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Targeted Inhibition of NF-kappa B to Facilitate Neural Repair in Alcoholic Encephalopathy

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Abstract: Recent evidence indicating that ethanol-induced neurodegeneration disrupts the function of neural stem cells (SCs) and other brain progenitor populations provides a strong pathogenetic rationale for developing drugs with neuroregenerative properties. The aim of the present investigation was to elucidate the effect of an inhibitor of Nuclear Factor kappa B (NF-kappa B, NF-κB) on both the neuropsychiatric profile and the neural progenitor cell pool within the brains of a preclinical model of alcoholic encephalopathy (AE). JSH-23 (Sigma-Aldrich, Germany), with activity guaranteed by the manufacturer, was used as an NF-κB inhibitor. The experimental protocol involved inducing alcoholic encephalopathy (AE) in C57BL/6 mice. To evaluate the effects of the NF-kappa B inhibitor JSH-23, we assessed cognitive performance via the open field test and the retention of a conditioned passive avoidance reflex (CPAR). Concurrently, *in vitro* assays were conducted to determine the content and functional activity of multipotent SCs and committed neuronal progenitors (NPs) derived from the subventricular zone (SVZ). Furthermore, we analyzed the secretion of progenitor-active growth factors by distinct neuroglial subpopulations. The administration of JSH-23 after AE induction corrected impairments in both exploratory behavior and CPAR reproducibility in mice with AE. This cognitive recovery was accompanied by a significant increase in the population of neuronal SCs and committed NPs within the SVZ. On day 7, these cell populations reached 152.6% and 211.1% of the corresponding levels in untreated AE animals. Furthermore, both progenitor types exhibited enhanced proliferative activity and differentiation intensity. This was paralleled by an increase in growth factor production by oligodendrocytes, which rose to 174.3% and 190.8% of control values (mice with AE without treatment) on days 3 and 7 following JSH-23 treatment. These results support further investigation into the possibility of using NF-κB blockers as a basis for the development of novel neuroregenerative therapies for AE.

Keywords: neurodegeneration; NF-kappa B inhibitors; neural stem cells; committed neuronal precursors; neuroglia; intracellular signal transduction; alcoholic encephalopathy.

1. Introduction

Alcohol-associated brain damage, specifically alcoholic encephalopathy (AE), represents a severe pathological condition that frequently results in profound, and in some cases irreversible, psychoneurological and somatic dysregulatory disorders [1,2]. The neurotoxic effects of ethanol induce a fundamentally altered biochemical environment, particularly within

nervous tissue. This disruption ultimately leads to the reorganization of the central nervous system and the emergence of a new functional activity pattern in its various structures [3-5]. Although a range of pharmacological agents (primarily targeting mature cells that survive under these pathological conditions) is currently available for treating this disease, effective therapy remains an extremely complex and often unattainable challenge. Consequently, there is a pressing need to develop fundamentally new approaches to AE pharmacotherapy. Such strategies must, above all, take into account the impairment of nervous tissue plasticity caused by the neurotoxic metabolites of ethanol [2,6,7].

Recent evidence indicates that ethanol-induced neurodegeneration disrupts the function of neural stem cells (SCs) and other brain progenitor populations, providing a strong pathogenetic rationale for developing drugs with neuroregenerative properties. Such therapies would operate by mobilizing this "deep reserve" of neuroplasticity, which primarily involves resident progenitors [8,9]. A particularly promising avenue for discovering these agents lies within the framework of the "Strategy for targeted regulation of intracellular signaling pathways in regenerative-competent cells" [10,11]. To date, experimental studies using models of various degenerative diseases have demonstrated, to varying degrees, the pronounced efficacy of this approach [10,12-15]. Furthermore, previous *in vitro* studies demonstrated that blocking Nuclear factor kappa B (NF-kappa B)-dependent intracellular signaling pathways significantly stimulates the realization of the growth potential of neural stem cells (SCs) and committed neuronal precursors (NPs) [2,14].

The role of NF-kappa B in the development of neuroinflammation, oxidative stress, neuronal apoptosis, and synaptic dysfunction, including that associated with chronic alcohol consumption, is also well established [16-18]. Furthermore, NF-kappa B inhibitors have been shown to block the production of pro-inflammatory cytokines (which inhibit neurogenesis) by microglial cells and to stimulate the secretion of neurotrophic factors by neuroglial cells [19,20].

The important role of this nuclear transcription factor in regulating numerous vital processes across different cell types [12,21,22] and in mediating various mechanisms of AE pathogenesis justifies further research into the potential of NF-kappa B as a therapeutic target for AE. Specifically, it is necessary to confirm in vivo that NF-kappa B inactivation can effectively control progenitor cell proliferation and differentiation. Furthermore, validating the therapeutic potential of such modifications to the cell renewal system in the context of alcohol-induced brain pathology is critically important. It appears likely that NF-kappa B blockade may stimulate the proneuroregenerative properties of progenitor cells both directly and by increasing neurotrophin production by neuroglial cells while inhibiting proinflammatory cytokine production by microglia. It is also possible that such changes in the brain may contribute to the correction of neuropsychiatric disorders associated with AE.

The aim of the present investigation was to elucidate the effects of an NF-kappa B inhibitor on both the neuropsychiatric profile and the neural progenitor cell pool in a preclinical model of AE.

2. Materials and Methods

2.1. Pharmacological agents and reagents.

NF-kappa B inhibitor JSH-23 (J4455) (Sigma-Aldrich, Germany); Serum-free MACS Neuro Medium; reagents for positive immunoseparation of cells: anti-CD56, anti-ACSA-2,

Anti-O4, and Anti-CD11b MicroBeads (Miltenyi Biotec, Germany); hydroxyurea, dimethyl sulfoxide (DMSO), trypsin-EDTA, trypan blue (Sigma-Aldrich, Germany).

2.2. Laboratory animals.

A total of 153 male C57BL/6 mice (Category 1), 2-2.5 months old and weighing 22-24 g, were used in this study. Mice were maintained in a temperature-controlled room (22 ± 1°C) with a 12-hour light/dark cycle (lights on at 8:00 AM). Animals were housed in groups of 8–10 animals in a standard polycarbonate cage (29 × 18 × 12 cm). Standard food was available ad libitum. The animals were obtained from the Tomsk National Research Medical Center. All experimental procedures were conducted in accordance with generally accepted principles for the humane treatment of laboratory animals. The study protocol was approved by the Ethics Committee of the Tomsk National Research Medical Center (Protocol No. GRIPh&RM-2025-03/07, dated March 7, 2025).

2.3. Experimental design.

All experimental procedures were conducted in accordance with ARRIVE guidelines. Animals were randomly assigned using a computer-generated randomization scheme. The investigators conducting the studies were blinded to group assignment. Exclusion criteria were defined a priori: animals with a latency of more than 60 seconds in the CPAR test during pre-training or with signs of illness (loss of more than 20% of body weight) were excluded from the analysis. The final sample sizes for each group are provided below in the descriptions of the relevant sections.

The animals were divided into 3 groups: 1) intact mice, 2) mice with AE, and 3) mice receiving the NF-kappa B inhibitor after modeling AE (n=51 in each group).

During the experiments, the preservation of their cognitive functions was studied using tests in the “open field” (on days 3, 7, 14, and 21) and the reproduction of the conditioned passive avoidance reflex (CPAR) of an electric painful stimulus (on days 7, 14, and 21) against the background of the introduction of JSH-23.

Using *in vitro* culture methods, we assessed the content and functional state of neural SCs and committed NPs in the subventricular zone of the brain (SVZ), as well as the production of growth factors (active against neural progenitors) by different fractions of neuroglia (on days 3 and 7). All studies were conducted in the morning.

2.3.1. AE model.

The model is validated for functional psychoneurological disorders. AE was induced in mice by daily intragastric administration of 0.2 ml of a 30% ethanol solution (equivalent to 3 g/kg/day) over a period of 16 weeks [2, 21]. Throughout this period, animals also had continuous access to a 5% ethanol solution in their home cages, rather than drinking water. Control animals (intact mice) received an equivalent volume of distilled water via the same intragastric route and had free access to clean drinking water.

During the AE modeling, no statistically significant decrease in body weight or changes in external features were observed in animals compared to intact control mice. No spontaneous mortality was observed.

2.3.2. NF-kappa B inhibitor.

JSH-23 specifically blocks nuclear translocation and DNA binding of the NF-kappa B p65 subunit without affecting IκB degradation (manufacturer's instructions). JSH-23 administration began 10 days after the final ethanol treatment. The JSH-23 administration began 10 days after the end of the ethanol treatment. A 10-day waiting period was necessary to eliminate ethanol and its immediate degradation products from the mice's bodies. This ensured that changes in parameters associated with persistent physiological changes, rather than toxic agents, were recorded [2,21]. The agent was injected subcutaneously at a dose of 5 mg/kg (dissolved in 0.2 mL of 0.2% DMSO) daily for 7 days. The first day of NF-kappa B inhibitor administration was designated as day "1". Control group animals (intact mice and those subjected to the AE model) received the vehicle (DMSO) following the same regimen and volume. The dosage and administration schedule of JSH-23 were selected based on a pilot study examining its psychopharmacological effects in an Alzheimer's disease model [23].

2.4. Study of psychoneurological status.

The effect of the pharmacological agent on cognitive function was evaluated using functional tests (n=15 per group: intact control, AE control, and experimental groups). The open-field test was used to record locomotor activity and various parameters of exploratory behavior (as detailed in the corresponding table). The open field setup was made of gray acrylic (40 × 40 × 30 cm). The test was conducted under dim white lighting (150 lux). Mice were transferred from the vivarium to the testing room at least 60 minutes before the experiment to allow them to acclimate to the environmental conditions (humidity 50–60%, temperature 24,0 ± 2°C). Each mouse was carefully placed in the center of the arena. Between trials, the arena floor was cleaned with a 70% ethanol solution and then dried to remove olfactory traces left by the previous animal. Animal behavior was recorded using a video camera mounted above and automatically analyzed using DeepLabCut/SLEAP software (Switzerland). The recorded indicators are shown in Table 1. To differentiate between psycho-emotional responses and cognitive function, data from the first minute were analyzed separately from those of minutes 2–3, as described previously [13,14,24].

Mnestic function was evaluated using the conditioned passive avoidance reflex (CPAR) test. This paradigm assesses the ability of animals to retain an acquired fear response. The apparatus consisted of a light (21 × 21 × 21 cm) and a dark (21 × 21 × 21 cm) compartment connected by a guillotine door. During training (day 1), after a 5‑s acclimation in the light side, the door was opened. The latency to enter the dark side (all four paws inside) was recorded, and upon entry, an unavoidable foot shock (0.5 mA, 2 s) was applied. During the experiments, all mice entered a dark compartment within 60 seconds, so there were no escapes of animals from the experiment. On days 3, 7, and 14 after training, the reproducibility of the CPAR was tested (the same mice were used at all times). The test exploits the conflict between this newly acquired avoidance reflex and the innate rodent preference for dark spaces. Successful retention of the reflex was determined by the mouse remaining in the light compartment throughout the 3-minute reflex maintenance test [13, 14].

2.5. Cell culture assays.

The JSH-23 effect on regenerative-competent cells (progenitors and neuroglial cells) was assessed by *in vitro* culture methods (n=36 in the control (intact control and control -

animals with AE) and experimental groups). Tissue fragments from the SVZ were placed in MACS Neuro Medium and enzymatically dissociated with 0.05% trypsin-EDTA for 15 min at 37°C with gentle agitation. The cell suspension was passed through a 40-μm cell strainer and washed twice by centrifugation.

2.5.1. Progenitor cells study.

The numbers of neural SCs and committed NPs were quantified by colony-forming assay in cultures of unfractionated cells and isolated CD56+ cells, respectively. CD56+ cells were enriched via positive immunomagnetic selection using PSA-NCAM microbeads and a MiniMACS Separator (Miltenyi Biotec, Germany) [8,15]. The purity of the obtained CD56+ cell population was confirmed by the manufacturer to be 95%- 97% based on flow cytometry data (FACS Canto II, BD Biosciences). Cell viability was determined using trypan blue. Following isolation, cells were cultured in MACS Neuro Medium at a density of 10^5 viable cells/ml under standard conditions (37°C, 5% CO_2, humidified atmosphere) for 5 days in a CO_2 incubator (Thermo Scientific 8000 DH, USA). Neural SC and committed NP content was assessed by their ability to form colonies, specifically neurospheres exceeding 100 cells (colony-forming units, CFU). Mitotic activity of CFU was determined using hydroxyurea (1 μM in the medium) to block cells in the S-phase of the cell cycle. Additionally, the specialization index (reflecting differentiation/maturation intensity) for both progenitor types was calculated as the ratio of cluster-forming units (ClFU; small neurospheres of 30–80 cells) to CFU.

2.5.2. Neuroglial cells study.

The individual neuroglial populations - astrocytes, oligodendrocytes, and microglial cells - were isolated from SVZ-derived cellular material via positive immunomagnetic separation using ACSA-2, O4, and CD11b antibodies, respectively (MiniMACS Cell Separator, Germany) [8, 14]. The purity of the obtained population was guaranteed by the manufacturer, as determined by flow cytometry (FACS Canto II, BD Biosciences), at 93%-97%. Conditioned media were subsequently harvested from these cells following a 2-day incubation period in MACS Neuro Medium under standard culture conditions. To evaluate the neurotrophic secretory capacity of each glial fraction - namely, their production of growth factors promoting colony formation - we quantified the neurosphere-stimulating activity (NSA) of the conditioned media in a bioassay. This was determined by the extent to which the media enhanced neurosphere formation in a test system. The content of individual neurotrophins was not assessed.

2.6. Statistical analysis.

Depending on the situation, one-way analysis of variance (ANOVA) followed by Dunnett's test (for comparison with the control group) or the Kruskal-Wallis test with a post hoc Mann-Whitney test (Bonferroni correction) was used. Before conducting ANOVA, normality of distribution (Shapiro-Wilk test) and homoscedasticity (Levene's test) were checked. Calculations were performed using STATISTICA 13.0. ckage. Results are expressed as the arithmetic mean and standard error of the mean (M ± SEM) in tabular form. Graphical data represent arithmetic means, with significance thresholds set at $p \leq 0.05$ [25].

3. Results and Discussion

3.1. Psychoneurological status.

Chronic ethanol exposure resulted in significant alterations in the psychoneurological profile of mice. At all time points examined, we observed elevated total locomotor activity, attributable predominantly to increased horizontal locomotion in the open field test. In contrast, vertical rearing and exploratory hole-poking were significantly reduced (Table 1). This behavioral simplification coincided with increased motor asymmetry. Notably, these alterations were present both during the initial minute and throughout minutes 2–3 of testing. Following established criteria [10], the early changes were interpreted as indicative of heightened anxiety, whereas the later changes reflected disruption of cognitive processing independent of locomotor deficits.

Table 1. Locomotor activity in the "open field" of intact C57BL/6 mice (1); in C57BL/6 mice with AE (2); and in C57BL/6 mice with the NF-kappa B inhibitor administration to mice with AE (3), arb. units (M±SEM).

Groups	Horizontal locomotor activity	Vertical locomotor activity	Hole-board exploration	Self-grooming	Defecation	Total locomotor activity	Asymmetry coefficient
Day 3							
First period (minute 1)							
1	13.93 ± 1.15	3.00 ± 0.48	4.87 ± 0.57	0.73 ± 0.21	0.27 ± 0.15	20.80 ± 1.95	0.57 ± 0.02
2	19.87 ± 2.05*	3.93 ± 0.66	3.40 ± 0.88	1.60 ± 0.31	1.33 ± 0.19*	30.13 ± 3.07 *	0.64 ± 0.03*
3	23.62 ± 1.45	3.50 ± 0.77	2.14 ± 0.52*	0.43 ± 0.17#	0.43 ± 0.17#	28.93 ± 1.71	0.70± 0.02*
Second period (minutes 2-3)							
1	27.87 ± 2.75	5.27 ± 0.67	5.67 ± 1.19	1.13 ± 0.27	0.63 ± 0.36	40.87 ± 4.35	0.69 ± 0.02
2	36.67 ± 1.91*	1.93 ± 0.40*	2.53 ± 0.59*	0.07 ± 0.07*	0.13 ± 0.09	41.33 ± 2.14	0.78 ± 0.05*
3	29.43 ± 4.45	6.50 ± 1.17#	4.93 ± 1.08#	1.36 ± 0.21#	0.41 ± 0.25	40.13 ± 5.31	0.79 ± 0.04*
Day 7							
First period (minute 1)							
1	14.67 ± 1.20	2.80 ± 0.40	1.80 ± 0.50	1.27 ± 0.23	0.60 ± 0.27	21.13 ± 1.36	0.63 ± 0.02
2	19.67 ± 1.17*	3.33 ± 0.60	2.00 ± 0.52	0.00 ± 0.00*	0.33 ± 0.13	26.33 ± 1.48*	0.78 ± 0.03*
3	18.64 ± 2.5	2.00 ± 0.31	1.14 ± 0.64	0.93 ± 0.25	0.27 ± 0.07	23.33 ± 2.86	0.81 ± 0.02*
Second period (minutes 2-3)							
1	12.47 ± 1.41	2.20 ± 0.30	1.53 ± 0.45	1.20 ± 0.22	0.60 ± 0.25	18.00 ± 1.96	0.60 ± 0.03
2	21.00 ± 2.99*	1.93 ± 0.96*	3.87 ± 0.99	0.73 ± 0.17	0.93 ± 0.25	28.47 ± 4.13*	0.70 ± 0.05
3	15.71 ± 2.40	3.36 ± 0.63	1.50 ± 0.95	1.71 ± 0.27	0.50 ± 0.18	22.20 ± 3.24	0.65 ± 0.05
Day 14							
First period (minute 1)							
1	15.07 ± 1.95	3.27 ± 0.42	1.07 ± 0.51	0.93 ± 0.18	0.13 ± 0.09	18.47 ± 1.24	0.67 ± 0.02
2	19.67 ± 1.63*	2.93 ± 0.59	1.53 ± 0.46	0.53 ± 0.19	0.20 ± 0.11	24.87 ± 2.02*	0.78 ± 0.02*
3	16.93 ± 1.3	3.13 ± 0.38	2.00 ± 0.32	0.27 ± 0.15	0.20 ± 0.11	20.53 ± 1.72	0.74 ± 0.02
Second period (minutes 2-3)							
1	18.47 ± 1.99	4.07 ± 0.77	3.87 ± 0.45	1.13 ± 0.19	0.33 ± 0.13	25.87 ± 2.44	0.68 ± 0.03
2	23.80 ± 1.84*	2.93 ± 0.66*	1.60 ± 0.39*	1.07 ± 0.27	0.53 ± 0.13	29.93 ± 1.62	0.79 ± 0.02*
3	15.00 ± 3.04#	4.86 ± 0.46#	2.43 ± 0.81	1.43 ± 0.36	0.64 ± 0.21	24.73 ± 5.01	0.51 ± 0.07#
Day 21							
First period (minute 1)							
1	15.07 ± 1.95	2.27 ± 0.32	2.07 ± 0.21	0.93 ± 0.18	0.13 ± 0.09	20.47 ± 2.24	0.71 ± 0.03
2	16.73 ± 2.81	1.13 ± 0.28*	1.00 ± 0.35*	0.40 ± 0.16*	0.13 ± 0.09	21.86 ± 3.10	0.76 ± 0.04
3	14.87 ± 1.67	3.00 ± 0.58	1.57 ± 0.30	0.70 ± 0.11	0.47 ± 0.13	18.60 ± 2.28	0.72 ± 0.03
Second period (minutes 2-3)							
1	13.60 ± 1.51	2.53 ± 0.35	1.60 ± 0.21	1.13 ± 0.39	0.80 ± 0.26	18.67 ± 1.10	0.66 ± 0.02
2	21.67 ± 2.01*	1.13 ± 0.46*	0.93 ± 0.34	0.67 ± 0.19	0.53 ± 0.19	24.93 ± 2.15*	0.74 ± 0.03*
3	15.87 ± 1.99#	2.73 ± 0.54#	2.13 ± 0.45	1.47 ± 0.17#	0.73 ± 0.13	19.33 ± 1.98#	0.64 ± 0.03#

$p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

In addition, chronic alcoholization of animals was accompanied by a drop in the level of CPAR reproduction on days 14 and 21 (up to 37.9% of the level of the same indicator in

intact mice on day 21) (Table 2). There were no statistically significant differences between the experimental groups in the latent period during recording of CPAR reproduction.

Table 2. CPAR parameters of the intact mice C57BL/6 (1); in C57BL/6 mice with AE (2); and C57BL/6 mice with the NF-kappa B inhibitor administration to mice with AE (3), (M±SEM)

Day	Groups	Proportion of animals with preserved reflex
7	1	0.93 ± 0.07
	2	0.60 ± 0.13*
	3	0.87 ± 0.09
14	1	0.87 ± 0.09
	2	0.40 ± 0.13*
	3	0.87 ± 0.09#
21	1	0.87 ± 0.09
	2	0.33 ± 0.13*
	3	0.87 ± 0.09#

$p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

3.2. NF-kappa B inhibitor effects on the functioning of progenitors.

The observed alterations in psychoneurological status and their subsequent pharmacological correction paralleled the detected changes in progenitor cell function. The investigation of distinct compartments within the neural tissue renewal system [9-11] during ethanol intoxication yielded noteworthy findings. The quantity of neural stem cells residing in the subventricular zone remained unaltered relative to controls (Figure 1A). However, the clonogenic CD56+ cell population, representing committed neuronal progenitors, exhibited a substantial contraction, decreasing to 75.9% and 63.2% of intact control values by days 3 and 7 post-exposure, respectively (Figure 2A). This was accompanied by suppressed mitotic activity affecting both progenitor classes: multipotent stem cells demonstrated proliferative reductions to 62.4% (day 3) and 54.7% (day 7) of initial levels (Figure 1B), while committed progenitors showed even greater impairment, falling to 46.1% and 37.9% of intact values on corresponding days (Figure 2B). Notably, the differentiation rate of both neural SCs and committed NPs remained unaffected throughout the experimental timeline (Figures 1C and 2C).

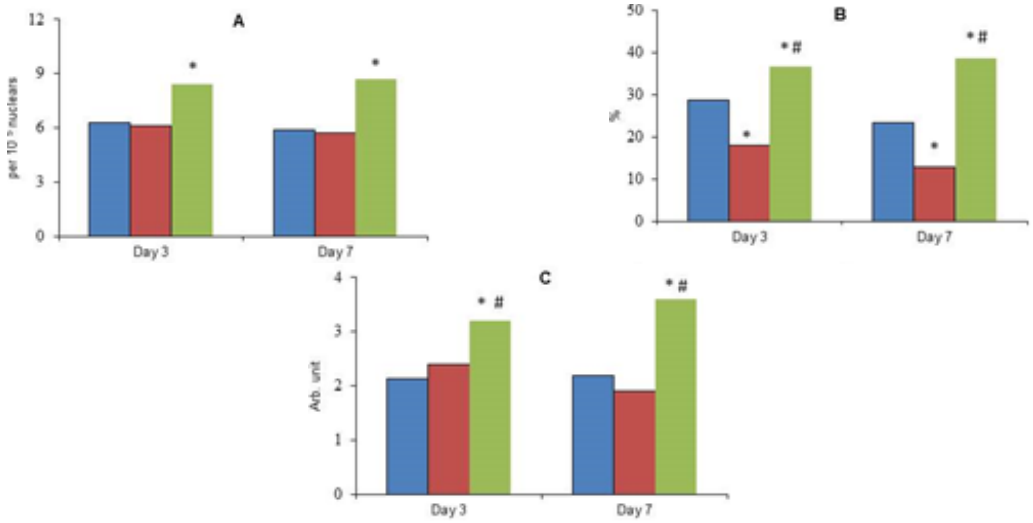

Figure 1. (A) Neural SC amount; **(B)** neural SC proliferative activity; **(C)** neural SC differentiation index. The abscissa axis shows the research timeframes, and the ordinate axis shows the units of measurement of the indicators (n=6). In intact C57BL/6 mice (blue bars), mice with AE (red bars), and with the NF-kappa B inhibitor administration to mice with AE; p < 0.05 in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

Here and in Figures 2-3: in intact C57BL/6 mice (blue bars), mice with AE (red bars), and with the NF-kappa B inhibitor administration to mice with AE; $p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

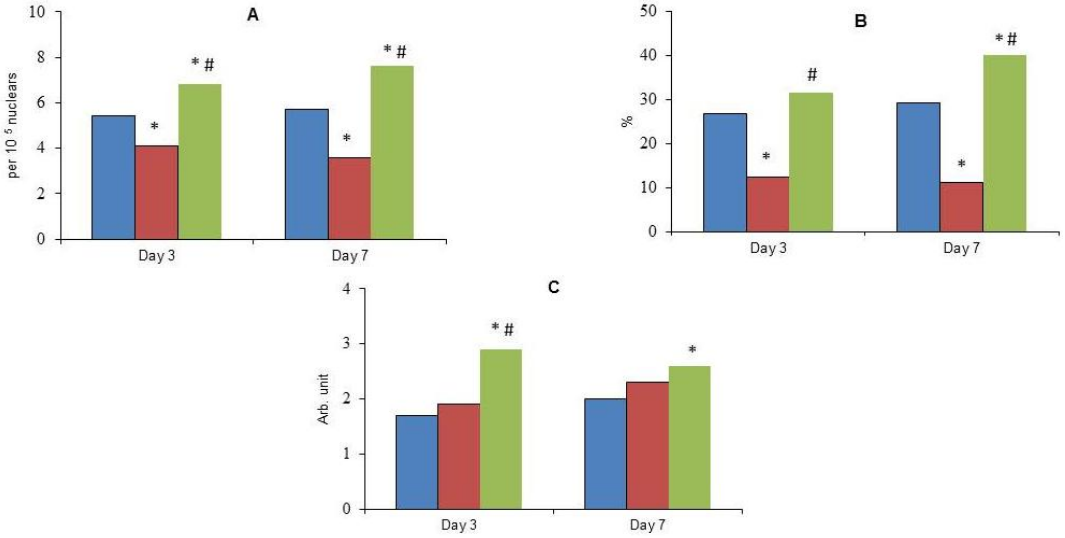

Figure 1. (A) Committed NP amount; **(B)** committed NP proliferative activity; **(C)** committed NP differentiation index. The abscissa axis shows the research timeframes, and the ordinate axis shows the units of measurement of the indicators (n=6). In intact C57BL/6 mice (blue bars), mice with AE (red bars), and with the NF-kappa B inhibitor administration to mice with AE; $p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

3.3. NF-kappa B inhibitor effects on the functioning of neuroglial cells.

The key observation highlighting progenitor dysfunction in this pathological model was that their failure occurred despite a marked increase in astrocytic neurotrophin secretion. Astrocyte-derived growth factor production rose significantly, reaching 154.5% and 172.2% of intact control levels on days 3 and 7, respectively (Figure 3). This is particularly noteworthy, given that astrocytes are recognized as the primary source of growth factors in the nervous system [26,27]. In contrast, the secretory activity of oligodendrocytes and microglia remained unchanged throughout the observation period (Figure 3).

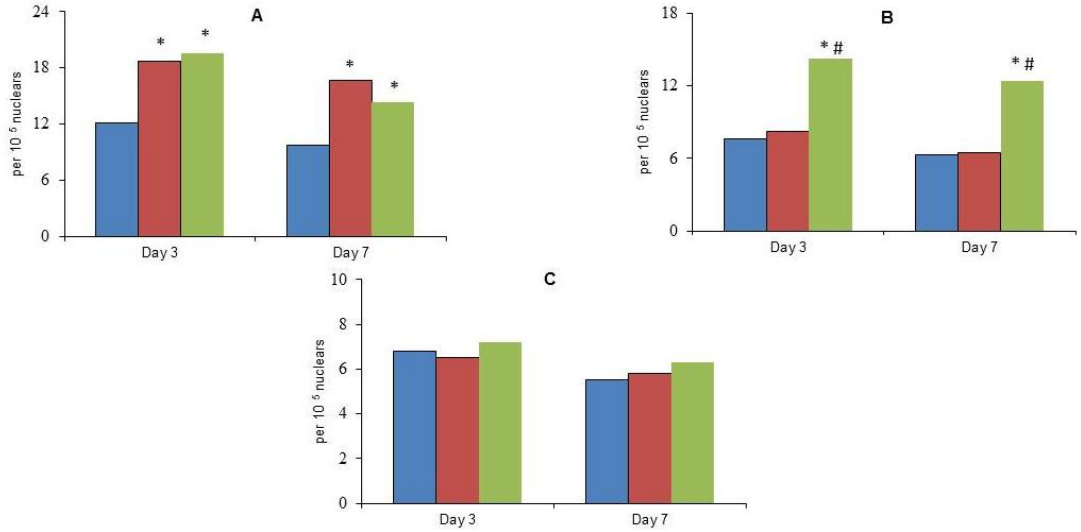

Figure 1. (A) NSA of conditioned media obtained from astrocytes; **(B)** oligodendrocytes; **(C)** microglial cells. The abscissa axis shows the research timeframes, and the ordinate axis shows the units of measurement of the indicators (n=6). In intact C57BL/6 mice (blue bars), mice with AE (red bars), and with the NF-kappa B inhibitor administration to mice with AE; $p < 0.05$ in comparison with * intact C57BL/6 mice, # C57BL/6 mice with AE.

The chronic ethanol exposure, therefore, resulted in the manifestation of pronounced central nervous system deficits consistent with alcoholic encephalopathy [2]. Concomitantly, we observed decompensation of neuroplasticity mechanisms that depend on the proliferative and differentiative capacities of both neural stem cells and committed neuronal progenitors. The committed progenitor population, which represents a more readily mobilizable reservoir for neural tissue renewal [15,21], demonstrated greater vulnerability to ethanol-induced toxicity than multipotent stem cells. In light of the robust, apparently compensatory astroglial response documented throughout the experimental period [13,26], it is plausible that progenitor failure stems from a combination of direct neurotoxic insult and excessive cytokine-driven signaling [27,28].

Treatment with the NF-kappa B inhibitor JSH-23 had minimal effects on exploratory parameters during the first minute of open-field testing (Table 1). Mice receiving the compound maintained elevated total locomotor activity, attributable predominantly to horizontal locomotion. However, by experimental day 3, analysis of the second observation interval (minutes 2–3) revealed significant augmentation of vertical rearing and hole-board exploration in the treatment group. These effects were further amplified on days 14 and 21, at which point behavioral parameters in JSH-23-treated animals were fully restored to levels observed in intact controls. It is noteworthy that statistically significant improvements relative to the AE control group - specifically, normalization of movement asymmetry, reduction in horizontal activity, and enhancement of vertical rearing - were manifest exclusively during the second observation period.

These findings indicate that NF-kappa B inactivation produced a more pronounced restoration of cognitive function than correction of the psychoemotional state (anxiety) in alcohol-exposed animals. The therapeutic effect on cognitive function was corroborated by conditioned reflex testing, which showed that JSH-23 treatment maintained CPAR retention at levels equivalent to those of intact controls (Table 2).

The observed behavioral improvements were consistent with the progenitor-stimulating effects of NF-kappa B inhibition previously documented *in vitro* [21,29], which we now confirm in vivo using a model of ethanol-induced brain pathology. The JSH-23 administration significantly expanded both progenitor populations in the SVZ: neural stem cell CFU increased to 137.7% (day 3) and 152.6% (day 7) of untreated AE control values, while committed NP CFU rose to 165.9% and 211.1% at the same time points. These expansions resulted from enhanced proliferative activity in both progenitor types. Additionally, targeted modulation of intracellular signaling substantially enhanced progenitor specialization. The neural SC differentiation index peaked on day 7 at 189.5% of control, while committed NP differentiation reached its maximum earlier, on day 3, at 152.6% of control (Figures 1, 2).

Changes in the functioning of neuroglial cells consisted of an increase in the secretion of neural growth factors by oligodendrocytes (up to 174.3% and 190.8% of the control on days 3 and 7, respectively) (Figure 3).

These experimental data indicate that JSH-23 administration produces substantial correction of alcohol-induced neuropsychiatric deficits. Inhibition of NF-kappa B-dependent intracellular signaling restored cognitive function, encompassing both exploratory behavior and mnemonic capacity. The emergence of these therapeutic effects coincided with significant alterations in the dynamics of the neural tissue renewal system [10, 11]. These observations validate previous *in vitro* findings demonstrating that NF-kappa B blockers enhance the realization of growth potential across diverse progenitor populations [21]. Under conditions of

ethanol-induced neurotoxicity, targeted modulation of NF-kappa B activity stimulated cell cycle progression and expanded key components of the brain's "deep regenerative reserve," namely multipotent stem cells and committed neuronal precursors [2, 9, 15] (Figure 4).

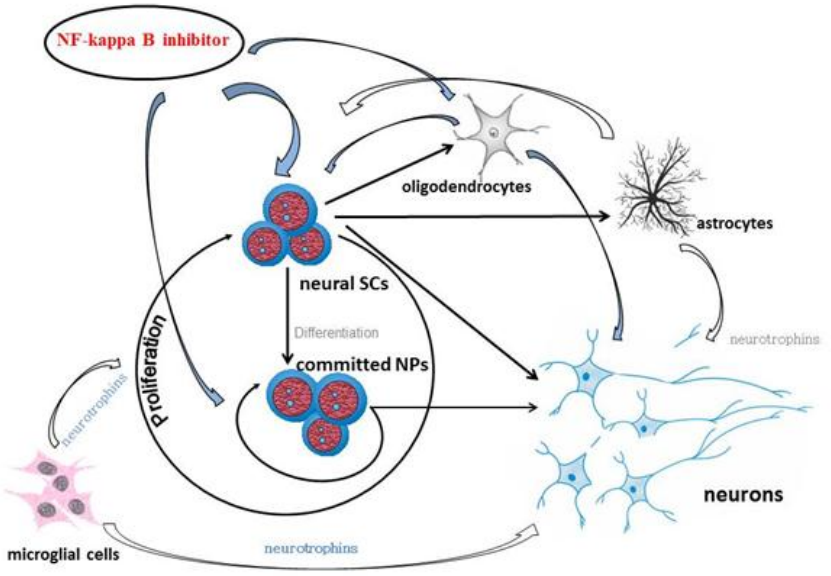

Figure 4. Effect of the NF-kappa B inhibitor on regenerative-competent cells in AE modeling. Thick lines and wide blue arrows – stimulation; thin lines and wide white arrows – inhibition.

This supports our assumption about the key role of neurogenesis stimulation in the restoration of CNS function in AE under an NF-kappa B blocker [21, 24].

The therapeutic utility of these targeted intracellular signaling modifiers in AE management is unlikely to be confined solely to their actions on regenerative-competent cells. Additional mechanisms likely contribute to enhanced neuroplasticity, notably the anti-inflammatory, microglia-mediated neuroimmune effects of NF-kappa B inhibition [11,16,30-32] and the pleiotropic activating effects on oligodendrocyte function extending beyond neurotrophin synthesis [33-35]. These results collectively support the feasibility of further exploring the potential of pharmacotherapy for AE using NF-kappa B inhibitors.

However, significant research is still needed, including undoubtedly studies of the pharmacokinetics, toxicity, dose-response relationship, and other characteristics of NF-kappa B inhibitors, specifically JSH-23. Furthermore, there is experimental evidence that disruption of NF-kappa B signaling can negatively affect neuronal bioenergetics and synaptogenesis [36–40]. Therefore, research on the effects of NF-kappa B inhibitors should be further extended to many other tissues of the body and cellular functions, including the immune system. Importantly, the functional significance of specific intracellular signaling pathways is highly context-dependent, with physiological roles potentially diverging substantially from those operative under pathogenic conditions [29,41,42].

4. Conclusions

These experimental data indicate that JSH-23 is capable of significantly correcting alcohol-induced neuropsychiatric disorders. The findings support further investigation into the potential of selective NF-kappa B blockers as a basis for the development of qualitatively novel targeted drugs with neuroregenerative activity for the treatment of AE. JSH-23 may be one candidate for such therapeutic development. Nonetheless, from both a fundamental

pharmacological and a practical perspective, it is of particular interest to further explore how NF-kappa B inactivation affects the functioning of primarily mature cells of the nervous tissue, including neurite and synaptogenesis, under conditions of ethanol-induced neurotoxicity.

Authorship Contributions

Conceptualization, G.N.Z.; methodology, G.N.Z. and L.A.M.; investigation, L.A.M. and T.Yu.P.; writing—original draft preparation, G.N.Z.; writing—review and editing, G.N.Z.; visualization, G.N.Z.; supervision, G.N.Z.; project administration, G.N.Z.; funding acquisition, G.N.Z. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was carried out in accordance with the principles of the humane treatment of animals (EU Directive 2010/63/EU). Permission was previously obtained from the local ethics committee of the Goldberg Research Institute of Pharmacology and Regenerative Medicine (Protocol No. GRIPh&RM-2025-03/07, dated March 7, 2025).

Informed Consent Statement

Not applicable.

Data Availability Statement

All data supporting the findings of this study are available in the article.

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Conflicts of Interest

The authors state there is no conflict of interest.

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