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ADIPOCYTE-DERIVED MESENCHYMAL STEM CELLS INJECTION ATTENUATES NEURONAL APOPTOSIS AND ENHANCES COGNITIVE RECOVERY AFTER MODERATE TRAUMATIC BRAIN INJURY IN RATS

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Background. *Traumatic brain injury (TBI) remains one of the leading causes of long-term disability worldwide. The secondary brain injury phase, which develops hours to days after primary trauma, is a critical therapeutic window for therapeutic interventions, however, no available therapy has been proven effective.* ***Objective.*** *To estimate the effect of adipose-derived mesenchymal stem cells (AD-MSCs) therapy on neuronal apoptosis, brain-derived neurotrophic factor (BDNF) level, and cognitive function in a rat model of moderate TBI.* ***Methods.*** *AD-MSCs from rat adipose tissue were isolated and analyzed using standardized techniques. Adult male Wistar rats were anesthetized, a left-sided craniectomy was performed and a moderate traumatic brain injury was induced by releasing a metal cylinder through a guiding tube. Following the impact, the incision was closed using absorbable sutures. At 24 hours following TBI, eight microinjections each consisting of $2{\cdot}10^5$ AD-MSCs in 5 µl PBS were administered in the pericontusional cortex. Control animals received equivalent volumes of sterile saline. Animals were euthanized on 7^{th} or 14^{th} day and the brain was collected for analysis. At the time point prior to euthanasia, rats underwent cognitive testing. Apoptotic index was evaluated by TUNEL assay, BDNF level by ELISA, cognitive performance by Barnes maze test.* ***Results.*** *Macroscopic brain examination revealed enhanced cortical regeneration and vascularization in AD-MSCs treated rats compared with controls. The apoptotic index was significantly lower in the AD-MSCs group on both 7^{th} and 14^{th} days. Cognitive performance improved markedly in the AD-MSCs group, with shorter escape times in the Barnes maze on both 7^{th} and 14^{th} days. In contrast, BDNF levels did not differ between groups at either time point.* ***Conclusion.*** *These findings demonstrate both neuroprotective and neuroregenerative effects of AD-MSCs and highlight its administration as a promising therapeutic strategy for mitigating secondary brain injury after TBI.*

K e y w o r d s: traumatic brain injury of rats, mesenchymal stem cells, apoptosis, brain-derived neurotrophic factor, cognitive function.

Traumatic brain injury (TBI) remains one of the leading causes of death and long-term disability worldwide, resulting in substantial medical, social, and economic burdens [1] .The complex pathophysiology of TBI involves primary and secondary brain injuries. Primary brain injury occurs immediately after the mechanical insult, leading to neuronal, axonal, glial, and vascular damage [2-5]. Secondary brain injury, which develops hours to days later [4, 5], results from neuroinflammatory cascades [5, 6], excitotoxicity [7, 8], oxidative stress [8, 9], and apoptotic cell death [4, 5, 10]. This

secondary injury phase is considered a critical therapeutic window for interventions aimed at preventing further neuronal damage and improving neurological outcomes [11].

Current management of TBI primarily focuses on maintaining optimal physiological conditions to minimize secondary injury. However, no available therapy has been proven effective in halting the progression of secondary brain damage or promoting substantial neural regeneration [4, 12, 13]. Therefore, novel treatment strategies targeting neuroprotection and neurorestoration are urgently needed.

Mesenchymal stem cells (MSCs) have gained attention as a promising therapeutic approach due to their multipotent differentiation capability, neuroprotective effects, and paracrine secretion of trophic and anti-inflammatory factors [14-16]. Among them, adipose-derived mesenchymal stem cells (AD-MSCs) are advantageous because they can be easily harvested, have strong proliferative potential, and exhibit both immunomodulatory and regenerative properties [17-19]. AD-MSCs secrete a variety of neurotrophic and angiogenic factors, including brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), and vascular endothelial growth factor (VEGF), which contribute to neuronal survival, angiogenesis, and tissue repair [20-23]. Previous study has also demonstrated that transplantation of AD-MSCs can enhance motor activity in animal models of TBI, suggesting their potential applicability in the management of TBI patients [24].

Therefore, this study aimed to evaluate the effects of intracortical AD-MSCs therapy on BDNF levels, neuronal apoptosis, and cognitive function in a TBI animal model, in order to elucidate the neuroprotective and neurorestorative potential of AD-MSCs in experimental brain injury.

Materials and Methods

Subjects and animal ethics. Adult male Wistar rats (*Rattus norvegicus*), aged 3 months and weighing 150–200 g, were obtained from the institutional animal facility. Animals were housed in standard laboratory conditions with free access to food and water, at a controlled temperature of 21 ± 2°C, relative humidity of 45 ± 15%, and a 12-h light/dark cycle. Animals with signs of head region infection were excluded. All experimental procedures involving animals were conducted in accordance with internationally recognized bioethical standards and principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and in accordance with institutional guidelines for the care and use of laboratory animals, with the approval of the relevant ethics committee (No. 1914/UN14.2.2.VII.14/LT/2024).

Experimental groups. The animals were randomly assigned to one of four experimental groups (Fig. 1), with at least seven animals in each group: group 1 (control day 7), which underwent TBI surgery and received an intracortical (IC) injection of 40 µl normal saline (NS), euthanized on day 7; group 2 (control day 14), which underwent TBI surgery and received an IC injection of 40 µl NS, euthanized on day 14; group 3 (AD-MSCs day 7), which underwent TBI surgery and received an IC injection of 2×10^5 AD-MSCs, euthanized on day 7; and group 4 (AD-MSCs day 14), which underwent TBI surgery and received an IC injection of 2×10^5 AD-MSCs, euthanized on day 14. Outcomes measured included BDNF levels, apoptosis index, and cognitive function at the respective termination time points. Cognitive function was evaluated before euthanasia, followed by euthanasia under anesthesia and brain tissue collection.

Isolation and processing of AD-MSCs. The AD-MSCs were processed using standardized enzymatic isolation and culture techniques. The procedure included isolation, culture/expansion, cell counting, viability assessment, characterization, and seeding. Adipose tissue from rats was enzymatically digested with collagenase to isolate AD-MSCs. The cells were incubated at 37°C in a 5% CO_2 atmosphere, and growth was monitored daily. When cultures reached approximately 80% confluency, cells were subcultured for expansion. Isolated MSCs were cultured in alpha-minimum essential medium (α-MEM) supplemented with penicillin–streptomycin and platelet-rich plasma (PRP). Cultures were maintained at 37°C in 5% CO_2. The growth medium was replaced every 3 days, and cells were passaged using 0.05% trypsin–ethylenediaminetetraacetic acid (EDTA) until reaching passage 12.

Cells were washed with phosphate-buffered saline (PBS), trypsinized, incubated for 5 min at 37°C, and trypsin activity was neutralized with culture medium. The cell suspension was centrifuged at 1200 rpm for 10 min at 20°C, and the pellet was resuspended in fresh medium. A 10 µl aliquot was mixed with 10 µl trypan blue, loaded onto a hemo-

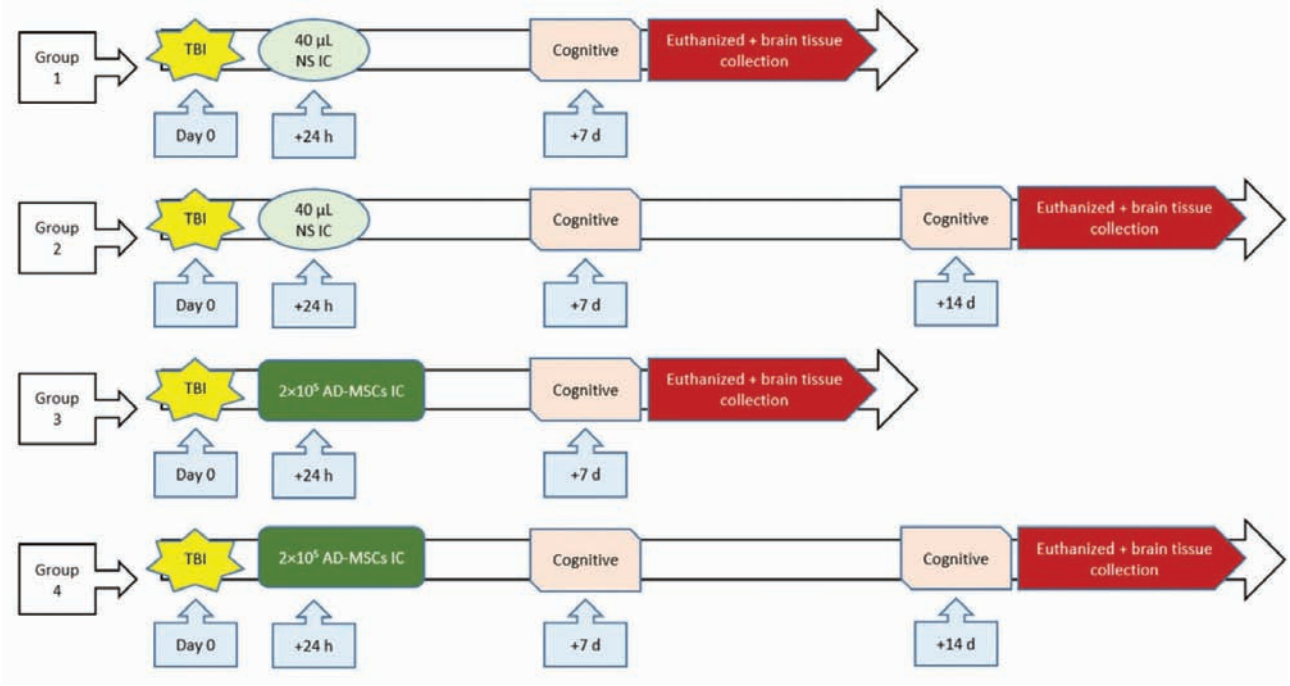

Fig. 1. Timeline of the experimental groups illustrating the sequence of TBI induction, intervention administration, cognitive evaluation, euthanasia, and subsequent brain tissue collection for apoptosis index and BDNF analysis

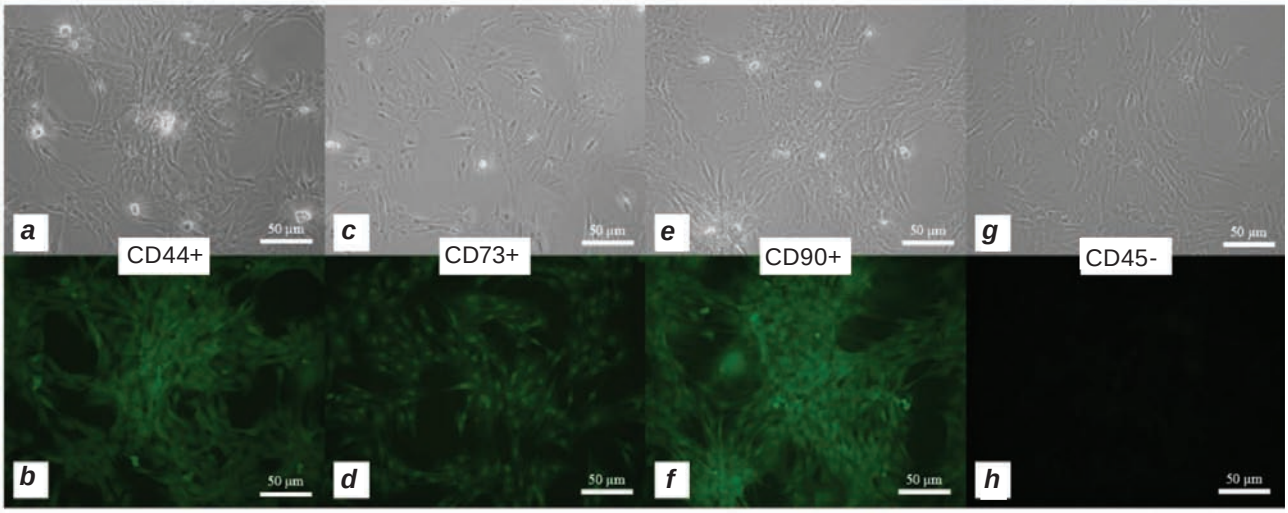

*Fig. 2. Immunophenotypic characterization of AD-MSCs prior to transplantation. (**a**, **b**) CD44+ cells; (**c**, **d**) CD73+ cells; (**e**, **f**) CD90+ cells; (**g**, **h**) CD45– cells. In each pair, the upper panel shows brightfield morphology, and the lower panel shows immunofluorescence staining (green) for the indicated marker*

cytometer, and counted under a light microscope. Cell viability was assessed during routine subculture. AD-MSCs were seeded in flasks with α-MEM and PRP-supplemented medium and monitored until reaching 80% confluency. Passaging was performed regularly up to passage 12. Flow cytometry was used to assess the expression of surface markers. AD-MSCs were positive for CD44, CD73, and CD90, and negative for CD45 (Fig. 2). Cells were incubated with appropriate antibodies and isotype control, and analyzed to confirm AD-MSCs identity and purity.

Surgical procedure and TBI induction. The surgical procedure and TBI induction were adapted and modified from a previously published study

[24]. Animals were anesthetized via intraperitoneal injection of ketamine (25 mg/kg), diazepam (2 mg/kg), and atropine (0.1 mg/kg), with a total volume of 2.5 ml/kg. When necessary, 25% of the initial dose was administered as a supplemental injection to achieve adequate analgesia. Animals were positioned prone on a surgical platform, and body temperature was maintained using a radiant heat source and monitored via rectal probe throughout the procedure.

Under aseptic conditions, a left-sided craniectomy measuring approximately 4×6 mm was performed, exposing the parietal and frontal cortex while preserving the arachnoid membrane. A moderate traumatic brain injury was induced using a controlled cortical impact model by releasing a 35-g solid metal cylinder (6 mm diameter) from a height of 20 cm through a guiding tube, achieving an impact velocity of approximately 2 m/s and generating a localized cortical contusion. Following the impact, the incision was closed using absorbable sutures. Tramadol (2 mg/kg, subcutaneous) was administered preoperatively and repeated as needed for postoperative pain control within the first 48 hours.

Intracortical administration. IC administration of AD-MSCs was performed 24 h following TBI. Animals designated to receive transplantation were randomly selected. After induction of anesthesia under sterile conditions, the original midline scalp incision was reopened to expose the contused cortical region.

A total dose of 2×10^5 AD-MSCs, adapted from a previous rat TBI model study [24], suspended in PBS, was delivered using a 26-gauge microinjection needle. Eight microinjections were administered in the pericontusional cortex, each containing 25,000 AD-MSCs in 5 µl PBS, injected over approximately 10 s at a depth of 2 mm below the cortical surface, resulting in a total volume of 40 µl per animal. Control animals underwent an identical procedure, but received equivalent volumes of sterile normal saline instead of AD-MSCs.

Cognitive function evaluation. Cognitive function was evaluated using the Barnes maze to assess spatial learning and memory, following a previously described protocol.[25] Rats underwent habituation and training sessions, followed by cognitive testing at the designated study time point prior to euthanasia. The primary outcome measured was escape latency (seconds), defined as the time required to locate the escape hole. Maze surfaces were cleaned between trials to eliminate olfactory cues and reduce bias.

Euthanasia and brain tissue collection. Animals were euthanized at the designated time points under deep anesthesia induced by intraperitoneal ketamine and xylazine. Adequate anesthesia was confirmed by the absence of reflex responses. Once unresponsive, a craniectomy was performed, and the brain was carefully removed and grossly inspected before further processing.

Brain tissue was divided coronally into anterior and posterior segments. The anterior portion was placed in 5 ml PBS for subsequent ELISA analysis, while the posterior portion was fixed in formalin for histopathological examination. Tissue samples were processed in accordance with standard laboratory protocols. Carcasses were subsequently destroyed by incineration using institutional animal disposal facilities.

Assessment of apoptosis index. Apoptotic cell death was evaluated using the TUNEL assay (In Situ Cell Death Detection Kit, Roche, Switzerland). Tissue sampling and histological processing followed standardized laboratory procedures and were performed by a qualified histologist under single-blind conditions. Following brain extraction, the posterior cerebrum was fixed in formalin, embedded in paraffin, and sectioned coronally into 5-µm-thick slices. TUNEL staining was performed according to the manufacturer's protocol, using a chromogenic reaction in which apoptotic nuclei were visualized as brown-stained cells under bright-field microscopy. Apoptotic cells were counted in three representative fields per section, and the apoptosis index was expressed as the percentage of TUNEL-positive cells relative to the total number of cells in each field.

Assessment of BDNF levels. BDNF concentrations were quantified using a sandwich ELISA in brain tissue lysates. The anterior cerebrum was homogenized and processed according to the manufacturer's protocol using a commercial rat BDNF ELISA kit (Elabscience, Cat. No. E-EL-R1235, USA).

Absorbance was measured at the specified wavelength using a microplate reader, and BDNF concentrations were calculated from a standard curve generated from serial dilutions of the kit standards. Results were expressed as pg/ml of tissue lysate. All procedures were performed following standard laboratory operating protocols to ensure assay reliability and reproducibility. To minimize measurement bias, ELISA processing and plate

reading were conducted by trained laboratory personnel blinded to the experimental groups.

Statistical analysis. Statistical analyses were performed using SPSS software (Version 23, IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize subject characteristics and variable distributions. Data normality was assessed using the Shapiro–Wilk test, given the sample size of fewer than 30 subjects per group. Homogeneity of variances was evaluated using Levene's test.

Comparisons between the control and treatment groups were conducted using an independent-samples *t*-test for normally distributed variables with equal variance. For data that did not meet normality assumptions, the Mann–Whitney U test was applied. A P-value < 0.05 was considered statistically significant.

Results

Baseline cognitive function. A total of 30 subjects were included in the study, consisting of 16 rats in the control group (group 1, n = 8; group 2, n = 8) and 14 rats in the AD-MSCs group (group 3, n = 7; group 4, n = 7). Shapiro–Wilk testing demonstrated that pre-treatment escape latency data were not normally distributed (P = 0.004). Therefore, values are presented as median ± interquartile range (IQR), and group comparisons were performed using the Mann–Whitney test.

At baseline, rats in the AD-MSCs group and control group demonstrated comparable escape latencies on the Barnes maze (Table 1). There was no statistically significant difference between the two groups (P > 0.05), indicating that both groups had homogeneous cognitive function prior to intervention.

Post-treatment gross brain morphology. Gross examination of brain tissue was performed at the time of euthanasia on days 7 and 14 (Fig. 3). At day 7, brains from the AD-MSCs group (Fig. 3, *a*) showed early evidence of tissue regeneration at the injury site, with newly formed tissue appearing similar in color and texture to surrounding healthy cortex. Areas of necrosis and encephalomalacia were minimal. In contrast, the control group (Fig. 3, *b*) demonstrated limited evidence of tissue repair, with a clearly demarcated cavitary defect and prominent necrotic-encephalomalacic changes.

By day 14, pronounced tissue regeneration was observed in the AD-MSCs group (Fig. 3, *c*), with tissue resembling normal cortex, and visible vascularization in the repair zone. Residual necrosis and atrophy were minimal. Conversely, the control group (Fig. 3, *d*) continued to exhibit a persistent lesion cavity with extensive encephalomalacia and

Table 1. Baseline cognitive function

Groups	Escape latencies, sec
AD-MSCs, n = 14	30.9 ± 84.0
Control, n = 16	45.7 ± 31.6
P-value	0.341

Table 2. Apoptosis index on day 7 and 14

Groups	Apoptosis index (D-7)	Apoptosis index (D-14)
AD-MSCs, n = 7	0.70 ± 0.38%	0.63 ± 1.04%
Control, n = 8	2.90 ± 2.18%	2.75 ± 2.45%
P-value	0.008*	0.01*

*Statistically significant mean difference at P < 0.05

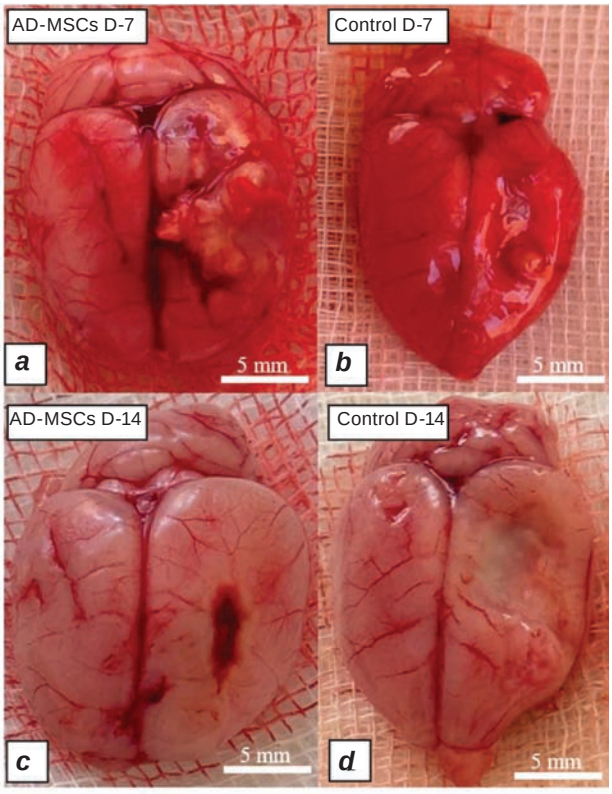

*Fig. 3. Post-treatment gross brain morphology: (**a**) AD-MSCs day 7, (**b**) control day 7, (**c**) AD-MSCs day 14, (**d**) control day 14*

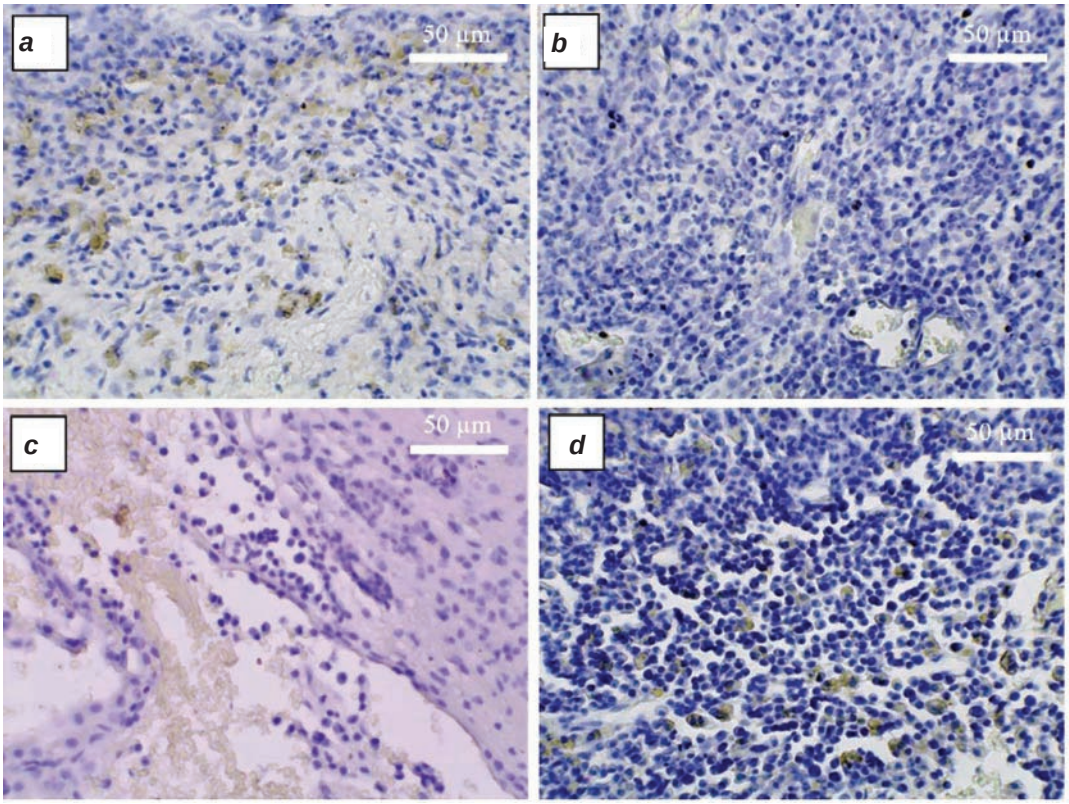

*Fig. 4. TUNEL staining of cortical brain tissue at 400× magnification. Brown-stained nuclei represent apoptotic cells. (**a**) Control day 7, (**b**) AD-MSCs day 7, (**c**) control day 14, (**d**) AD-MSCs day 14*

cortical atrophy, consistent with chronic post-injury tissue loss.

Apoptosis index. The apoptosis index of cortical neurons was evaluated using TUNEL staining on days 7 and 14, as summarized in Table 2. Shapiro–Wilk tests indicated a non-normal distribution for apoptosis data at both time points (day 7: $P = 0.002$; day 14: $P = 0.032$), therefore, values are reported as median ± IQR and analyzed with the Mann–Whitney test.

On day 7, the AD-MSCs group demonstrated a significantly lower apoptosis index compared with the control group. Similarly, on day 14, the AD-MSCs group showed markedly reduced apoptosis compared with the control group. These findings indicate a sustained anti-apoptotic effect of AD-MSCs administration following TBI.

Representative TUNEL images are shown (Fig. 4). Brown-stained nuclei indicate apoptotic cells. Fewer TUNEL-positive cells were observed in the AD-MSCs group at both time points compared with the control group, supporting the quantitative findings.

Table 3. BDNF levels on days 7 and 14

Groups	BDNF levels D-7, pg/ml	BDNF levels D-14, pg/ml
AD-MSCs, $n = 7$	57.8 ± 69.8	1.3 ± 3.19
Control, $n = 8$	94.4 ± 155.8	0.6 ± 0.8
P-value	0.728	0.269

Table 4. Cognitive function on days 7 and 14

Groups	Escape latencies D-7, sec	Escape latencies D-14, sec
AD-MSCs, $n = 7$	49.2 ± 64.5	34.1 ± 78.4
Control, $n = 8$	300 ± 194.5	219.1 ± 228.9
P-value	0.002*	0.014*

Note. *Statistically significant mean difference at $P < 0.05$

BDNF levels. BDNF levels in brain tissue were measured on days 7 and 14 (Table 3). Data were non-normally distributed (Shapiro-Wilk, $P = 0.005$).

Thus, data are presented as median ± IQR and analyzed using the Mann-Whitney test. On both days, no significant differences were observed between the AD-MSCs and control groups, indicating similar BDNF levels across treatments.

Post-treatment cognitive function. Cognitive function following treatment was assessed on days 7 and 14 using the Barnes maze test (Table 4). On day 7, Shapiro–Wilk testing showed a non-normal data distribution (P = 0.001). Therefore, data were presented as median ± IQR and analyzed using the Mann-Whitney test. The AD-MSCs group demonstrated significantly faster escape latency compared with the control group (P = 0.002), indicating improved cognitive recovery at this early time point.

On day 14, the AD-MSCs group continued to show significantly shorter escape latency than the control group (P = 0.014), confirming sustained cognitive improvement following AD-MSC therapy. These findings indicate that AD-MSCs administration resulted in a significant improvement in cognitive recovery post-TBI compared with the control condition.

Discussion

Neuroprotection and tissue preservation following AD-MSCs therapy. This study demonstrated that AD-MSCs therapy significantly reduced neuronal apoptosis on days 7 and 14 following TBI compared to control animals. Consistent with the apoptosis findings, gross morphological evaluation revealed reduced tissue loss and evidence of tissue regrowth in AD-MSCs–treated brains. In particular, AD-MSCs–treated subjects exhibited preservation of cortical architecture, narrowing of the injury cavity, and visible vascular structures suggestive of neuroregenerative activity. In contrast, control animals displayed extensive necrosis, cavitation, and cortical atrophy throughout the observation period. Collectively, these results support the neuroprotective potential of AD-MSCs in attenuating neuronal apoptosis and promoting tissue preservation following TBI [21, 26, 27].

Neuronal survival is critical in TBI recovery due to the limited regenerative capacity of central nervous system neurons, making apoptosis a key determinant of long-term functional outcomes [28]. Following primary mechanical trauma, TBI triggers secondary pathophysiological cascades including glutamate-mediated excitotoxicity, intracellular calcium overload, mitochondrial dysfunction, oxidative stress, transcriptional dysregulation, and inflammatory activation, ultimately driving apoptotic cell death [4].

AD-MSCs therapy counteracts these pathways by exerting neuroprotective and immunomodulatory effects. Prior studies have shown that AD-MSCs therapy attenuates glutamate excitotoxicity, reduces oxidative stress, stabilizes the blood–brain barrier, modulates inflammatory cytokines, and inhibits apoptotic signaling [21, 26, 29-31]. The present findings align with this body of evidence, reinforcing the concept that AD-MSCs therapy preserves neuronal viability and reduces injury progression after TBI.

Beyond acute neuroprotection, the neuroregenerative process, including enhanced angiogenesis, neurogenesis, oligodendrogenesis, and axonal/dendritic remodeling, also represents a crucial therapeutic strategy in TBI management [4]. In line with this, the observable neovascularization and tissue regeneration in AD-MSCs–treated brains in the present study further support the regenerative potential of AD-MSCs therapy.

Overall, AD-MSCs therapy appears to serve not only as neuroprotective agents shielding neural tissue in the acute post-injury phase, but also as biological mediators that promote structural repair and lesion resolution. These complementary actions highlight the therapeutic potential of AD-MSCs in improving neural survival and preserving brain integrity following TBI.

Cognitive improvement. In the present study, cognitive performance at day 7 and day 14 post-TBI was significantly higher in AD-MSCs–treated rats compared with the control group. These findings strengthen the growing evidence that AD-MSCs function as potent neurorestorative agents capable of enhancing neurological recovery. By supporting the integrity of the central nervous system and promoting neurovascular remodeling, AD-MSCs may facilitate key regenerative processes, including angiogenesis, neurogenesis, oligodendrogenesis, and axonal and dendritic growth, following TBI. This improvement is consistent with previous studies demonstrating the neurorestorative capacity of AD-MSCs and their ability to enhance neurological function after injury [16, 24, 26, 32, 33].

The cognitive enhancement observed in this study may be attributed to the ability of AD-MSCs to mitigate the multifactorial cascade of secondary brain injury, including glutamate excitotoxicity, neuroinflammation, oxidative stress, and neuronal

apoptosis [21, 26, 29-31]. By attenuating these processes, AD-MSCs likely create a permissive microenvironment for early and robust neuroregeneration, ultimately supporting improved functional recovery.

Supporting this interpretation, previous preclinical study reported enhanced motor performance in a TBI rat model following intracortical allogeneic AD-MSCs administration, accompanied by cell engraftment in perilesional regions and increased hippocampal cell density consistent with neurogenesis [24]. Similarly, previous preclinical study demonstrated that intracortical AD-MSCs therapy increased neuronal nuclei (NeuN) expression, a marker of mature neurons, and significantly improved modified neurological severity scores (mNSS) compared to the control [26]. Collectively, these findings suggest that AD-MSCs therapy effectively suppress neuronal apoptosis and accelerate neurorestorative processes, leading to improved neurobehavioral recovery, including cognitive function, after TBI.

BDNF levels and interpretative considerations. In the present study, BDNF concentrations at day 7 and day 14 post-TBI did not differ significantly between the AD-MSCs and control groups. This finding contrasts with several prior preclinical studies reporting that AD-MSCs upregulate neurotrophic factors, including BDNF, contributing to neuronal survival and regeneration following brain injury [21, 27, 30, 31, 34].

One possible explanation relates to BDNF gene methylation following TBI, which may suppress BDNF transcription in both treatment and control groups [35]. Supporting this, previous work using real-time polymerase chain reaction (RT-PCR) demonstrated that although AD-MSCs exhibit significantly higher BDNF and nerve growth factor (NGF) gene expression compared to Schwann cells, only NGF protein levels remained significantly elevated on ELISA, whereas BDNF protein levels did not differ [31]. Incorporating RT-PCR in future studies may therefore help clarify post-injury gene-to-protein regulatory dynamics.

The timing of sampling may also contribute. Experimental data show that BDNF expression increases sharply within the first 24 h post-injury and declines thereafter, with no significant elevation beyond 36 h [36]. In contrast, BDNF protein in this study was assessed at days 7 and 14, potentially missing the transient early peak.

Nevertheless, macroscopic tissue findings in the AD-MSCs group demonstrated more robust and sustained tissue regeneration and vascularization compared to the control group, consistent with the well-established paracrine secretion of neurotrophic factors by AD-MSCs, including BDNF, NGF, neurotrophin (NT)-3, and NT-4/5 [37]. NGF, in particular, may play a compensatory role, promoting neuroprotection and neurogenesis via AMP-activated protein kinase (AMPK) activation [38].

Importantly, the absence of a significant difference in BDNF levels does not negate the therapeutic benefit of AD-MSCs. In this study, the AD-MSCs group demonstrated significantly lower neuronal apoptosis and better cognitive outcomes, reinforcing that the secondary brain injury cascade is multifactorial and not solely dependent on BDNF regulation. Previous research has shown that AD-MSCs exert broad neuroprotective effects through multiple mechanisms, including reducing pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), suppressing astrocyte and microglial activation as reflected by lower expression of glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule 1 (IBA-1), and inhibiting neuronal apoptosis evidenced by increased expression of NeuN and an elevated B-cell lymphoma-2 to Bcl-2-associated X protein ratio (Bcl-2/Bax) [26]. AD-MSCs also attenuate tissue edema and limit nitric oxide (NO) production following TBI [26]. Collectively, these pathways likely contributed to the enhanced functional recovery observed in the AD-MSC group, even in the absence of significant BDNF elevation.

The novelty of this study lies in being among the first to investigate intracortical AD-MSCs administration in a moderate TBI rat model, with comprehensive evaluation of BDNF levels, neuronal apoptosis, and cognitive performance. This integrated analysis underscores both the neuroprotective and neurorestorative potential of AD-MSCs and supports their role in promoting brain repair and cognitive functional recovery. Nevertheless, this study has limitations, as long-term risks and the detailed fate of transplanted cells were not evaluated, and further investigations are needed to clarify these aspects.

Conclusion. AD-MSCs therapy post-TBI in male Wistar rats reduced neuronal apoptosis and improved cognitive function, despite no significant change in BDNF levels. These results highlight the neuroprotective and neurorestorative potential of AD-MSCs, supporting their role in promoting brain repair and functional recovery.

Author contributions. GFPK – study conception, study design, data acquisition, data analysis, data interpretation, manuscript editing, and review. TGBM – study conception, study design, data interpretation, manuscript review. NNSB - study conception, study design, data interpretation, manuscript review. TA – study conception, data acquisition, data analysis, manuscript review, ABSS – data acquisition, data analysis, manuscript review.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ВВЕДЕННЯ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН, ОТРИМАНИХ ІЗ АДИПОЦИТІВ, ЗМЕНШУЄ АПОПТОЗ НЕЙРОНІВ ТА СПРИЯЄ ВІДНОВЛЕННЮ КОГНІТИВНИХ ФУНКЦІЙ ПІСЛЯ ЧЕРЕПНО-МОЗКОВОЇ ТРАВМИ СЕРЕДНЬОГО СТУПЕНЯ У ЩУРІВ

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Вступ. Черепно-мозкова травма залишається однією з провідних причин тривалої інвалідності у всьому світі. Фаза вторинного ураження мозку, яка розвивається через кілька годин або днів після первинної травми, є критичним періодом для терапевтичних втручань, однак жодна з доступних терапій поки не виявилася ефективною. **Мета.** Оцінити вплив терапії мезенхімальними стовбуровими клітинами, виділеними з адипозної тканини (AD-MSC), на апоптоз нейронів, рівень нейротрофічного фактора мозку (BDNF), та когнітивні функції після черепно-мозкової травми середнього ступеня у щурів. **Методи.** AD-MSC щурів було виділено та проаналізовано за допомогою стандартизованих методів. Дорослих самців щурів породи Вістар анестезували, виконували кранітомію з лівого боку та індукували помірну черепно-мозкову травму шляхом скидання металевого циліндра через направляючу трубку. Після удару розріз зашивали нитками, що розсмоктуються. Через 24 години після ЧМТ у периконтузійну кору вводили вісім мікроін'єкцій, кожна з яких містила 2×10^5 AD-MSC у 5 мкл PBS. Тваринам контрольної групи вводили еквівалентні об'єми стерильного фізіологічного розчину. Евтаназію тварин проводили на 7-й або 14-й день, після чого вилучали мозок для аналізу. Перед евтаназією щурам проводили когнітивні тести. Індекс апоптозу оцінювали за допомогою тесту TUNEL, рівень BDNF – методом ELISA, когнітивні функції за допомогою тесту «лабіринт Барнса». **Результати.** Макроскопічне дослідження мозку виявило посилену регенерацію кори та васкуляризацію у щурів, які отримували AD-MSC, порівняно з контрольною групою. Індекс апоптозу був значно нижчим у групі AD-MSC як на 7-й, так і на 14-й день. Когнітивні функції помітно покращилися в групі AD-MSC, про що свідчило скорочення часу проходження лабіринту Барнса як на 7-й, так і на 14-й день. Натомість рівні BDNF не відрізнялися між групами в обидва моменти часу. **Висновок.** Ці результати демонструють як нейропротекторні, так і нейрорегенеративні ефекти AD-MSC та свідчать про перспективність їхнього застосування як терапевтичної стратегії для зменшення вторинних уражень мозку після черепно-мозкової травми.

Ключові слова: черепно-мозкова травма у щурів, мезенхімальні стовбурові клітини, апоптоз, нейротрофічний фактор мозку, когнітивні функції.

References

1. Maas AIR, Menon DK, Manley GT, Abrams M, Åkerlund C, Andelic N, Aries M, Bashford T, Bell MJ, Bodien YG. et al. Traumatic brain injury: progress and challenges in prevention, clinical care, and research. *Lancet Neurol.* 2022; 21(11): 1004-1060.

2. Williams OH, Tallantyre EC, Robertson NP. Traumatic brain injury: pathophysiology, clinical outcome and treatment. *J Neurol*. 2015; 262(5): 1394-1396.
3. Werner JK, Stevens RD. Traumatic brain injury: recent advances in plasticity and regeneration. *Curr Opin Neurol.* 2015; 28(6): 565-573.
4. Xiong Y, Zhang Y, Mahmood A, Chopp M. Investigational agents for treatment of traumatic brain injury. *Expert Opin Investig Drugs.* 2015; 24(6): 743-760.
5. Liu YW, Li S, Dai SS. Neutrophils in traumatic brain injury (TBI): friend or foe? *J Neuroinflammation.* 2018; 15(1): 146.
6. Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. *Exp Neurol*. 2016; 275(Pt 3): 316-327.
7. Liu Z, Chopp M. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. *Prog Neurobiol*. 2016; 144: 103-120.
8. Fang J, Han D, Hong J, Tan Q, Tian Y. The chemokine, macrophage inflammatory protein-2γ, reduces the expression of glutamate transporter-1 on astrocytes and increases neuronal sensitivity to glutamate excitotoxicity. *J Neuroinflammation.* 2012; 9: 267.
9. Pineau I, Sun L, Bastien D, Lacroix S. Astrocytes initiate inflammation in the injured mouse spinal cord by promoting the entry of neutrophils and inflammatory monocytes in an IL-1 receptor/MyD88-dependent fashion. *Brain Behav Immun*. 2010; 24(4): 540-553.
10. Lu KT, Wang YW, Yang JT, Yang YL, Chen HI. Effect of interleukin-1 on traumatic brain injury-induced damage to hippocampal neurons. *J Neurotrauma*. 2005; 22(8): 885-895.
11. Ng SY, Lee AYW. Traumatic brain injuries: pathophysiology and potential therapeutic targets. *Front Cell Neurosci.* 2019; 13: 528.
12. Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, Borlongan CV. Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr Dis Treat. 2015; 11: 97-106.
13. Galgano M, Toshkezi G, Qiu X, Russell T, Chin L, Zhao LR. Traumatic brain injury: current treatment strategies and future endeavors. *Cell Transplant*. 2017; 26(7): 1118-1130.
14. Zhou Y, Shao A, Xu W, Wu H, Deng Y. Advance of stem cell treatment for traumatic brain injury. *Front Cell Neurosci.* 2019; 13: 301.
15. Wislet-Gendebien S, Laudet E, Neirinckx V, Rogister B. Adult bone marrow: which stem cells for cellular therapy protocols in neurodegenerative disorders? *J Biomed Biotechnol*. 2012; 2012: 601560.
16. Mashkouri S, Crowley MG, Liska MG, Corey S, Borlongan CV. Utilizing pharmacotherapy and mesenchymal stem cell therapy to reduce inflammation following traumatic brain injury. *Neural Regen Res.* 2016; 11(9): 1379-1384.
17. Ghasemi N. Transdifferentiation of human adipose-derived mesenchymal stem cells into oligodendrocyte progenitor cells. *Iran J Neurol*. 2018; 17(1): 24-30.
18. Czerwiec K, Zawrzykraj M, Deptuła M, Skoniecka A, Tymińska A, Zieliński J, Kosiński A, Pikuła M. Adipose-derived mesenchymal stromal cells in basic research and clinical applications. *Int J Mol Sci*. 2023; 24(4): 3888.
19. Suchanecka M, Grzelak J, Farzaneh M, Azizidoost S, Dari MAG, Józkowiak M, Data K, Domagała D, Niebora J, Kotrych K, Czerny B, Kamiński A, Torlińska-Walkowiak N, Bieniek A, Szepietowski J, Piotrowska-Kempisty H, Dzięgiel P, Mozdziak P, Kempisty B. Adipose derived stem cells - Sources, differentiation capacity and a new target for reconstructive and regenerative medicine. *Biomed Pharmacother*. 2025; 186: 118036.
20. Sumarwoto T, Suroto H, Mahyudin F, Utomo DN, Romaniyanto, Tinduh D, Notobroto HB, Sigit Prakoeswa CR, Rantam FA, Rhatomy S. Role of adipose mesenchymal stem cells and secretome in peripheral nerve regeneration. *Ann Med Surg (Lond).* 2021; 67: 102482.
21. Kokai LE, Marra K, Rubin JP. Adipose stem cells: biology and clinical applications for tissue repair and regeneration. *Transl Res*. 2014; 163(4): 399-408.
22. Bateman ME, Strong AL, Gimble JM, Bunnell BA. Concise Review: Using Fat to Fight Disease: A Systematic Review of Nonhomologous Adipose-Derived Stromal/Stem Cell Therapies. *Stem Cells.* 2018; 36(9): 1311-1328.
23. Li P, Guo X. A review: therapeutic potential of adipose-derived stem cells in cutaneous wound

healing and regeneration. *Stem Cell Res Ther.* 2018; 9(1): 302.
24. Mastro-Martínez I, Pérez-Suárez E, Melen G, González-Murillo Á, Casco F, Lozano-Carbonero N, Gutiérrez-Fernández M, Díez-Tejedor E, Casado-Flores J, Ramírez-Orellana M, Serrano-González A. Effects of local administration of allogenic adipose tissue-derived mesenchymal stem cells on functional recovery in experimental traumatic brain injury. *Brain Inj.* 2015; 29(12): 1497-1510.
25. Rosenfeld CS, Ferguson SA. Barnes maze testing strategies with small and large rodent models. *J Vis Exp.* 2014; (84): e51194.
26. Jiang W, Luo H, Zhao M, Fan Q, Ye C, Li X, He J, Lai J, He S, Chen W, Xian W, Chen S, Chen Z, Li D, Chen R, Wang B. Evaluation of canine adipose-derived mesenchymal stem cells for neurological functional recovery in a rat model of traumatic brain injury. *BMC Vet Res.* 2024; 20(1): 110.
7. Salgado AJ, Reis RL, Sousa NJ, Gimble JM. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. *Curr Stem Cell Res Ther.* 2010; 5(2): 103-110.
28. Zhao Y, Gibb SL, Zhao J, Moore AN, Hylin MJ, Menge T, Xue H, Baimukanova G, Potter D, Johnson EM, Holcomb JB, Cox CS Jr, Dash PK, Pati S. Wnt3a, a protein secreted by mesenchymal stem cells is neuroprotective and promotes neurocognitive recovery following traumatic brain injury. *Stem Cells.* 2016; 34(5): 1263-1272.
29. Khaksari M, Rajizadeh MA, Bejeshk MA, Soltani Z, Motamedi S, Moramdi F, Islami M, Shafa S, Khosravi S. Does inhibition of angiotensin function cause neuroprotection in diffuse traumatic brain injury? *Iran J Basic Med Sci*. 2018; 21(6): 615-620.
30. Zhang HT, Liu ZL, Yao XQ, Yang ZJ, Xu RX. Neural differentiation ability of mesenchymal stromal cells from bone marrow and adipose tissue: a comparative study. *Cytotherapy.* 2012; 14(10): 1203-1214.
31. Reid AJ, Sun M, Wiberg M, Downes S, Terenghi G, Kingham PJ. Nerve repair with adipose-derived stem cells protects dorsal root ganglia neurons from apoptosis. *Neuroscience*. 2011; 199: 515-522.
32. Weston NM, Sun D. The potential of stem cells in treatment of traumatic brain injury. *Curr Neurol Neurosci Rep.* 2018; 18(1): 1.
33. Xue S, Zhang HT, Zhang P, Luo J, Chen ZZ, Jang XD, Xu RX. Functional endothelial progenitor cells derived from adipose tissue show beneficial effect on cell therapy of traumatic brain injury. *Neurosci Lett.* 2010; 473(3): 186-191.
34. Berg J, Roch M, Altschüler J, Winter C, Schwerk A, Kurtz A, Steiner B. Human adipose-derived mesenchymal stem cells improve motor functions and are neuroprotective in the 6-hydroxydopamine-rat model for Parkinson's disease when cultured in monolayer cultures but suppress hippocampal neurogenesis and hippocampal memory function when cultured in spheroids. *Stem Cell Rev Rep.* 2015; 11(1): 133-149.
35. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. *Arch Med Sci*. 2015; 11(6): 1164-1178.
36. Wurzelmann M, Romeika J, Sun D. Therapeutic potential of brain-derived neurotrophic factor (BDNF) and a small molecular mimics of BDNF for traumatic brain injury. *Neural Regen Res.* 2017; 12(1): 7-12.
37. Liu Y, Zhao C, Zhang R, Pang Y, Li L, Feng S. Progression of mesenchymal stem cell regulation on imbalanced microenvironment after spinal cord injury. *Stem Cell Res Ther.* 2024; 15(1): 343.
38. Figiel-Dabrowska A, Radoszkiewicz K, Rybkowska P, Krzesniak NE, Sulejczak D, Sarnowska A. Neurogenic and Neuroprotective Potential of Stem/Stromal Cells Derived from Adipose Tissue. *Cells.* 2021; 10(6): 1475.