± 0.17	0.40 ± 0.19	65.87 ± 2.62	0.56 ± 0.01
2	14.69 ± 1.97*	2.62 ± 0.55*	1.69 ± 0.43*	2.38 ± 0.89*	0.77 ± 0.23	22.15 ± 2.53*	0.67 ± 0.04*
3	12.14 ± 3.28*	3.79 ± 1.01*	3.50 ± 0.64*#	1.57 ± 0.37*	0.71 ± 0.24	21.71 ± 4.72*	0.49 ± 0.05#
Day 3							
Frist period (minute 1)							
1	19.87 ± 1.28	11.87 ± 0.87	6.33 ± 0.73	0.07 ± 0.07	0.47 ± 0.22	38.60± 2.40	0.52 ± 0.01
2	12.62 ± 2.16*	2.15 ± 0.75*	1.77 ± 0.35*	1.15 ± 0.30*	0.08 ± 0.08	17.77 ± 2.76*	0.70 ± 0.06*
3	12.77 ± 0.88*	1.54 ± 0.46*	2.69 ± 0.28*#	1.00 ± 0.34*	0.00 ± 0.00	18.00 ± 1.53*	0.65 ± 0.04*
Second period (minutes 2-3)							
1	28.93 ± 1.85	14.87 ± 0.98	8.67 ± 0.97	1.47 ± 0.31	0.40 ± 0.19	54.33 ± 2.47	0.53 ± 0.02
2	16.92 ± 1.20*	3.54 ± 0.90*	1.31 ± 0.47*	2.62 ± 0.90	0.77 ± 0.17	25.15 ± 2.34*	0.70 ± 0.05*
3	14.62 ± 2.67*	2.62 ± 0.59*	4.69 ± 1.05#	2.62 ± 0.35	1.00 ± 0.32	25.54 ± 3.96*	0.53± 0.04#
Day 7							
Frist period (minute 1)							
1	13.93 ± 0.86	11.33 ± 0.60	3.53 ± 0.40	0.33 ± 0.19	0.33 ± 0.19	29.53 ± 1.43	0.47 ± 0.01
2	10.58 ± 1.02*	0.58 ± 0.26*	1.42 ± 0.40*	1.08 ± 0.34	0.17 ± 0.11	13.83 ± 2.54*	0.72 ± 0.07*
3	7.79 ± 1.86*	0.86 ± 0.33*	1.43 ± 0.50*	2.71 ± 0.32*#	0.14 ± 0.10	12.93 ± 2.26*	0.46 ± 0.07#
Second period (minutes 2-3)							
1	20.61 ± 1.20	9.93 ± 0.76	2.67 ± 0.42	2.00 ± 0.31	1.07 ± 0.30	34.27 ± 3.42	0.52 ± 0.02
2	16.08 ± 1.07*	2.83 ± 0.37*	1.25 ± 0.35	1.67 ± 0.33	0.83 ± 0.24	22.67 ± 2.79*	0.72 ± 0.03*
3	15.43 ± 2.09	4.29 ± 0.42*#	4.50 ± 1.11#	2.50 ± 0.56	0.43 ± 0.14	27.14 ± 3.86	0.58 ± 0.03#

Groups	Horizontal locomotor activity	Vertical locomotor activity	Hole-board exploration	Self-grooming	Defecation	Total locomotor activity	Asymmetry coefficient
Day 14							
Frist period (minute 1)							
1	19.47 ± 1.69	13.67 ± 1.43	4.00 ± 0.57	0.53 ± 0.19	0.40 ± 0.16	38.07 ± 3.29	0.52 ± 0.02
2	12.83 ± 1.73*	0.83 ± 0.27*	1.75 ± 0.45*	1.25 ± 0.41	0.42 ± 0.19	17.08 ± 2.17*	0.74 ± 0.03*
3	10.73 ± 1.26*	2.00 ± 0.36*#	1.36 ± 0.34*	0.95 ± 0.21	0.18 ± 0.12	14.73 ± 1.67*	0.73 ± 0.02*
Second period (minutes 2-3)							
1	28.73 ± 2.93	15.47 ± 1.06	5.53 ± 0.62	1.93 ± 0.32	0.73 ± 0.21	52.40 ± 4.21	0.54 ± 0.02
2	14.42 ± 1.27*	2.58 ± 0.45*	1.50 ± 0.34*	1.50 ± 0.44	0.42 ± 0.15	20.42 ± 1.59*	0.70 ± 0.03*
3	11.82 ± 1.58*	3.18 ± 0.63*	1.55 ± 0.41*	1.91 ± 0.37	1.18 ± 0.33	19.64 ± 2.29*	0.60 ± 0.03#
Day 21							
Frist period (minute 1)							
1	20.07 ± 1.26	14.53 ± 1.09	3.60 ± 0.61	0.73 ± 0.23	0.47 ± 0.17	39.40 ± 2.58	0.51 ± 0.01
2	13.08 ± 1.27*	0.58 ± 0.34*	0.83 ± 0.21*	1.25 ± 0.48	0.25 ± 0.13	16.00 ± 1.67*	0.83 ± 0.03*
3	13.45 ± 2.75*	1.64 ± 0.51*	2.36 ± 0.69	0.73± 0.27	0.36 ± 0.20	18.55 ± 3.75*	0.71 ± 0.03*#
4	8.13 ± 1.37*	0.50 ± 0.14*	0.73 ± 0.23*	0.80 ± 0.28	0.27 ± 0.15	10.13 ± 1.63*	0.71 ± 0.08*
Second period (minutes 2-3)							
1	28.6 ± 1.90	15.00 ± 0.87	5.60 ± 0.61	1.20 ± 0.24	0.53 ± 0.24	50.93 ± 2.85	0.56 ± 0.01
2	9.33 ± 2.17*	1.25 ± 0.35*	0.67 ± 0.26*	1.00 ± 0.37	0.42 ± 0.15	12.67 ± 2.44*	0.73 ± 0.06*
3	10.91 ± 2.28*	1.73 ± 0.49*	2.00 ± 0.36*#	2.18 ± 0.26#	0.82 ± 0.23	17.64 ± 2.87*	0.45± 0.08#
4	6.13 ± 0.95*	0.47 ± 0.27*	1.78 ± 0.30*#	1.07 ± 0.18	1.07 ± 0.27	10.47 ± 1.15*	0.56 ± 0.05#

Here and in table 2: $p < 0.05$ in comparison with *male C57BL/6 mice at the age of 2 months, #aged male C57BL/6 mice.

Table 2. Parameters of conditioned passive avoidance response in 2-month-old male C57BL/6 mice (1); aged male C57BL/6 mice (2); aged male C57BL/6 mice treated with the NF-κB inhibitor (3), (M±SEM).

Day	Groups	Percentage of animals with reflex,%
7	1	92,86 ± 7.14
	2	35.71 ± 13.29*
	3	78.57 ± 11.38#
14	1	85.71 ± 9.71
	2	28.57 ± 12.53*
	3	78.57 ± 11.38#
21	1	78.57 ± 11.38
	2	14.29 ± 9.71*
	3	64.29 ± 13.29#

3.2. Effects of the NF-κB inhibitor on neuropsychiatric disorders in aged mice.

The administration of the NF-κB inhibitor significantly corrected the exploratory behavior in aged mice. There was an increase in vertical locomotor activity (on days 7, 14) and hole-board exploration (on days 1, 3, 7, 21) in the open field test. The changes in these parameters were recorded in the first (1 min) and the second (2-3 min) observation period. This indicates the influence of the pharmacological agent not only on the cognitive activity of the animals but also on its anxiolytic effects and a decrease in the excitability of aged mice (as evidenced by the change in indicators in 1 min) [14]. At the same time, it is possible that increased anxiety in aged mice was an integral part of the manifestations of disorders within the framework of cognitive impairment [28]. In any case, the detected correction of the exploratory behavior was a manifestation of the therapeutic effects of the NF-κB inhibitor on age-related disorders of CNS functioning but not on locomotor activity [26]. This was confirmed by a significant decrease in the asymmetry coefficient of their actions on all days (Table 1).

In addition, using the NF-κB inhibitor significantly increased the level of CPAR reproduction in aged mice (Table 2). This effect was especially significant on the 14th and 21st days of the experiment: up to 275.0% and 449.9% of those in control (in old mice not treated with an NF-κB inhibitor), respectively.

Thus, NF-κB inactivation in aged C57BL/6 mice (representing an adequate model of AD [21]) led to a pronounced enhancement of their learning and memory and the correction of maladaptive exploratory behavior.

3.3. Effect of the NF-κB inhibitor on progenitor functioning in aged mice.

The course administration of the NF-κB inhibitor to aged C57BL/6 mice was accompanied by a significant increase in the content of NSCs in the SVZ (up to 264.2%, 255.5%, and 180.5% on days 3, 7, and 14, respectively) and NCPs (up to 181.4%, 145.1% and 125.4% of similar values in untreated aged mice on days 3, 7, and 14, respectively) (Figure 1A and 2A). At the same time, an increase in the mitotic activity of progenitors was noted. Moreover, a more pronounced change in this parameter was also observed in NSCs (with a maximum of up to 334.7% on the 3rd day from the initial control level) (Figures 1B and 2B). In addition, the intensity of specialization of multipotent progenitors dropped sharply (to 39.0%, 45.4%, and 60.9% of those in aged mice that did not receive the inhibitor on days 3, 7, and 14, respectively) (Figures 1C and 2C).

Thus, NF-κB inactivation stimulated the proliferation of nervous tissue progenitors in aged C57BL/6 mice, accompanied by an increase in cell populations of the brain cell renewal system [7, 9]. At the same time, the functioning of NSCs under conditions of the NF-κB blockade was characterized by a significant decrease in the intensity of differentiation. This, according to previously obtained data [5, 7, 13], can be considered a factor that increases the efficiency of neurogenesis in AD due to the de novo formation of functionally more complete cells than with extremely rapid specialization of progenitors.

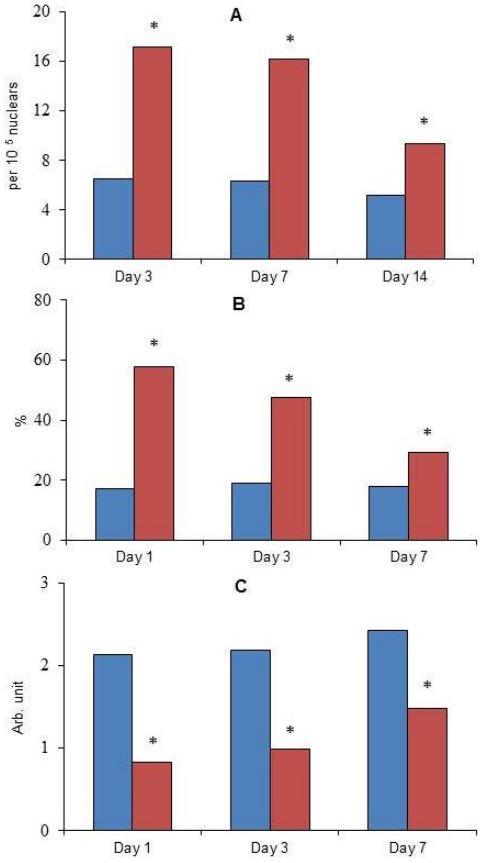

Figure 1. (A) Amount of NSC**, (B)** NSC proliferative activity**,** and **(C)** NSC differentiation index**.** Here and in Figures 2-3: in aged male C57BL/6 mice (blue columns); aged male C57BL/6 mice treated with the NF-κB inhibitor (red columns); * - differences at $p < 0.05$.

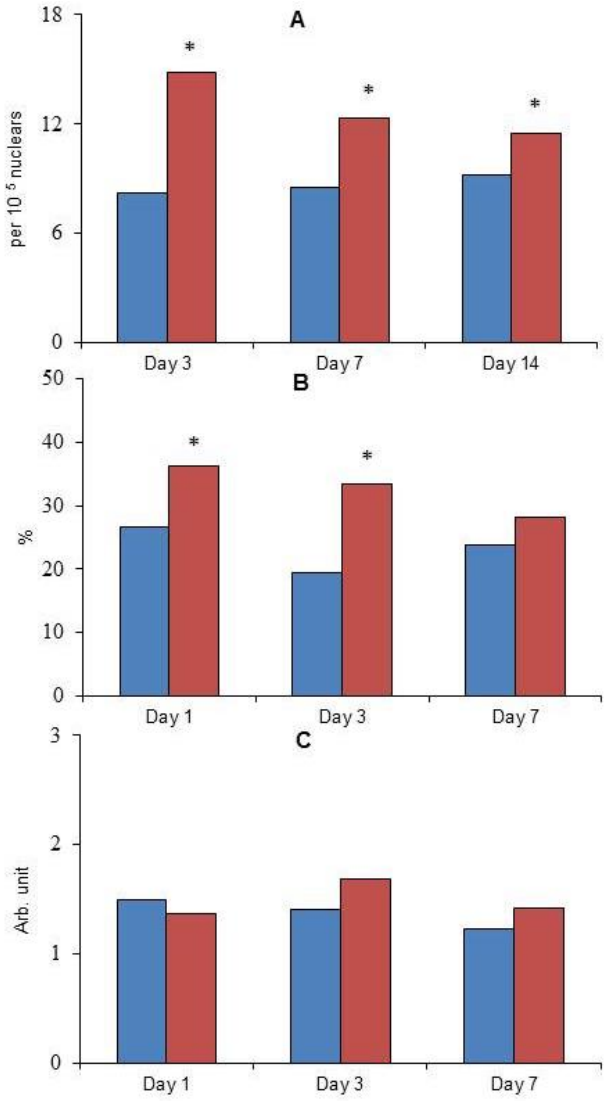

Figure 2. (A) Amount of NCP**, (B)** NCP proliferative activity**,** and **(C)** NCP differentiation index**.**

3.4. Effect of the NF-κB inhibitor on the functioning of neuroglial cells in aged mice.

An ambiguous effect of the NF-κB blockade on the production of neurotrophic growth factors by neuroglial cells of different types was revealed. The violation of signal transduction through NF-κB in ACSA-2+ cells (astrocytes) in aged mice reduced the NSA of their conditioned media on days 3 and 7 (up to 60.5% and 75.0% of the control values, respectively) (Figure 3A). These changes in the production of neurotrophic factors that seem to be necessary for neurogenesis by astrocytes [19, 29] should not be considered as an unambiguously negative effect of the NF-κB inhibitor on the functioning of the CNS. It is known that the development of AD is accompanied by reactive astrogliosis, which plays a pathogenic role in the progression of neurodegeneration [30]. Therefore, the discovered phenomenon should be considered, on the contrary, as one of the mechanisms of the revealed therapeutic effect of the studied target agent.

At the same time, the NF-κB blockade in O4+ and CD11b+ cells was accompanied by an increase in the NSA of their conditioned media on all days of the experiment (Figures 3B and 3C). The most significant was the increase in the production of neurotrophic growth factors by microglial cells: up to 136.9%, 163.4%, and 131.2% on days 3, 7, and 14 of similar parameters in aged mice that were not injected with the NF-κB inhibitor.

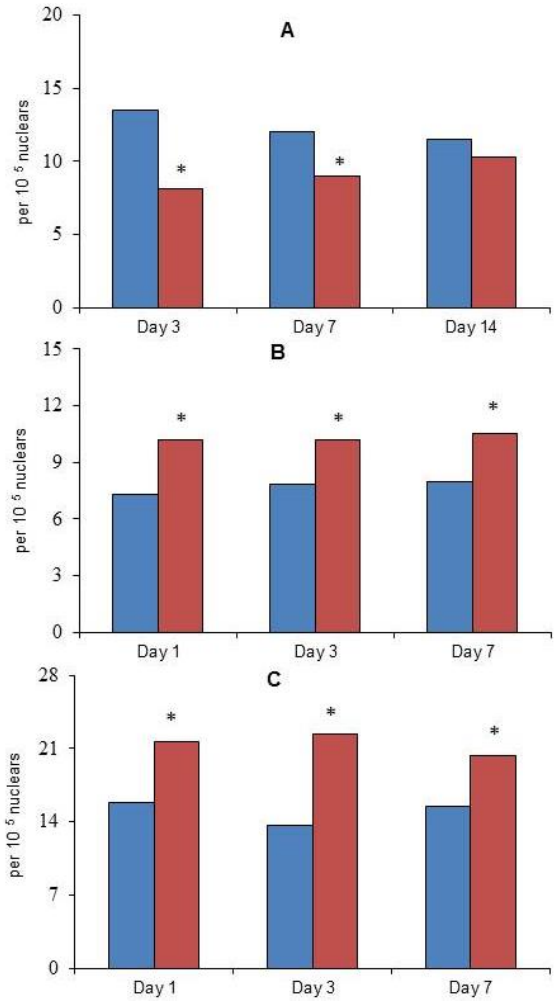

Figure 3. NSA of conditioned media of **(A)** astrocytes**, (B)** oligodendrocytes**,** and **(C)** microglial cells**.**

The revealed change in the secretory function of oligodendrocytes is difficult to interpret unambiguously. The contribution of these cells to the production of neurotrophic growth factors of the entire nervous tissue is not great [17, 31]. However, it is possible that the detected changes in the secretory function of oligodendrocytes under the influence of the NF-κB blocker may reflect the correction of their activity in relation to functioning in general. In this case, taking into account the responsibility of these cells for myelination and the formation of the blood-brain barrier (which is significantly impaired in AD [32, 33]), the revealed increase in their activity during NF-κB inactivation is undoubtedly therapeutically justified.

The situation is similar to the observed effect of the pharmacological agent on microglial cells. These cells produce the least amount of neurotrophins [34]. Accordingly, the proportion of cytokines they produce is of the least importance for neurogenesis. However, it should be taken into account that the detected change in the colony-forming capacity of conditioned media from microglial cells is an integrative indicator that depends on the production of both direct neurotrophins and factors inhibiting the proliferation of progenitors (primarily, pro-inflammatory cytokines [35]). Therefore, the revealed phenomenon of an increase in neurotrophic activity in microglial cells could be associated with a decrease in the production of pro-inflammatory cytokines (inhibiting the proliferation of progenitors [7, 14]). This seems to be the most convincing since the anti-inflammatory effects of NF-κB inhibitors are known [36], which is often the key argument for the expediency of their use in certain types of neurodegenerative diseases. Currently, there is no doubt about the pathogenic role of the maladaptive reaction of microglia and neuroinflammation in AD [37].

In general, the study's results confirm the data on impaired cognitive activity and the appearance of signs of dementia in aged C57BL/6 mice [21-23]. Moreover, the development of the identified disorders in the functioning of the CNS as a consequence of irreversible disorganization of brain structures [4, 20] is obviously based on the high intensity of the processes of endogenous formation of extracellular amyloid aggregates in the nervous tissue in these experimental animals with age [21]. This circumstance makes this mouse model quite suitable for simulating AD [21, 22, 35] to develop and study approaches to its pharmacotherapy.

The experimental data indicate the effectiveness of correction of signs of senile dementia and maladaptive exploratory behavior in aged C57BL/6 mice using the NF-κB inhibitor (as part of the implementation of the "Strategy for targeted regulation of intracellular signal transduction in regenerative-competent cells" [12, 13, 38]). The inactivation of a nuclear transcription factor led to a significant improvement of indicators reflecting the cognitive and mnestic activity of the CNS [14, 22].

At the same time, it was found that the development of these pharmacological effects occurs against the background of a pronounced change in the functioning of the system of cellular renewal of the nervous tissue (Figure 4). The NF-κB targeting in nervous tissue progenitors under conditions of neurotoxic exposure to endogenous Aβ [21] led to the progression of the cell cycle and an increase in NSCs and NCPs compartments in the brain. The decrease in the intensity of differentiation of multipotent progenitors revealed in this case should also be considered as a correction of impairments in their activity under the influence of neurotoxic Aβ aggregates [4, 5, 7] since it is known that an excessively high rate of NSC specialization, especially under conditions of disturbed tissue homeostasis, is often the cause of aberrant development of nerve cells [5, 7, 13].

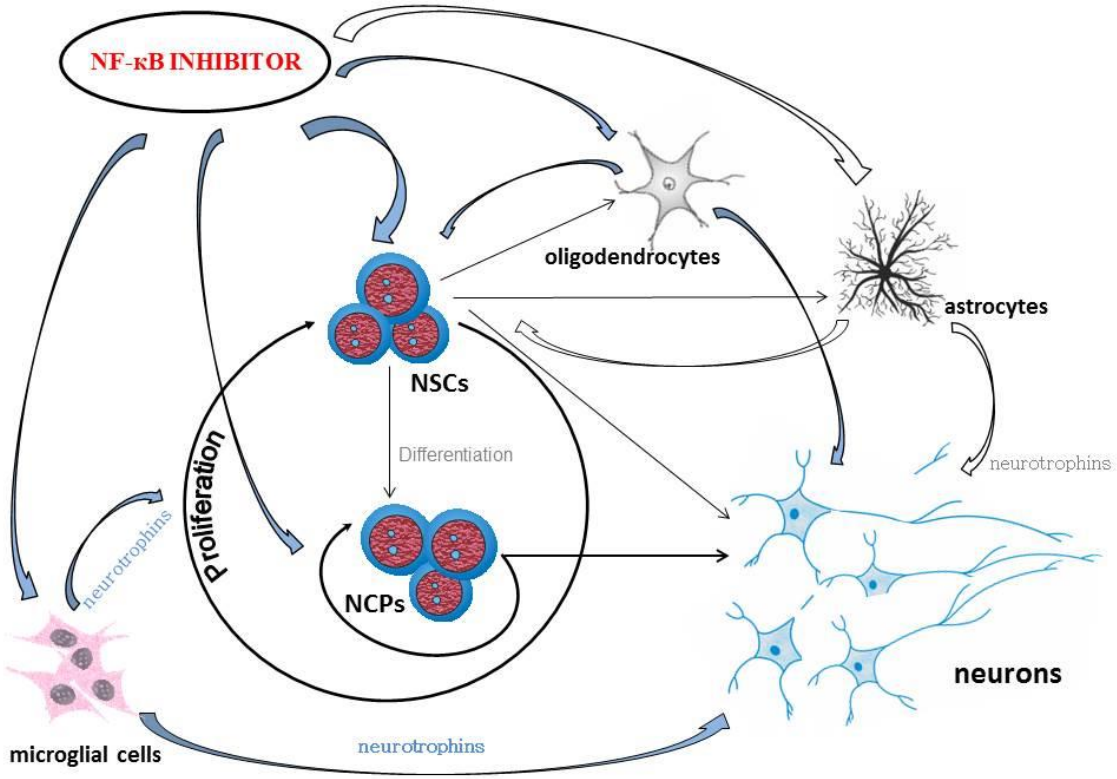

Figure 4. Effects of the NF-κB inhibitor on the functioning of the system of cellular renewal of the nervous tissue in Alzheimer's disease. Thick lines and wide blue arrows - stimulating effect; thin lines and wide white arrows - inhibitory action.

In addition, it was found that an important role in implementing the therapeutic effects of the NF-κB inhibitor is played by the influence of a pharmacological agent on the functioning of neuroglial cells of various types. Blockade of NF-κB in neuroglial cells can not only stimulate the realization of the growth potential of nervous tissue progenitors (due to the production of neurotrophins by oligodendrocytes and, possibly, microglial cells), but also promote effective neurogenesis by correcting the manifestations of reactive astrogliosis [30] and neuroinflammation [7, 39].

4. Conclusions

The findings indicate the prospects for developing fundamentally new approaches to the treatment of Alzheimer's disease using NF-κB inhibitors. In this case, pharmacological effects will be realized due to the restoration (stimulation and coordination) of the activity of different compartments of the system of cellular renewal of the nervous tissue. For the first time, the creation of Alzheimer's drugs with such a mechanism of action is proposed.

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Conflicts of Interest

The authors declare no conflict of interest.

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