ОГЛЯДИ

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Study and treatment of brain ischemic-reperfusion injury

S.V. Konovalov, V.M. Moroz, M.V. Yoltukhivskyy, I.V. Gusakova

Vinnytsia National Pirogov Memorial Medical University; e-mail: serhii.konovalov555@gmail.com

Ischemic stroke, caused by impaired blood flow to the brain, remains one of the medical conditions associated with high mortality and long-term disability. Ischemia followed by recanalization of the occluded vessel (via thrombolysis or thrombectomy) triggers a cascade of biochemical events in the affected brain regions, resulting in ischemia-reperfusion injury and neuronal death. Conventional treatment of ischemic stroke, involving the use of antithrombotic and neuroprotective agents, is not always sufficiently effective or safe. Regenerative medicine, particularly stem cell transplantation, has emerged as a promising therapeutic approach for cerebral ischemia-reperfusion injury. Numerous experimental studies, along with some clinical trials, have investigated various types of stem cells. Stem cell–based therapy holds the potential to develop more effective and comprehensive strategies targeting neuroregeneration in the ischemia-damaged brain. This review aims to provide an updated perspective on acute cerebral ischemia-reperfusion, with particular emphasis on the underlying pathogenesis and morphological changes in both the ischemic core and penumbra, as well as the therapeutic prospects of stem cell–based interventions for cerebroprotection in acute ischemic stroke.

Key words: brain ischemia-reperfusion; pathogenesis; morphological changes; mesenchymal stromal cells.

INTRODUCTION

Cerebral stroke is a pathological condition that belongs to emergency states. Among the different types of stroke, ischemic strokes occur much more frequently, accounting for 65-87% of cases, while hemorrhagic strokes (including intracerebral hematomas and subarachnoid hemorrhages) represent only 10-20% [1].

A characteristic feature of stroke course is the rapid and often irreversible development of pathophysiological and biochemical processes, frequently resulting in neuronal death and even the destruction of entire brain regions. These processes include [2]:

- a decrease in cerebral blood flow;
- a reduction in the synthesis of macroergic substances, such as ATP (adenosine triphosphate);
- dysfunction of active ion transport;
- activation of the glutamate cascade, involving excessive glutamate release from presynaptic terminals of ischemic neurons into the intercellular space;
- calcium release from the intracellular stores;
- activation of multiple enzymes - phospholipase, xanthine oxidase, and calpain protease - as well as accumulation of arachidonic acid;
- oxidative stress with accumulation of free radicals and reactive forms of oxygen;
- inflammation and genetically programmed neuron death.

The use of advanced diagnostic methods for acute cerebrovascular accidents, particularly neuroimaging, has enabled the study of cerebral ischemia and its progression at the molecular level, contributing to the development of modern concepts of ischemic stroke pathogenesis [3, 4].

Pathological mechanisms of cerebral ischemia-reperfusion injury

Contemporary understanding of ischemic stroke pathogenesis involves the concept of a threshold level of cerebral blood flow. When blood flow drops below this critical threshold, oxygen supply becomes insufficient, resulting in ischemia [5]. Impaired venous outflow also plays a significant role in this process [2]. The resulting hypoxia triggers an acute energy deficit in neurons, initiating the "ischemic cascade", a sequence of molecular and cellular events that ultimately leads to irreversible damage to nervous tissue [6]. Researchers have distinguished a specific sequence of metabolic disturbances that occur during the progression of acute brain ischemia. The initial response is triggered when cerebral blood flow falls below 55 ml/100 g/min, leading to the suppression of protein synthesis [7]. A further decrease to below 35 ml/100 g/min activates anaerobic glycolysis. When cerebral perfusion drops below 20 ml/100 g/min, neuronal bioelectrical activity, a requisite to hold energy stockpiles, is inhibited, this is accompanied by the excessive release of excitatory neurotransmitters such as glutamate and aspartate [8]. When cerebral blood flow critically declines to 10-12 ml/100 g/min or less (the lower ischemic threshold), anoxic depolarization of cell membranes occurs within 6-8 min, ATP synthesis ceases, membrane function is disrupted, and neurons lose potassium while accumulating calcium, sodium, and water through osmosis [8]. These processes leads to necrosis of neurons, glial cells, and endothelial cells, leading to the infarct core formation. Clinically, this is manifested by signs of neurological deficit [5, 7]. Surrounding the infarct core is a border zone that forms within the first hours of cerebral ischemia. In this region, cerebral blood flow is reduced to approximately 18-20 ml/100 g/min (the upper ischemic threshold). Here, although synaptic transmission is impaired, cellular energy supply and ion pump function remain intact [9]. Cells in this zone are less severely affected by ischemia and remain viable for a limited period (typically 3-6 h), with cell death primarily occurring via apoptosis. This area is known as the ischemic penumbra [10, 11]. In the penumbra, the deficits in glucose and oxygen are less severe, allowing cells to generate a limited amount of ATP. Cells in the "ischemic penumbra" may either progress to irreversible damage, thus expanding the infarct area, or recover normal function if perfusion is rapidly restored [12]. The time during which these cells remain viable and capable of recovery is referred to as the "therapeutic window", the critical period when treatment is most effective [13]. According to current understanding, neurodestruction caused by ischemia is accompanied by pathobiochemical cascades complex. These begin primarily with impaired energy metabolism and mitochondrial dysfunction, leading to the excessive production of reactive oxygen species and nitric oxide, excitotoxicity, disruption of the blood-brain barrier, inflammation, and the expression of pro-apoptotic proteins. Together, these processes result in neuronal death and the impairment of sensory, motor, and cognitive functions [13, 14].

The formation of an ischemic focus in acute cerebral blood flow disorders is accompanied by destructive and degenerative changes in the cytoarchitecture of nervous tissue. This is evidenced by a reduction in neuronal area and density, as well as a decrease in nucleic acid content. Reduced cerebral blood supply, resulting from either cerebral artery occlusion or central hemodynamic disturbances, limits the delivery of oxygen and glucose to the brain. In the necrotic core, where oxygen and glucose levels are critically low, glycolysis is activated, but ATP production is insufficient. This is accompanied by excessive lactate accumulation, depletion of ATP reserves, and failure of the sodium-potassium pump, all of which contribute to mitochondrial dysfunction. It is now well established that increased lactate production leads to both intracellular and extracellular acidosis [15].

Acidosis occurring and spreading within the penumbra zone of cerebral infarction promotes

the interaction of acid-sensing ion channel 1a (ASIC1a) with receptor-interacting protein kinase 1 (RIP1) and its activation. RIP1 is critical effector of inflammatory responds and activates cell death through both caspase-dependent apoptosis and caspase-independent necroptosis [16]. In addition, the acidic environment, along with calpain activation, increases lysosomal membrane permeability, allowing the release of lysosomal proteases, cathepsins and hydrolases, into the cytosol, leading to autolysis [17].

Nerve cells also lose K^+ ions and remain in a state of sustained depolarization due to the opening of voltage-dependent sodium and calcium channels, as well as the activation of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [14, 18]. This leads to elevated intracellular concentrations of Na^+ and Ca^{2+}, resulting in increased osmotic pressure and enhanced water influx into the cells, causing cytotoxic swelling in brain tissue [8, 19].

Cells swell and eventually undergo plasma membrane rupture, releasing their contents, including neurotransmitters, into the interstitial space. This allows glutamate to bind to NMDA receptors on neighboring and even distant cells. The accumulation of glutamate and aspartate in the synaptic cleft leads to overactivation of ionotropic glutamate receptors. As a result, intracellular calcium concentration increases, while potassium ions accumulate in the extracellular space [13, 14]. A high intracellular Ca^{2+} level activate various enzymes, including phospholipases, calpains, and proteases, which contribute to cytoskeletal degradation [14]. This form of cell death, known as oncosis (or necrosis), affects not only neurons but also astrocytes and oligodendrocytes [10].

To develop modern approaches to pathogenetic therapy for ischemic stroke, it is essential to determine which type of cellular destruction prevails during acute cerebral blood flow impairment. From the standpoint of evidence-based medicine, thrombolytic therapy is currently the standard treatment for ischemic stroke [12].

However, the restoration of perfusion in the ischemic zone can exacerbate metabolic disturbances, this leads to the development of reperfusion injury in brain tissue. Destruction of desmosomes and increased spacing between neurons facilitate the spread of both free radicals and secondary messengers, affecting neighboring intact cells and thereby expanding the lesion area [6, 11]. Under conditions of acute cerebral blood flow disruption, apoptotic cell death predominates over necrotic one, as it can induce all the factors involved in secondary neuronal damage. In ischemia-reperfusion (IR) conditions, IR modelling clinically reflects the post-perfusion cerebral injury after thrombolysis, most neurons undergo apoptosis [20].

Apoptotic cell death is not followed by inflammation development, as membrane integrity is not disturbed. During apoptosis, cells undergo DNA fragmentation, degradation of cytoskeletal and nuclear proteins, protein cross-linking, and the formation of apoptotic bodies, which are subsequently subjected to phagocytosis. Thus, apoptotic neuronal death is considered a ‘lesser evil’ compared to necrosis, offering certain advantages for the brain despite the overall reduction in cell number [21].

Multiple mechanisms contribute to cell destruction in the penumbra zone. Reactive oxygen species (ROS), along with glutamate binding to NMDA and other glutamate receptors, increase intracellular Ca^{2+} concentration. This increase in calcium promotes mitochondrial permeability and the release of cytochrome C and apoptosis-inducing factor into the cytoplasm, initiating both caspase-dependent and caspase-independent apoptotic pathways [22]. Excessive intracellular calcium and a sharp rise in oxidative processes also stimulate nitric oxide synthesis. When ROS production exceeds the capacity of the antioxidant defense system, oxidative stress develops [23].

Increased intracellular calcium activates calpains, which cleave pro-apoptotic Bcl-2 family protein, promoting their mitochondrial

translocation with formation of pores in the outer mitochondrial membrane. This ensures the release of subsequent cytochrome C in the intrinsic or mitochondrial pathway of apoptosis [22].

Necroptosis is a form of regulated necrosis in which RIP1 phosphorylates and activates RIP3, leading to the expression and activation of mixed-lineage kinase domain-like protein (MLKL). Phosphorylated MLKL then oligomerizes at the plasma membrane, ultimately disrupting membrane integrity [24]. Apoptosis-inducing factor (AIF), released from mitochondria following the opening of the mitochondrial permeability transition pore, translocates to the nucleus where it induces chromatin degradation and activates poly(ADP-ribose) polymerase (PARP). The PARP product, poly(ADP-ribose), inhibits hexokinase, resulting in bioenergetic collapse and triggering a distinct form of cell death known as parthanatos [21, 22].

The principal sources of reactive oxygen species (ROS) in brain tissue are nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, and monoamine oxidase (MAO) [25]. Accumulation of ROS affects the endoplasmic reticulum, leading to calcium depletion within it and Ca^{2+} influx into the cytoplasm, ultimately resulting in calcium overload and the initiation of apoptosis [26]. ROS also activate microglia, which begin releasing interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α within minutes after the onset of acute oxygen and glucose deprivation [27]. Astrocytes become activated as well, promoting their proliferation and the secretion of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases [23]. These cytokines promote aggregation of inflammatory cells and enhance inflammatory cytokines output, further worsening brain dysfunction [28]. Moreover, cytokines amplify the expression of chemokines such as chemokine ligand 1 (CXCL1) and monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) in endotheliocytes, leading to infiltration of damaged tissue by peripheral monocytes/macrophages, thereby intensifying the inflammatory response [29].

Activation of microglia, together with excessive stimulation of NMDA receptors (excitotoxicity), leads to the binding of TNF-α to its receptors on the cell surface, recruitment of the Fas-associated death domain (FADD), and subsequent activation of procaspase-8, thereby initiating the autolytic cascade of the extrinsic apoptotic pathway [21, 27].

The subsequent stages of the ischemic cascade involve gene expression and the development of delayed consequences of ischemia, such as local inflammatory responses and microvascular disturbances, which lead to damage to the blood-brain barrier and neuronal death [19]. Endothelial dysfunction, along with structural and functional alterations of the vascular wall associated with arterial hypertension, atherothrombosis, and hypercoagulability, increases permeability of the blood-brain barrier, exacerbates microcirculatory disturbances, promotes blood clot formation, and raises the risk of ischemic brain damage [30]. Neuronal loss within the infarct zone can also cause the death of distant neurons through transneuronal degeneration [22], resulting from disrupted synaptic interactions and excessive NMDA receptor activation, which in turn enhances the expression of pro-apoptotic factors such as Puma, APAF-1, and caspase-9 [31].

Therefore, a key strategy in cytoprotection involves the development and clinical implementation of novel approaches aimed at preventing or limiting apoptosis. In most cases, inhibition of this form of cell death is considered beneficial, and selective suppression of apoptosis plays a critical role in the realization of cytoprotective mechanisms. Attenuating the ischemic cascade by protecting neurons in the penumbra represents a promising direction for neuroprotective therapy [32]. Thus, it can be concluded that selectively preserving programmed cell death pathways in the circumstances of acute cerebral circulatory disorders is a suboptimal cytoprotective strategy. Instead, the search for new therapeutic agents

capable of simultaneously inhibiting both programmed and non-programmed cell death pathways is promising.

Cell therapy of cerebral ischemia, implementation prospects

Stem cell transplantation has become a new impetus in the therapy of ischemic stroke, acting as a key component of regenerative strategies. Initially, stem cells were used as a therapy for replacing lost cells. Now the views of scientists are focused on the ability of stem cells to release substances that interfere with multiple pathogenetic cascades and contribute to the survival, transmigration, differentiation, and functional integration of transplanted cells into the brain in acute ischemia [33]. This shift was driven by the results of experiments on animal models and several clinical trials [34].

Neural stem cells (NSCs) can be obtained from the inner cell mass of a blastocyst between 5 and 7 days after conception, prior to implantation in the uterus. At this stage, they are referred to as embryonic stem cells (ESCs), which have the potential to differentiate into NSCs. Alternatively, if cells are isolated from the fetal nervous system between 7 and 21 days after conception, they are classified as multipotent neural stem cells capable of differentiating into neurons, astrocytes, and oligodendrocytes [35].

NSCs exhibit several precedences over other types of stem cells by virtue of:

- a strong resemblance to brain tissue [36],
- a lower risk of rejection, both for inter-individual and inter-species transplanted NSC cells, compared to neurocytes [37],
- significant chemotaxis, when NSCs can migrate toward the site of injury [38].

Recent studies have revealed that chemokine factor-1α (SDF-1α/CXCL12), derived from stromal cells, tends to accumulate in the ischemic zone, where it interacts with CXCR4 receptors on NSCs or with monocyte chemotactic protein-3/cinnamoyl-coenzyme A reductase, promoting the migration of these cells to the affected region [39]. Endogenous proliferation of NSCs in the subventricular zone and dentate gyrus region, as well as the migration of these cells to the area of ischemic damage, increases following NSC transplantation within the first two days after stroke onset [40]. In this manner, they are able to replace damaged neural cell types and also produce neuroprotective and regenerative growth factors [41]. By releasing nerve growth factor, brain-derived neurotrophic factor, and glial cell line-derived neurotrophic factor (GDNF), transplanted NSCs attenuate the pro-inflammatory cascade and thereby suppress secondary injury processes [42].

This mechanism of transplanted NSC action is known as the “bystander effect.” The bystander effect can also be observed following intravenous administration of NSCs. However, intraparenchymal transplantation of fetal NSCs in rodent models of ischemic stroke has been associated with reduced expression of IL-1β, IL-6, intracellular adhesion molecule-1 (ICAM-1), TNF-α, MCP-1, and vascular cell adhesion molecule-1 (VCAM-1), thereby enhancing their immunomodulatory ability [43]. However, NSCs, while retaining their multipotent differentiation potential, exhibit a tendency to early senescence [44].

NSCs can, at least theoretically, be obtained from the subventricular and subgranular zones of the human brain. However, their abundance decreases with age, and in addition, in vitro culturing changes their cellular state and promotes immune rejection [37].

Another approach involves the generation of induced pluripotent stem cells (iPSCs) through retroviral transduction of the OSKM genes: octamer-binding transcription factor 4 (Oct4), sex-determining region Y-box 2 (Sox2), Krüppel-like factor 4 (Klf4), and the avian myelocytomatosis virus oncogene homologue (c-Myc) [45]. In the presence of specific inducers and proteins, these iPSCs can differentiate into NSCs [46]. However, the resulting iPSCs may carry chromosomal aberrations, which contribute to their tumorigenic and immunogenic potential [47]. The transdifferentiation of non-neural cells (such as astrocytes or pericytes, whether

differentiated or proliferating) into neuronal cells is a promising method for obtaining NSCs. This can be achieved through retrovirus-mediated co-expression of various transcription factors [48]. This approach bypasses the pluripotent cell stage and thereby reduces the risk of neoplasia [49]. Nevertheless, the use of NSCs raises numerous ethical, religious as well as scientific concerns [33].

Cell therapy using mesenchymal stromal cells (MSCs) has shown beneficial effects on endogenous neuroregenerative mechanisms in response to ischemic damage to brain structures [50].

MSCs are defined by the Committee on Mesenchymal Stromal Cells of the International Society for Cell and Gene Therapy (ISCT) according to the following criteria:

- plastic adherence;
- expression of the CD73, CD90, CD105 markers;
- lack of expression of the hematopoietic and endothelial CD11b, CD14, CD19, CD34, CD45, CD79a, and HLA-DR markers;
- capability of differentiation into adipocyte, chondrocyte, and osteoblast lineages *in vitro* [51].

Numerous preclinical studies have demonstrated the capacity of MSCs to alleviate tissue damage and promote functional recovery through multiple mechanisms, including immunomodulation, proangiogenic signaling, secretion of neurotrophic factors, and neuronal differentiation [50]. MSCs also offer several advantages over other stem cells, such as easier methods of isolation, low tumorigenic risk, and the absence of ethical concerns [52]. MSCs can be obtained from various sources, including bone marrow, tooth buds and pulp, adipose tissue, liver, umbilical cord, umbilical cord blood, and placenta [47].

MSCs lack human leukocyte antigen class II (HLA-II) molecules, which makes them less immunogenic. In ischemic stroke, MSCs reduce microglial activation, protect mitochondrial function, and inhibit neuronal apoptosis during the acute phase [53]. They also suppress the release of pro-inflammatory cytokines, promote angiogenesis, and reduce blood-brain barrier damage in the subacute phase [54]. MSCs are pluripotent cells derived from adult tissues, making them ethically preferable for both preclinical and clinical research [55]. The mechanisms underlying the beneficial effects of stromal cell transplantation include bystander effects, paracrine signaling, and extracellular vesicle-mediated restorative actions [56].

Various types of stem cells have been studied as monotherapy in animal models of ischemic stroke. For example, mesenchymal stem cells derived from the human amniotic membrane (amniotic mesenchymal stem cells, AMSCs) have been transplanted into rats with experimentally induced ischemic stroke, where they modulate the immune response by suppressing pro-inflammatory cytokine expression and increasing the expression of the CD200 factor. Additionally, rats receiving AMSC-derived MSCs show reduced microglial activation in the penumbra and improved behavioral responses. Therefore, CD200 modulates pro-inflammatory cytokines level and microglial activation, thus increasing the potential for recovery after ischemic brain injury [57].

The efficacy of human amniotic epithelial cells (hAECs) has been investigated in animal models of cerebral ischemia, including both young and aged mice (7-14 weeks and 20-22 months old, respectively, of both sexes) as well as adult marmots. When administered intravenously 1.5 h after stroke induction, hAECs migrated to the ischemic zone and reduced infarct volume, inflammation, and functional deficits. Administration of hAECs 1 to 3 days post-stroke also resulted in improved functional recovery in mice of both sexes and age groups. Thus, systemic hAEC administration demonstrates notable neuroprotective effects and facilitates recovery mechanisms [58].

The protective effect of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) has been demonstrated in

rats with transient cerebral ischemia [59]. Intravenous administration of hUCB-MSCs prevented the induction of tissue inhibitors of metalloproteinases and matrix metalloproteinases [59].

Bone marrow-derived cells have been extensively studied in recent years, they include mesenchymal stem cells (MSCs), bone marrow mononuclear cells (BM-MNCs), multipotent adult progenitor cells (MAPCs), endothelial progenitor cells (EPCs), and multilineage-differentiating stress-enduring (Muse) cells [60]. Autologous BM-MNCs are relatively easy to obtain and can be transplanted immediately after extraction from the bone marrow, which makes them particularly suitable for patients in the acute or subacute phases of ischemic stroke [61]. EPCs represent another class of bone marrow-derived stem cells capable of readily differentiating into mature vascular endothelial cells; however, their limited numbers restrict clinical application [34, 61]. Additionally, EPC-based therapies raise certain ethical concerns [62].

Thus, most preclinical studies show promising results for the use of stem cells in the treatment of ischemic stroke, but the outcomes of the relatively few clinical trials have been less encouraging [50]. This discrepancy may be attributed to the stark contrast in regenerative capacity between young, generally healthy animal models and elderly patients with multiple chronic diseases. For example, Xu et al. [63] reported a reduction in the proliferative and angiogenic potential of EPCs and MSCs in individuals with coronary heart disease and metabolic disorders.

Disruption of the blood-brain barrier is a secondary pathological manifestation of ischemic stroke and represents a key target for novel treatments of acute cerebral circulation disorders. An electron microscopy study demonstrated the ability of human bone marrow-derived endothelial progenitor cells (hBMEPCs) to restore the blood-brain barrier in adult Sprague–Dawley rats subjected to transient middle cerebral artery occlusion. In this study, β-galactosidase-prelabeled hBMEPCs were administered intravenously 48 h after the occlusion. Ultrastructural analysis of microvessels on day 5 post-ischemia revealed typical blood-brain barrier pathology in untreated rats. In contrast, rats that received hBMEPC transplantation showed restoration of the vascular network in the motor cortex and striatum, with no evident perivascular edema [64].

EPCs, along with the chemokine CXCL12, support neurogenesis and angiogenesis in the brain by secreting trophic factors. CXCL12 attracts oligodendrocyte progenitor cells (OPCs), neural progenitor cells, and EPCs to the affected area. Increased vascular density and preservation of myelin sheath integrity were also reported in animals that received this treatment [65].

In other studies, adipose-derived mesenchymal stem cells (ADMSCs) have demonstrated therapeutic potential in animal models of ischemic stroke by promoting functional recovery through the upregulation of markers associated with brain repair. Burrow et al. [66] reported that ADMSCs proliferate more rapidly and reach higher population doubling levels than bone marrow-derived MSCs from the same donor. Consequently, ADMSCs exhibit greater proliferative capacity and retain their differentiation potential more effectively than bone marrow MSCs, making them a promising cell type for the development of cell-based therapies in regenerative medicine.

Among MSCs, Wharton's jelly-derived MSCs (WJ-MSCs) have attracted significant attention. It is worth noting that MSCs derived from bone marrow or adipose tissue, in contrast to perinatal organs, have some limitations in terms of their use, such as an invasive procurement procedure, a higher risk of infectious disease transmission, the age of the donor, and the limited proliferative potential of MSCs [67]. WJ-MSCs have several benefits over other MSCs due to simple and non-invasive methods of their obtaining and significant yield compared to other sources, a low risk of tumorigenicity, a

lower tendency to induce a "graft-versus-host" reaction after their administration, and absence of ethical problems [52]. They also demonstrate strong proliferative potential, rapid growth rates, and the ability to retain multipotency over more passages in vitro compared to bone marrow- or adipose-derived MSCs [68]. Semenova et al. [67] reported that MSCs obtained from different regions of the umbilical cord exhibit varying properties. Among them, WJ-MSCs showed a high and stable proliferation potential and phenotype, which can be considered as a promising base of stem cells for forward clinical application.

Key factors influencing the successful use of MSCs include effective dosage, delivery route, and optimal timing of transplantation [69]. Therefore, the efficacy of MSCs largely depends on the method of administration. Guo et al. [55] suggest that intraparenchymal transplantation is the most effective delivery route for stroke patients, as it allows for the highest concentration of MSCs to be delivered directly to the infarcted area, thereby maximizing neurological recovery. However, the surgical procedure itself carries the risk of brain damage and serious complications in stroke patients. Moreover, intracerebral transplantation is limited by the number of cells that can be safely injected without increasing intracranial volume, a limitation that systemic administration can overcome [34].

In addition to the intraparenchymal route, MSCs can be administered less invasively via intravenous, intra-arterial, intranasal, or intrathecal delivery. Among these, intra-arterial transplantation is considered effective; however, its efficacy largely depends on the dose of stem cells delivered, as cell number significantly influences both migration rate and infarct volume. Notably, lower doses of intra-arterially administered MSCs have been associated with better therapeutic outcomes [70]. Although this route offers improved functional recovery, it carries the risk of cerebral microvascular embolism, the formation of intra-arterial emboli, which may further impair local cerebral blood flow [71].

Intravenous transplantation is an effective and minimally invasive delivery method that avoids the serious side effects discussed above [72]. However, following intravenous administration, the majority of transplanted cells accumulate in the liver, spleen, kidneys, and especially the lungs; only about 4% of the injected cells reach the ischemic brain tissue [55].

In the RESSTORE01 study, improvement in functional recovery was observed following the use of adipose-derived mesenchymal stem cells (ADMSCs) in adult male Sprague–Dawley rats with middle cerebral artery occlusion. Intravenous administration of ADMSCs at a dose of $2 \cdot 10^6$ cells led to behavioral recovery, which was evaluated using the Rogers Functional Assessment Scale (assessing sensory responses, reflexes, and basic motor functions), as well as performance in the cylinder and sticker tests over a 42-day follow-up period. Tissue curing was assessed using histological markers for angiogenesis (RECA-1), gliosis (GFAP), and glial scar formation [73]. In particular, the neuroprotective effects of ADMSC in the ischemic brain injury were found by Tsupykov et al. [74].

Both NSCs and MSCs from various sources have been widely studied in animal models of ischemic stroke. NSCs have been shown to migrate to the peri-infarct zone and differentiate into neuronal cell types, while MSCs exert more rapid effects primarily through the "bystander effect." Transplantation of NSCs within the first 7 days after ischemic injury (during the acute or subacute phase) significantly reduced infarct size, likely by inhibiting apoptosis and secondary tissue damage, as well as preserving neuronal circuitry. A meta-analysis also showed that relatively low doses of NSCs (below $1 \cdot 10^6$ cells/kg) delivered into the brain parenchyma led to substantially better functional recovery, possibly due to effective migration to the injury site and attenuation of ischemic progression [75]. However, unlike MSCs, NSCs carry a potential risk of tumorigenesis [76].

MSCs lack HLA-II molecules, this makes them less immunogenic. In the acute phase of

ischemic stroke, MSCs have been shown to inhibit neuronal apoptosis, preserve mitochondrial function, and reduce microglial activation in rats [53]. In the subacute phase, they promote angiogenesis, reduce blood–brain barrier disruption, and inhibit the release of pro-inflammatory cytokines [54].

MSCs are pluripotent cells derived from adult human tissues, making them ethically preferable for both preclinical and clinical research [55]. The mechanisms underlying the beneficial effects of stromal cell transplantation include bystander effects, paracrine mechanisms, and extracellular vesicle-mediated repair [56]. In animal models of cerebral ischemia, the effective MSCs dose for achieving significant functional and histological recovery has been determined to be $1 \cdot 10^6$ cells per animal [77]. In a study by Lin [78], a higher dose of MSCs ($4 \cdot 10^6$), administered intravenously to mice with middle cerebral artery occlusion, did not result in a significantly greater reduction in infarct size compared to a lower dose ($1 \cdot 10^6$). In addition, allogeneic transplantation more effectively reduced the extent of ischemic injury than xenotransplantation, likely due to reduced immunological rejection [79].

According to Guo et al. [55], MSCs transplantation can be performed within a time window of up to 7 days after the onset of cerebral ischemia, but transplantation within the first 6 hours appears to yield significantly better recovery of sensorimotor indicators. In other studies, the interval from ischemia onset to stem cell transplantation ranged from 10 min [80] to up to 3 weeks [81]. Therefore, MSC transplantation within 7 days after ischemic stroke may be considered the optimal treatment window; however, earlier transplantation led to better neurological recovery, reduced infarct volume, and required fewer donor cells ($1 \cdot 10^6$).

Prospects for further development

However, several aspects of cell therapy remain not fully understood, including the choice of cell type, cell dose, and administration route. Therefore, closer integration between basic and clinical research is essential. Recent studies indicate that MSCs isolated from bone marrow can exhibit varying functional and molecular phenotypes depending on the methods used [61], highlighting challenges in maintaining consistent cell quality during preparation. Additionally, each stem cell type has its own advantages and disadvantages, and currently it remains unclear which cell type is most effective for treating cerebral ischemia in acute cerebrovascular events.

С.В. Коновалов, В.М. Мороз, М.В. Йолтухівський, І.В. Гусакова

ДОСЛІДЖЕННЯ ТА ЛІКУВАННЯ ІШЕМІЧНО-РЕПЕРФУЗІЙНОГО УРАЖЕННЯ ГОЛОВНОГО МОЗКУ

Вінницький національний медичний університет ім. М.І. Пирогова; e-mail: serhii.konovalov555@gmail.com

Ішемічний інсульт, основною причиною якого є порушення кровопостачання головного мозку, залишається одним із захворювань, які супроводжуються високою смертністю та інвалідізацією. Ішемія з подальшою реканалізацією оклюзованої судини (через тромболізис чи тромбектомію) супроводжується каскадом біохімічних реакцій у ішемізованих ділянках головного мозку, наслідком яких є виникнення ішемічно-реперфузійних ушкоджень та загибель клітин мозку. Традиційна терапія ішемічного інсульту, яка базується на використанні антитромботичних та нейропротекторних засобів, не завжди є ефективною та безпечною. Регенеративна медицина з трансплантацією стовбурових клітин при церебральній ішемії-реперфузії нині перспективний напрямок у лікуванні ішемічного інсульту. У багатьох експериментальних дослідженнях та в деяких клінічних випробуваннях застосовуються стовбурові клітини різних типів. Клітинна терапія на основі стовбурових клітин дає змогу розробити більш ефективний та безпечний терапевтичний підхід, направлений на нейровідновлення при ішемічно-реперфузійному уражені головного мозку. Метою цього огляду було узагальнення сучасних поглядів на проблему гострої ішемії-реперфузії головного мозку, зокрема патогенезу та морфологічним змінам у нервовій тканині, які виникають у фокусі ішемії та ділянці ішемічної напівтіні (пенумбри), а також перспективам використання клітинної терапії стовбуровими клітинами при гострому ішемічному інсульті (протекції і регенерації). Ключові слова: ішемія-реперфузія головного мозку; патогенез; морфологічні зміни; мезенхімальні стромальні клітини.

REFERENCES

1. Kuriakose D, Xiao Z. Pathophysiology and treatment of stroke: Present status and future perspectives. Int J Mol Sci. 2020 Oct 15;21(20):7609. doi: 10.3390/ijms21207609.
2. Du Z, Xing L, Lin M, Sun Y. Estimate of prevalent ischemic stroke from triglyceride glucose-body mass index in the general population. BMC Cardiovascul Disord. 2020 Nov 12;20(1):483. doi: 10.1186/s12872-020-01768-8.
3. Jadhav AP, Desai SM, Liebeskind DS, Wechsler LR. Neuroimaging of acute stroke. Neurol Clin. 2020 Feb;38(1):185-99. doi: 10.1016/j.ncl.2019.09.004.
4. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2019 Dec;50(12):e344-e418. doi: 10.1161/STR.0000000000000211.
5. Darwish EAF, Abdelhameed-El-Nouby M, Geneidy E. Mapping the ischemic penumbra and predicting stroke progression in acute ischemic stroke: the overlooked role of susceptibility weighted imaging. Insights Imag. 2020 Jan 13;11(1):6. doi: 10.1186/s13244-019-0810-y. .
6. Chamorro Á, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. Lancet Neurol. 2016 Jul;15(8):869-81. doi: 10.1016/S1474-4422(16)00114-9.
7. Li M, Liao L, Tian W. Extracellular vesicles derived from apoptotic cells: An Essential link between death and regeneration. Front Cell Dev Biol. 2020 Oct 2;8:573511. doi: 10.3389/fcell.2020.573511.
8. Balch MHH, Nimjee SM, Rink C, Hannawi Y. Beyond the brain: The systemic pathophysiological response to acute ischemic stroke. J Stroke. 2020 May;22(2):159-72. doi: 10.5853/jos.2019.02978. Epub 2020 May 31.
9. Griñán K, Arboix A, Massons J, Díez L, Vergés E, Gil F, Arboix-Alió J, Sánchez-López MJ, García-Eroles L. Cardioembolic stroke: Risk factors, clinical features, and early outcome in 956 consecutive patients. Rev Invest Clin. 2020 May 7;73(1):23-30. doi: 10.24875/RIC.20000227.
10. Fricker M, Tolkovsky AM, Borutaite V, Coleman M, Brown GC. Neuronal cell death. Physiol Rev. 2018 Apr 1;98(2):813-80. doi: 10.1152/physrev.00011.2017.
11. Jurcau A, Ardelean IA. Molecular pathophysiological mechanisms of ischemia/reperfusion injuries after recanalization therapy for acute ischemic stroke. J Integr Neurosci. 2021 Sep 30;20(3):727-44. doi: 10.31083/j.jin2003078.
12. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. DEFUSE 3 Investigators. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. N Engl J Med. 2018 Feb 22;378(8):708-18. doi: 10.1056/NEJMoa1713973.
13. He Z, Ning N, Zhou Q, Khoshnam SE, Farzaneh M. Mitochondria as a therapeutic target for ischemic stroke. Free Radic Biol Med. 2020 Jan;146:45-58. doi: 10.1016/j.freeradbiomed.2019.11.005.
14. Liu L, Kearns KN, Eli I, Sharifi KA, Soldozy S, Carlson EW, Scott KW, Sluzewski MF, Acton ST, Stauderman KA, Kalani MYS, Park M, Tvrdik P. Microglial calcium waves during the hyperacute phase of ischemic stroke. Stroke. 2021 Jan;52(1):274-83. doi: 10.1161/STROKEAHA.120.032766.
15. Chavda V, Lu B. Reverse electron transport at mitochondrial complex i in ischemic stroke, aging, and age-related diseases. Antioxidants (Basel). 2023 Apr 6;12(4):895. doi: 10.3390/antiox12040895.
16. Webster JD, Kwon YC, Park S, Zhang H, Corr N, Ljumanovic N, Adedeji AO, Varfolomeev E, Goncharov T, Preston J, Santagostino SF, Patel S, Xu M, Maher J, McKenzie BS, Vucic D. RIP1 kinase activity is critical for skin inflammation but not for viral propagation. J Leukoc Biol. 2020 Jun;107(6):941-52. doi: 10.1002/JLB.3MA1219-398R.
17. Annoni F, Peluso L, Gouvêa Bogossian E, Creteur J, Zanier ER, Taccone FS. Brain protection after anoxic brain injury: is lactate supplementation helpful? Cells. 2021 Jul 6;10(7):1714. doi: 10.3390/cells10071714.
18. Belov Kirdajova D, Kriska J, Tureckova J, Anderova M. Ischemia-triggered glutamate excitotoxicity from the perspective of glial cells. Front Cell Neurosci. 2020 Mar 19;14:51. doi: 10.3389/fncel.2020.00051.
19. Sarvari S, Moakedi F, Hone E, Simpkins JW, Ren X. Mechanisms in blood-brain barrier opening and metabolism-challenged cerebrovascular ischemia with emphasis on ischemic stroke. Metab Brain Dis. 2020 Aug;35(6):851-68. doi: 10.1007/s11011-020-00573-8.
20. Shvedskyi VV. Experimental violation of cerebral blood circulation against the background of alloxan diabetes mellitus: characteristics of the model. Med Visn. 2012;16(61):150-6 doi: https://doi.org/10.24061/222694
21. Radak D, Katsiki N, Resanovic I, Jovanovic A, Sudar-Milovanovic E, Zafirovic S, Mousad SA, Isenovic ER. Apoptosis and acute brain ischemia in ischemic stroke. Curr Vasc Pharmacol. 2017;15(2):115-22. doi: 10.2174/1570161115666161104095522.
22. Shafie M, Yu W. Recanalization therapy for acute ischemic stroke with large vessel occlusion: Where we are and what comes next? Transl Stroke Res. 2021 Jun;12(3):369-81. doi: 10.1007/s12975-020-00879-w.
23. Orellana-Urzúa S, Rojas I, Líbano L, Rodrigo R. Pathophysiology of ischemic stroke: Role of oxidative stress. Current Pharm Des. 2020;26(34):4246-60. doi: 10.2174/1381612826666200708133912.
24. Dondelinger Y, Declercq W, Montessuit S, Roelandt R, Goncalves A, Bruggeman I, et al. MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. Cell Rep. 2014 May

22;7(4):971-81. doi: 10.1016/j.celrep.2014.04.026.
25. Granger DN, Kvietys PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. Redox Biol. 2015 Dec;6:524-51. doi: 10.1016/j.redox.2015.08.020.
26. Cao SS, Kaufman RJ. Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. Antioxid Redox Sign. 2014 Jul 20;21(3):396-413. doi: 10.1089/ars.2014.5851.
27. Jurcau A, Simion A. Neuroinflammation in cerebral ischemia and ischemia/reperfusion injuries: From pathophysiology to therapeutic strategies. Int J Mol Sci. 2021 Dec 21;23(1):14. doi: 10.3390/ijms23010014.
28. Mo ZT, Liao YL, Zheng J, Li WN. Icariin protects neurons from endoplasmic reticulum stress-induced apoptosis after OGD/R injury via suppressing IRE1α-XBP1 signaling pathway. Life Sci. 2020 Aug 15;255:117847. doi: 10.1016/j.lfs.2020.117847
29. Remus EW, Sayeed I, Won S, Lyle AN, Stein DG. Progesterone protects endothelial cells after cerebrovascular occlusion by decreasing MCP-1- and CXCL1-mediated macrophage infiltration. Exp Neurol. 2015 Sep;271:401-8. doi: 10.1016/j.expneurol.2015.07.010.
30. Candelario-Jalil E, Dijkhuizen RM, Magnus T. Neuroinflammation, stroke, blood-brain barrier dysfunction, and imaging modalities. Stroke. 2022 May;53(5):1473-86. doi: 10.1161/STROKEAHA.122.036946.
31. Choi DW. Excitotoxicity: Still hammering the ischemic brain in 2020. Front Neurosci. 2020 Oct 26;14:579953. doi: 10.3389/fnins.2020.579953.
32. Zhang L, Wang LM, Chen WW, Ma Z, Han X, Liu CM, Cheng X, Shi W, Guo JJ, Qin JB, Yang XQ, Jin GH, Zhang XH. Neural differentiation of human Wharton's jelly-derived mesenchymal stem cells improves the recovery of neurological function after transplantation in ischemic stroke rats. Neural Regen Res. 2017 Jul;12(7):1103-10. doi: 10.4103/1673-5374.211189.
33. Borlongan CV. Concise review: Stem cell therapy for stroke patients: are we there yet? Stem Cell Transl Med. 2019 Sep;8(9):983-8. doi: 10.1002/sctm.19-0076.
34. Kawabori M, Shichinohe H, Kuroda S, Houkin K. Clinical trials of stem cell therapy for cerebral ischemic stroke. Int J Mol Sci. 2020 Oct 6;21(19):7380. doi: 10.3390/ijms21197380.
35. Svendsen CN, ter Borg MG, Armstrong RJ, Rosser AE, Chandran S, Ostenfeld T, Caldwell MA. A new method for the rapid and long term growth of human neural precursor cells. J Neurosci Method. 1998 Dec 1;85(2):141-52. doi: 10.1016/s0165-0270(98)00126-5.
36. Golas MM. Human cellular models of medium spiny neuron development and Huntington disease. Life Sci. 2018 Sep 15;209:179-196. doi: 10.1016/j.lfs.2018.07.030.
37. Jiao Y, Liu YW, Chen WG, Liu J. Neuroregeneration and functional recovery after stroke: advancing neural stem cell therapy toward clinical application. Neural Regen Res. 2021 Jan;16(1):80-92. doi: 10.4103/1673-5374.286955.
38. Addington CP, Dharmawaj S, Heffernan JM, Sirianni RW, Stabenfeldt SE. Hyaluronic acid-laminin hydrogels increase neural stem cell transplant retention and migratory response to SDF-1α. Matrix Biol. 2017 Jul;60-1:206-216. doi: 10.1016/j.matbio.2016.09.007.
39. Gójska-Grymajło A, Zieliński M, Wardowska A, Gąsecki D, Pikuła M, Karaszewski B. CXCR7+ and CXCR4+ stem cells and neuron specific enolase in acute ischemic stroke patients. Neurochem Int. 2018 Nov;120:134-9. doi: 10.1016/j.neuint.2018.08.009.
40. Ryu S, Lee SH, Kim SU, Yoon BW. Human neural stem cells promote proliferation of endogenous neural stem cells and enhance angiogenesis in ischemic rat brain. Neural Regen Res. 2016 Feb;11(2):298-304. doi: 10.4103/1673-5374.177739.
41. Rahman AA, Amruta N, Pinteaux E, Bix GJ. Neurogenesis after stroke: A therapeutic perspective. Transl Stroke Res. 2021 Feb;12(1):1-14. doi: 10.1007/s12975-020-00841-w.
42. Huang L, Wong S, Snyder EY, Hamblin MH, Lee JP. Human neural stem cells rapidly ameliorate symptomatic inflammation in early-stage ischemic-reperfusion cerebral injury. Stem Cell Res Ther. 2014 Nov 23;5(6):129. doi: 10.1186/scrt519.
43. Eckert A, Huang L, Gonzalez R, Kim HS, Hamblin MH, Lee JP. Bystander effect fuels human induced pluripotent stem cell-derived neural stem cells to quickly attenuate early stage neurological deficits after stroke. Stem Cell Transl Med. 2015 Jul;4(7):841-51. doi: 10.5966/sctm.2014-0184.
44. Anderson L, Burnstein RM, He X, Luce R, Furlong R, Foltynie T, Sykacek P, Menon DK, Caldwell MA. Gene expression changes in long term expanded human neural progenitor cells passaged by chopping lead to loss of neurogenic potential *in vivo*. Exp Neurol. 2007 Apr;204(2):512-24. doi: 10.1016/j.expneurol.2006.12.025.
45. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76. doi: 10.1016/j.cell.2006.07.024.
46. Jurcău MC, Andronie-Cioara FL, Jurcău A, Marcu F, Ţi DM, Pacalău N, Nistor-Cseppentö DC. The link between oxidative stress, mitochondrial dysfunction and neuroinflammation in the pathophysiology of Alzheimer's disease: Therapeutic implications and future perspectives. Antioxidants (Basel). 2022 Oct 31;11(11):2167. doi: 10.3390/antiox11112167.
47. Derakhshankhah H, Sajadimajd S, Jafari S, Izadi Z, Sarvari S, Sharifi M, Falahati M, Moakedi F, Muganda WCA, Müller M, Raoufi M, Presley JF. Novel therapeutic strategies for Alzheimer's disease: Implications from cell-based therapy and nanotherapy. Nanomedicine. 2020 Feb;24:102149. doi: 10.1016/j.nano.2020.102149.
48. Karow M, Camp JG, Falk S, Gerber T, Pataskar A, Gac-Santel M, et al. Direct pericyte-to-neuron reprogramming via unfolding of a neural stem cell-like program. Nat Neurosci. 2018 Jul;21(7):932-40. doi: 10.1038/s41593-018-0168-3.

49. An N, Xu H, Gao WQ, Yang H. Direct conversion of somatic cells into induced neurons. Mol Neurobiol. 2018 Jan;55(1):642-51. doi: 10.1007/s12035-016-0350-0.
50. Chung JW, Chang WH, Bang OY, Moon GJ, Kim SJ, Kim SK, Lee JS, Sohn SI, Kim YH. STARTING-2 Collaborators. Efficacy and safety of intravenous mesenchymal stem cells for ischemic stroke. Neurology. 2021 Feb 16;96(7):e1012-e23. doi: 10.1212/WNL.0000000000011440.
51. Viswanathan S, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, Nolta J, Phinney DG, Sensebe L. Mesenchymal stem versus stromal cells: International society for cell & gene therapy (ISCT®) mesenchymal stromal cell committee position statement on nomenclature. Cytotherapy. 2019 Oct;21(10):1019-24. doi: 10.1016/j.jcyt.2019.08.002.
52. Patel AA, Mohamed AH, Rizaev J, Mallick AK, Qasim MT, Abdulmonem WA, Jamal A, Hattiwale HM, Kamal MA, Ahmad F. Application of mesenchymal stem cells derived from the umbilical cord or Wharton's jelly and their extracellular vesicles in the treatment of various diseases. Tissue Cell. 2024 Aug;89:102415. doi: 10.1016/j.tice.2024.102415.
53. Li Z, Ye H, Cai X, Sun W, He B, Yang Z, Xu P. Bone marrow-mesenchymal stem cells modulate microglial activation in the peri-infarct area in rats during the acute phase of stroke. Brain Res Bull. 2019 Nov;153:324-33. doi: 10.1016/j.brainresbull.2019.10.001.
54. Yoshida Y, Takagi T, Kuramoto Y, Tatebayashi K, Shirakawa M, Yamahara K, Doe N, Yoshimura S. Intravenous administration of human amniotic mesenchymal stem cells in the subacute phase of cerebral infarction in a mouse model ameliorates neurological disturbance by suppressing blood brain barrier disruption and apoptosis via immunomodulation. Cell Transplant. 2021 Jan-Dec;30:9636897211024183. doi: 10.1177/09636897211024183.
55. Guo Y, Peng Y, Zeng H, Chen G. Progress in mesenchymal stem cell therapy for ischemic stroke. Stem Cells Int. 2021 Jun 15;2021:9923566. doi: 10.1155/2021/9923566.
56. Brooks B, Ebedes D, Usmani A, Gonzales-Portillo JV, Gonzales-Portillo D, Borlongan CV. Mesenchymal stromal cells in ischemic brain injury. Cells. 2022 Mar 17;11(6):1013. doi: 10.3390/cells11061013.
57. Kong T, Park JM, Jang JH, Kim CY, Bae SH, Choi Y, Jeong YH, Kim C, Chang SW, Kim J, Moon J. Immunomodulatory effect of CD200-positive human placenta-derived stem cells in the early phase of stroke. Exp Mol Med. 2018 Jan 12;50(1):e425. doi: 10.1038/emm.2017.233.
58. Evans MA, Lim R, Kim HA, Chu HX, Gardiner-Mann CV, Taylor KWE, et al. Acute or delayed systemic administration of human amnion epithelial cells improves outcomes in experimental stroke. Stroke. 2018 Mar;49(3):700-9. doi: 10.1161/STROKEAHA.117.019136.
59. Chelluboina B, Nalamolu KR, Mendez GG, Klopfenstein JD, Pinson DM, Wang DZ, Veeravalli KK. Mesenchymal stem cell treatment prevents post-stroke dysregulation of matrix metalloproteinases and tissue inhibitors of metalloproteinases. Cell Physiol Biochem. 2017;44(4):1360-9. doi: 10.1159/000485533.
60. Uchida H, Morita T, Niizuma K, Kushida Y, Kuroda Y, Wakao S, Sakata H, Matsuzaka Y, Mushiake H, Tominaga T, Borlongan CV, Dezawa M. Transplantation of unique subpopulation of fibroblasts, muse cells, ameliorates experimental stroke possibly via robust neuronal differentiation. Stem Cells. 2016 Jan;34(1):160-73. doi: 10.1002/stem.2206.
61. Stroncek DF, Jin P, McKenna DH, Takanashi M, Fontaine MJ, Pati S, Schäfer R, Peterson E, Benedetti E, Reems JA. Human mesenchymal stromal cell (MSC) characteristics vary among Laboratories when manufactured from the same source material: A report by the cellular therapy team of the biomedical excellence for safer transfusion (BEST) collaborative. Front Cell Dev Biol. 2020 Jun 16;8:458. doi: 10.3389/fcell.2020.00458.
62. Johnson TC, Siegel D. Directing stem cell fate: The synthetic natural product connection. Chem Rev. 2017 Sep 27;117(18):12052-86. doi: 10.1021/acs.chemrev.7b00015.
63. Xu S, Qiu Y, Tao J. The challenges and optimization of cell-based therapy for cardiovascular disease. J Transl Int Med. 2021 Dec 31;9(4):234-8. doi: 10.2478/jtim-2021-0017.
64. Garbuzova-Davis S, Haller E, Lin R, Borlongan CV. Intravenously transplanted human bone marrow endothelial progenitor cells engraft within brain capillaries, preserve mitochondrial morphology, and display pinocytotic activity toward blood-brain barrier repair in ischemic stroke rats. Stem Cells. 2017 May;35(5):1246-1258. doi: 10.1002/stem.2578.
65. Li Y, Chang S, Li W, Tang G, Ma Y, Liu Y, Yuan F, Zhang Z, Yang GY, Wang Y. cxcl12-Engineered endothelial progenitor cells enhance neurogenesis and angiogenesis after ischemic brain injury in mice. Stem Cell Res Ther. 2018 May 11;9(1):139. doi: 10.1186/s13287-018-0865-6.
66. Burrow KL, Hoyland JA, Richardson SM. Human adipose-derived stem cells exhibit enhanced proliferative capacity and retain multipotency longer than donor-matched bone marrow mesenchymal stem cells during expansion *in vitro*. Stem Cells Int. 2017;2017:2541275. doi: 10.1155/2017/2541275.
67. Semenova E, Grudniak MP, Machaj EK, Bocian K, Chroscinska-Krawczyk M, Trochonowicz M, Stepaniec IM, Murzyn M, Zagorska KE, Boruczkowski D, Kolanowski TJ, Oldak T, Rozwadowska N. Mesenchymal stromal cells from different parts of umbilical cord: approach to comparison & characteristics. Stem Cell Rev Rep. 2021 Oct;17(5):1780-95. doi: 10.1007/s12015-021-10157-3.
68. Liau LL, Ruszymah BHI, Ng MH, Law JX. Characteristics and clinical applications of Wharton's jelly-derived mesenchymal stromal cells. Curr Res Transl Med. 2020

Jan;68(1):5-16. doi: 10.1016/j.retram.2019.09.001.
69. Cherkashova EA, Namestnikova DD, Gubskiy IL, Revkova VA, Sukhinich KK, Mel'nikov PA, Chekhonin VP, Gubsky LV, Yarygin KN. Dose-dependent effects of intravenous mesenchymal stem cell transplantation in rats with acute focal cerebral ischemia. Bull Exp Biol Med. 2022 Aug;173(4):514-8. doi: 10.1007/s10517-022-05573-5.
70. Grudzenski S, Baier S, Ebert A, Pullens P, Lemke A, Bieback K, Dijkhuizen RM, Schad LR, Alonso A, Hennerici MG, Fatar M. The effect of adipose tissue-derived stem cells in a middle cerebral artery occlusion stroke model depends on their engraftment rate. Stem Cell Res Ther. 2017 Apr 26;8(1):96. doi: 10.1186/s13287-017-0545-y.
71. Cui LL, Kerkelä E, Bakreen A, Nitzsche F, Andrzejewska A, Nowakowski A, Janowski M, Walczak P, Boltze J, Lukomska B, Jolkkonen J. The cerebral embolism evoked by intra-arterial delivery of allogeneic bone marrow mesenchymal stem cells in rats is related to cell dose and infusion velocity. Stem Cell Res Ther. 2015 Jan 27;6(1):11. doi: 10.1186/scrt544.
72. Rascón-Ramírez FJ, Esteban-García N, Barcia JA, Trondin A, Nombela C, Sánchez-Sánchez-Rojas L. Are we ready for cell therapy to treat stroke? Front Cell Dev Biol. 2021 Jun 23;9:621645. doi: 10.3389/fcell.2021.621645.
73. Mu J, Bakreen A, Juntunen M, Korhonen P, Oinonen E, Cui L, Myllyniemi M, Zhao S, Miettinen S, Jolkkonen J. Combined adipose tissue-derived mesenchymal stem cell therapy and rehabilitation in experimental stroke. Front Neurol. 2019 Mar 26;10:235. doi: 10.3389/fneur.2019.00235.
74. Tsupykov OM, Lushnikova IV, Ustymenko AM, Kyryk VM, Nikandrova YA, Patseva MA, Yatsenko KV, Butenko GM, Skibo GG. Protective effects of adipose-derived multipotent mesenchymal stromal cells of mice on periventricular leukomalacia model *in vitro*. Cell Organ Transplantol. 2017; 5(1):28-32. doi:10.22494/cot.v5i1.66
75. Huang H, Qian K, Han X, Li X, Zheng Y, Chen Z, Huang X, Chen H. Intraparenchymal neural stem/progenitor cell transplantation for ischemic stroke animals: A meta-analysis and systematic review. Stem Cells Int. 2018 Oct 2;2018:4826407. doi: 10.1155/2018/4826407.
76. Stonesifer C, Corey S, Ghanekar S, Diamandis Z, Acosta SA, Borlongan CV. Stem cell therapy for abrogating stroke-induced neuroinflammation and relevant secondary cell death mechanisms. Prog Neurobiol. 2017 Nov;158:94-131. doi: 10.1016/j.pneurobio.2017.07.004.
77. Oh SH, Choi C, Noh JE, Lee N, Jeong YW, Jeon I, Shin JM, Kim JH, Kim HJ, Lee JM, Kim HS, Kim OJ, Song J. Interleukin-1 receptor antagonist-mediated neuroprotection by umbilical cord-derived mesenchymal stromal cells following transplantation into a rodent stroke model. Exp Mol Med. 2018 Apr 13;50(4):1-12. doi: 10.1038/s12276-018-0041-1.
78. Lin W, Hsuan YC, Lin MT, Kuo TW, Lin CH, Su YC, Niu KC, Chang CP, Lin HJ. Human umbilical cord mesenchymal stem cells preserve adult newborn neurons and reduce neurological injury after cerebral ischemia by reducing the number of hypertrophic microglia/macrophages. Cell Transplant. 2017 Nov;26(11):1798-810. doi: 10.1177/0963689717728936.
79. Chen L, Zhang G, Gu Y, Guo X. Meta-Analysis and Systematic Review of Neural Stem Cells therapy for experimental ischemia stroke in preclinical studies. Sci Rep. 2016;6:32291. doi: 10.1038/srep32291.
80. Mochizuki N, Takagi N, Kurokawa K, Onozato C, Moriyama Y, Tanonaka K, Takeo S. Injection of neural progenitor cells improved learning and memory dysfunction after cerebral ischemia. Exp Neurol. 2008 May;211(1):194-202. doi: 10.1016/j.expneurol.2008.01.027.
81. Somaa FA, Wang TY, Niclis JC, Bruggeman KF, Kauhausen JA, Guo H, McDougall S, Williams RJ, Nisbet DR, Thompson LH, Parish CL. Peptide-based scaffolds support human cortical progenitor graft integration to reduce atrophy and promote functional repair in a model of stroke. Cell Rep. 2017 Aug 22;20(8):1964-77. doi: 10.1016/j.celrep.2017.07.069.

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