Repairing the Brain with Mesenchymal Stem Cells for Stroke Recovery

By

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A thesis submitted to the Department of School of Pharmacy in partial fulfillment of the requirements for the degree of

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing my degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted for any other degree or diploma at a university or other institution.
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Approval

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Ethics Statement

This paper is done by my own work and under the supervision of Md. Tanvir Kabir, Senior Lecturer, School of Pharmacy, Brac University, and I have given proper credit from where I have used ideas and information. This thesis paper is submitted for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Honors), so it does not involve any human or animal trial. No animals were used or harmed while doing this project.

Abstract

Worldwide, stroke is the most frequent cerebrovascular infarction causing death and disability. Despite advancements in Surgical and pharmacological treatments, therapy of neurological restoration after a stroke goes minimal, resulting in a significant reduction in quality of life. Two procedures have been approved by the Food and Drug Administration (FDA): intravenous thrombolytic therapy and endovascular thrombectomy. However, because of the tight therapeutic window, only a small fraction of stroke patients receives these therapies. Due to their potential to reverse the neurological consequences, advances in stem cell treatment have given patients with stroke fresh hope. Various kinds of stem cells have been utilized to treat stroke experimentally in animal experiments and clinical studies throughout the last few decades, from which mesenchymal stem cells have shown the most promising and safe results.

Keywords: Stroke; Thrombectomy; Rehabilitation; Stem cell therapy; Mesenchymal stem cells

Dedication

This project is dedicated to the respected Dean of School of Pharmacy Eva Rahman Kabir Ph.D. ma'am and my respective supervisor Md. Tanvir Kabir sir.

Acknowledgment

All praises go to the Almighty Allah for giving me enough patience and courage to complete my project work. I am thankful to my respective supervisor Md. Tanvir Kabir sir guided me with his vast knowledge and inspired me to complete my project work. Without his guidance and direction, this project would not have been completed. I am also grateful to my department head, Eva Rahman Kabir Ph.D. ma'am, for her support and contribution to my undergraduate life.

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List of Acronyms

FDA	Food and Drug Administration
MI	Myocardial Infarction
CNS	Central Nervous System
CDV	Cardiovascular Disease
CI	Cerebrovascular Infraction
SCs	Stem cells
ESC	Embryonic Stem Cell
BM	Bone Marrow
BM-MSC	Bone Marrow mesenchymal stem cell
MNC	Mononuclear Cells
iPSC	Induced pluripotent stem cells

Chapter 1

Introduction

1.1 Background

Stroke holds the second leading position for death and the world's third-leading reason of disability (disability-adjusted life years lost – DALYs measurement) (Singh et al., 2020a). This neurological condition occurs due to acute injury of the vascular part of the brain. Cerebrovascular accidents are predominantly stratified to either ischemic stroke or hemorrhagic stroke. IS is the most typical kind of stroke, responsible for about 85 percent of all acute strokes, which transpires when the blood vessels such as internal carotid arteries (70% of arterial blood to the brain) and vertebral arteries (30% of arterial blood to the brain) in charge for carrying blood up to the brain gets interrupted or clogged (Acute Stroke - StatPearls - NCBI Bookshelf, n.d.). Ischemic stroke (IS) depletes energy and causes excitotoxicity and neuroinflammation in the domain of brain injury. Moreover, Hemorrhagic strokes, which are caused by a blood vessel rupture, are responsible for 15% of all acute strokes globally. Paralysis, hemiparesis, chronic pain, and psychomotor and behavioral signs are examples of motor and cognitive abnormalities that can last a long time and prevent the patient's appropriate societal reintegration, in addition to the expensive healthcare burden of stroke. Two procedures have been authorized by the Food and Drug Administration (FDA): endovascular thrombectomy and intravenous thrombolytic therapy. However, the limited therapeutic range limits their use, so only a few stroke patients get these therapies (H. Liu et al., 2021). As a result, there is an urgent need to discover therapeutic treatments that last longer than the initial few hours following a stroke. This necessitates a paradigm change in treating damaged or impaired brain tissue, from neuroprotection to neuro-restoration. Numerous research utilizing animal models that simulate various neurological disorders with brain injury have found that stem cell-based therapy may successfully boost recovery in two ways. The first mode, called

the bystander effect, is thought to be induced by releasing several substances that result in immunomodulation, decreased brain-blood barrier damage, promotion of endogenous neurogenesis, neural plasticity, and angiogenesis (Eckert et al., 2015). The second method is rooted in cell replacement, which is supported by recent literature illustrating the capability of implanted pluripotent stem cells (PSCs) to distinguish between various types of neurons morphologically, create a relation between neurons and host circuitry, and connect into the broken central nervous system (Chang et al., 2013). These advancements bring stem cell treatment for stroke patients closer to a clinical application.

1.2 Global Impact of Stroke

The World Health Organization (WHO) claims that globally around 15 million people go through a stroke every year. Five million will suffer and die, and the other five million will be forever crippled. (WHO EMRO | Stroke, Cerebrovascular Accident | Health Topics, n.d.).

(Feigin et al., 2021), showed that the overall amount of stroke-related DALYs owing to threat had grown significantly globally during the last three decades (From 91.5 million in 1990 to 125 million in 2019, the number of patients has increased around 33.5 million), with divaricate pattern in the High-income and low-income to upper-middle-income areas, according to the World Bank. The higher-income category had a little fall, while the lower-income to upper-middle-income group saw a spike. Moreover, the escalation resulted not only from growth in population and old age but also from a significant enhancement of several alarming factors such as high blood sugar, high BMI, alcohol intake, high systolic blood pressure, physically inactive, kidney malfunction, particulate contamination in the environment and high temperature. Additionally, the systematic review for the Global Burden of Disease Study 2019 study showed findings of Ischaemic stroke comprising the largest portion compared to all new strokes, subarachnoid hemorrhage with 9.7%, intracerebral hemorrhage with 27.6% and 62.4%

are all incidental strokes in 2019, and it differed respectively to income group. Furthermore, an alarming pattern had been observed with a linear interpolation with the current burden of stroke that there will be roughly 200 million stroke survivors, 13 million stroke fatalities per year, 25 million new strokes, and 300 million DALYs by the end of 2050. (Feigin et al., 2021b).

1.3 Stroke in Bangladesh

Stroke holds the third leading position cause of death in Bangladesh. Bangladesh's death rate from stroke is ranked 84th globally by the World Health Organization. Stroke prevalence was reported to be 0.3 percent; however, no data on stroke incidence had been documented. Hypertension is the leading cause of ischemic and hemorrhagic stroke in Bangladesh, according to hospital-based research undertaken over the last several decades (Islam et al., 2013). Moreover, by 2015 Bangladesh became a low-middle income country with an enhanced per capita gross domestic product GDP of 350 billion US dollars with health expenditure of 2.484% of GDP according to the world bank (World Bank 2020).

According to World Health Rankings, up-to-date World Health Organization WHO data published in 2018 ranked Bangladesh 41 as the Stroke Deaths escalated to 15.31% or 118,918 of total deaths (*Stroke in Bangladesh*, n.d.).

It was found that 72% had an ischemic stroke in a multi-centric hospital related to specifically small vessel lacunar disease and a seasonal number of hemorrhagic stroke. Stroke mortality may be linked to the severity of the stroke, late or slow diagnosis, and inconsistency or gaps in primary care. Currently, there are two governmental five private hospitals for acute stroke care. For subacute stroke care by neurologists,' there are 23 governmental, seven private hospitals in different parts of the country for better health care.

The Bangladesh Rehabilitation Assistance Committee (BRAC), a nongovernmental

organization (NGO) known to greatly aid stroke rehabilitation assistance to patients with financial aid and enhance awareness, runs numerous educational programs. The Center for the Rehabilitation of the Paralyzed (CRP), another NGO, also provides numerous therapies such as speech, physiotherapy, occupational therapy, language therapy, and the aware public of symptoms of a stroke.

In Bangladesh, more has to be done in stroke care. More stroke physicians and rehabilitation services are needed, particularly in rural locations. For stroke survivors, community resources must be improved. Stroke preventive public education efforts must continue to be effective. The burden of stroke in Bangladesh can be minimized using these strategies.

Chapter 2

Types of Strokes

Stroke is a medical condition that causes the flow of oxygen, nutrients, and glucose to be limited and sometimes decreased in some brain regions. The obstructed zones cell response is an inescapable result of cerebral Ischemia. At these wound locations, regulated systems are activated, producing intricately ordered signaling sequences. However, an uncontrolled route, referred to as accidental cell death, is also induced, and the process is physiologically uncontrollable. Cerebrovascular events, such as stroke, are classified into three broad categories: ischaemic, hemorrhagic, and transient ischaemic stroke (TIA).

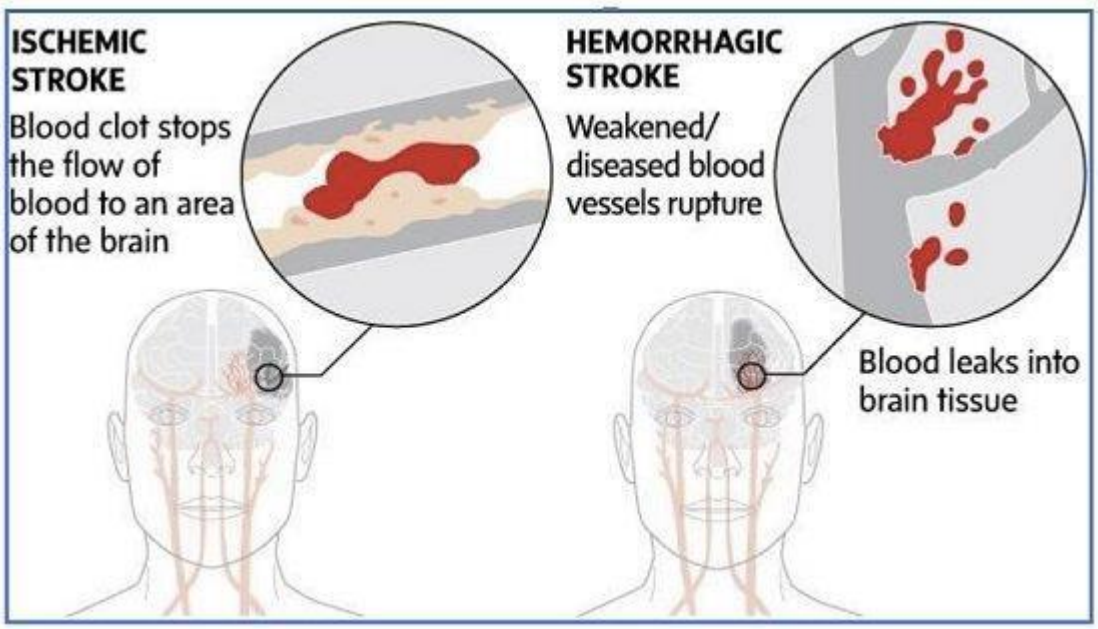


Figure 1: Types of brain stroke.

Source: (*Ischemic Stroke Detection Using EEG Signals*, n.d.)

2.1 Ischemic Stroke

Ischemic stroke (IS) is the most common form of stroke, roughly for about approximately 85-87% of all acute strokes, which transpires when the blood vessels such as internal carotid arteries (70% of arterial blood to the brain) and vertebral arteries (30% of arterial blood to the

brain) in charge for carrying blood up to the brain gets interrupted or clogged (Acute Stroke - PubMed, n.d.). Ischemic stroke (IS) depletes energy and causes excitotoxicity and neuroinflammation in the domain of brain injury. Ischemic stroke is further sub-divided into embolic, thrombotic, and watershed stroke. An embolism occurs when a clot breaks off from the walls of the left atrium or ventricle of the heart and travels up via the carotid artery and resides in the cerebral artery of the brain, blocking the blood, depriving that particular brain tissue of oxygen, nutrients. Moreover, cholesterol buildup in carotid arteries or basilary arteries known as atherosclerosis may obstruct the anterior cerebral arteries, causing brain tissue death. Secondly, thrombotic stroke occurs when a clot forms locally in the cerebral artery due to the accumulation of fatty cholesterol deposits known as atherosclerosis which eventually cracks open, resulting in a blood clot above it and enhances in size that prohibits blood flow in the cerebral base. Atherosclerosis mainly occurs in large vessels such as the middle cerebral artery, carotid artery, and basal artery. Moreover, in smaller vessels, when atherosclerosis happens, known as lacunar infarct/stroke, the vessel cannot withstand the high blood pressure, and as the walls of the vessels thicken up, blood cannot pass through, brain tissue starts to die. When total blood reaching the brain reduces due to large atheroma or atherosclerosis deposits in the internal carotid artery, it results in hyperfusion where a large region of the brain gets deprived of blood supply with oxygen and nutrients. Thus, the tissue dies. That particular area is known as a watershed area, which is called a watershed ischemic stroke.

2.2 Haemorrhagic Stroke

It was observed that hemorrhagic strokes, simulated by the rupture of a blood vessel account for 15 percent of acute strokes worldwide (Acute Stroke - PubMed, n.d.). Hemorrhagic strokes can be sub-divided into extra and intra axial hemorrhage. However, when bleeding occurs inside the skull, outside the brain tissue known as an extra-axial hemorrhage, further classified as epidural, subdural, and subarachnoid hemorrhage. In epidural bleeding occurs between the

skull and dura mater due to head trauma such as skull fracture (70-95%), motor vehicle accident, or assault. Its clinical manifestation includes altered consciousness, headache, vomiting, seizures, aphagia, and prolonged lucid intervals. Subdural hemorrhage results when bleeding occurs between the dura mater and arachnoid membrane. The causes of subdural hemorrhage are similar to an epidural, except its clinical manifestation includes coma. Thirdly, spontaneous subarachnoid hemorrhage occurs due to berry's aneurysm of a cerebral artery resulting in rupture of the blood vessel. Berry's aneurysm or arteriovenous aneurysm is a congenital disability of tunica media, and its common site is the circle of wills consisting of the junction of anterior carotid arteries and anterior communication branches where the blood by-passes the capillary system entering directly from the artery to the narrowed lumen vein with high blood pressure resulting in damage. Intra axial hemorrhage, on the other hand, takes place within the confined space inside the brain tissue and is further categorized into an intraventricular and intracerebral hemorrhage, which, along with subarachnoid, accounts for 5% of strokes (Tadi and Lui, 2021). It usually occurs in the middle cerebral artery in the lenticular striate branch. The most common reason for intracerebral hemorrhage is enhanced hypertension caused by high lipid and hyaline in the blood vessel resulting in dilation of vessel walls; a condition is known otherwise lipohyalinosis. Moreover, vessel wall dilation is also called an aneurysm which causes rupture of the vessel resulting in intracranial bleeding due to intracranial pressure. Intraventricular hemorrhage is a secondary phenomenon when intracerebral or subarachnoid rupture extends to ventricles, and the bleeding is confined within the ventricular system of the brain.

2.3 Transient Ischemic Attack (TIA)

Around 20 to 25% 20 to 25% of ischemic strokes are proclaimed by transient ischemic symptoms, where these symptoms remain for seconds to minutes and usually last for less than an hour. A revised version was established of TIA definition from symptom-based (lasting <24

hours) to tissue-based in relationship to the recognition or pinpointing of cerebral infraction imaging of patients undergoing symptoms of over 10 minutes' time interval and patients reaching hospital within 6 hours afterward the inception of symptoms requires emergency revascularization. An ischemic abrasion tends not to be visible in MRI of a patient with TIA, and a minute ischemic abrasion in imaging is regarded as a minor ischemic stroke. The clinical manifestation of a TIA and minor ischemic stroke are treated as the same. The primary distinction between stroke and TIA revolves around TIA typically resolving within a small time interval (less than 24 hours). The symptoms of TIA include difficulty in speech, any motor or coordination such as walking or loss of balance, sudden weakness in the face such as facial droop, and a severe headache.

Chapter 3

The discovery of neurogenesis in the average adult brain

Numerous scientists found stem cells inside the adult brain, which took many decades. Dr. Joseph Altman and Dr. Gopal D. Das, working together in the 1960s, offered preliminary proof indicating the existence of neural stem cells in the brain, which numerous additional researchers in the 1980s supported. Researchers took note once two distinct groups were capable of extracting stem cells even from brains and showed that these cells displayed the stem cell features indicated above. In 1989, Dr. Sally Temple identified stem cells from rat embryonic brains and cultivated them in cell culture plates in the lab. Some of the stem cells she discovered may turn into neurons, while others could become astrocytes. All the pluripotent cells have the potential to produce new neural stem cells. Scientists believe that comparable stem cells may be discovered in the fetal brains of other animals, including humans, due to her research. Drs. Brent Reynolds and Samuel Weiss discovered that mature mouse brains contained stem cells in 1992. They cultivated cells from mature mouse brains in a plate adding "growth factors," crucial chemicals. They demonstrated that the isolated cells could self-renew and develop neurons or astrocytes in the influence among these growth factors, indicating that they were neural stem cells. Dr. Eriksson and his associates found neurogenesis in the mature human brain in 1998-1999. For the first time, neural stem cells have been extracted from an adult human brain (Swayne et al., 2016).

Chapter 4

Stem Cells and Stem Cell therapy

In recent years, progenitor cells, commonly known as stem cells, have been extensively investigated in research for their intriguing properties as anti-apoptotic and anti-inflammatory agents. Simultaneously, they promote angiogenesis and neurogenesis or increase the environment with trophic factors. Cell therapy is stratified into two methods, the substitution of cells that are pathologically affected and simulation of endogenous procedures with self-renewal tissue. Due to their extraordinary adaptability, stem cells (SCs) recently gained considerable interest. Their primary traits are their capability for self-renewal and the ability to change their phenotypic or progeny into specialized or uniform cellular divisions. SCs are classified according to their source: embryonic stem cells (ESCs) and adult stem cells (ASCs) (Rascón-Ramírez et al., 2021a).

4.1 Types of Stem Cells

Stem cells are stratified according to their origin, mainly embryonic stem cells (ESCs) and adult stem cells (ASCs). ESCs are the up-to-date well studied pluripotent SCS, which are segregated from the inner cell mass of the blastocysts. ASCs are primarily attained from the subsisting pool in the grown tissues or cells in the birth cord ASCs examples include cells existing in the bone marrow, such as (i) mesenchymal stem cells (BM-MSC), which consist of the ability to differentiate into adipose cells, chondrocytes, bone (ii) hematopoietic stem cells (BM-HSC), which consists of the quality of enhancing cells of the hematopoietic system, (iii) endothelial SCs (Rascón-Ramírez et al., 2021b).

Moreover, mononuclear cells (MNC) were discovered, characterized by inadequate granules in the cytoplasm and non-rounded or non-detached nuclei in the cells. Numerous studies found these cells' capabilities with promising therapeutic potentials, less complicated isolation, and

cell. Mononuclear cells isolation can be performed from the peripheral blood or BM (PBMC) and consist of variable quantities of hematopoietic and mesenchymal stem cells (Rascón-Ramírez et al., 2021b).

4.2 The Selection of the Route of Administration

When designing clinical studies, one of the primary issues to address is the method of delivery. This is frequently tied to the pathogenic phase of the stroke targeted by the therapy, including other factors such as treatment's degree of safety, the kind of cells used, or the dosage. Parenteral administration may be accomplished in a variety of ways including intraperitoneal (IP), intra-arterial (IA), intravenous (IV), or intranasal (IN).

4.2.1 Intravenous (IV)

IV administration is often the most frequently employed mode of delivery in cell-based therapies for ischemic stroke clinical trials. This technique, similar to blood transfusion, includes infusion via a central or local venous catheter. Complications like pulmonary embolisms or thrombi are conceivable due to the buildup of infused cells. Currently, 14 trials have been reported in which IV administration was employed. A pilot study monitored 12 recurrent stroke patients for 24 months after receiving IV injections of enlarged BM-MSCs. Subsequently, after a year, the same research team conducted a similar clinical experiment employing CD34+ cells obtained by cytometry from bone marrow. The experimental group demonstrated a tendency toward improvement when compared to the controls. A controlled clinical experiment used one of the most significant cell dosages reported: 10 million CD34+ cells per kilogram of body weight. Since patients received treatment in the acute phase, cells were separated using cytometry from the BM aspirate. The MNC group had significantly more activity in the central premotor and parietal regions than the controls, but the disparities were

insignificant. Two trials have shown the safety and tolerability of autologous BM-MNCs IV transplantation (Rascón-Ramírez et al., 2021b).

According to a 2014 study, 117 of 120 randomized subacute stroke victims maintained an annual follow-up. PET and electroencephalography were used to assess safety parameters, and there was no evidence of neoformations consistent with mass of tumor, toxicity or the initiation of the cellular cycle. Another multicenter trial reported its findings in 2017 after randomly assigning 129 patients to receive adult stem cells or a placebo. They demonstrated that patients who underwent cell treatment had more good outcomes (as judged by several measures) and substantial differences in their favor. In 2016, Bashin's group released the results of a fourth clinical study in which BM-MNCs were examined in an experimental group of both the sample and control group of 10 people. For the first time, the procedure incorporated an evaluation of paracrine transmission as a proxy for treatment effectiveness in addition to the conventional neurological scales. The scientists assessed the quantities of vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF). However, they could not establish a link between their levels and the number of CD34+ cells. In nine chronic patients, UC-MSCs were employed in a phase I/II experiment that was aleatory, controlled, and double-blind. Six and ten weeks following enrollment, patients received two doses of 4.57 107 MSCs. The treatment's effectiveness was evaluated because the experimental group improved much more than the controls. The amount of BM extracted was approximately 2 ml/kg to inject a maximum of 10 million cells/kg of MNC, but four patients got between 8 and 9 million cells. Numerous adverse events (AEs) were documented throughout the annual follow-up session, altering the exclusion criteria. Three individuals had to expand infarctions, while one experienced a hypotensive episode (Rascón-Ramírez et al., 2021b)

4.2.2 Intra-Arterial (IA)

A femoral or radial artery puncture is used to cannulate the contralateral middle cerebral artery in the IA mode of delivery. In general, this technique has limited advantages because of its more invasiveness than IV administration and lack of precision compared to IC administration. The first pilot research to use this strategy was published in 2011, and it was an estimation experiment that did not have a control group. Following experiments with IA delivery were conducted promptly, including eight experimental patients and nine non-randomized, blinded control subjects. 1.559 108 cells in total were injected, of which 3.38 106 were CD34+. Five to nine days after a stroke (subacute), the cells were delivered, and the patients were observed for six months. Two participants in this research had seizures three months following therapy, even though there seemed to be a link between biological measures and neurological scales. Clinical trials designed experimentally may be critical for acquiring a deeper understanding of the effectiveness of stroke therapies such as stem cell therapy. This research established precedents for the parameters used in the following clinical trials' design. It emphasized the need to develop a more consistent experimental design capable of removing intra-assay variability (Rascón-Ramírez et al., 2021b).

4.2.3 Intracerebral (IC)

One of the study's most remarkable findings was the lack of problems during surgery, especially considering the inherent hazards of this invasive technique. This research provided further evidence for the safety of this method of administration since no adverse events or problems were seen in hitherto unknown functions. One of the earliest allogeneic transplantation experiments employed a commercial strain of immortalized human neural stem cells from ReNeuron (CTX0E03). This single-site, open-label trial comprised 13 patients who did not have any adverse events (AEs) associated with the operation or the transplanted cells.

In recent research, every one of the three sectors received a single dose of 2.5, 5.0, or 10 106 cells when BM-MSCs were transplanted. Furthermore, seven patients reported nine major adverse events after an annual follow-up, with no clear correlation between cell dosage and SAE frequency. The purpose of doing this experiment was to determine the stability and safety of the progenitor cells in the therapy of persistent motor stroke-induced hemiparesis. Treatment was well tolerated at all dosages, and MRI investigations revealed cavity filling due to the development of new brain tissue (Rascón-Ramírez et al., 2021b).

Chapter 5

Mechanism of action of MSC-therapy

There are still many things to learn about the mechanisms of action of MSC-based stroke treatment, which is now being investigated. MSCs are thought to protect against stroke by a variety of methods, including direct differentiation into various types of cells, paracrine actions, and mitochondrial transfer, according to several experimental investigations.

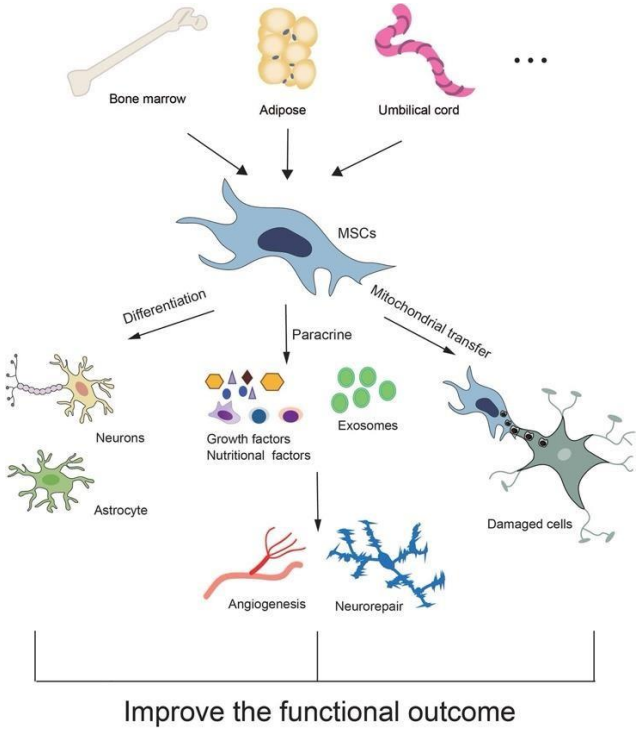


Figure 2 : Possible route of action of mesenchymal stem cell therapy in the treatment of stroke.

Source: (Li et al., 2021)

5.1 Mesenchymal-Stem Cell Differentiation

MSCs are pluripotent adult mesenchymal cells that have the potential to self-renew and differentiate into a variety of cell types (Klimczak & Kozlowska, 2016). MSCs are actively transplanted or homed to the wounded site to heal the damage in the case of injury and inflammation. (Shi et al., 2012). MSCs can divide and proliferate in a particular milieu of a tissue or organ, differentiate and mature into the very same type of cell as the body tissue,

including neuronal cells, and effect healing when placed in that microenvironment (Clevers et al., 2014; Klimczak & Kozlowska, 2016). In one study, it was shown that MSCs obtained from the human umbilical cord might develop into neuron-like cells while maintaining their immunomodulatory as well as antioxidant properties (Li et al., 2012).

5.2 Paracrine Effect

In recent years, there has been increasing evidence that the capacity of MSCs to develop into neuron cells is insufficient to explain the majority of the advantages associated with MSC-based stroke treatment. The immune system's modulation, as well as the encouragement of angiogenesis, are examples of paracrine function. A large number of paracrine components work together to build a complicated exocrine factor network that helps to maintain the stability of cells while also enhancing the regeneration response. Both endothelial cell proliferation, as well as migration, are induced by a simple fibroblast growth factor as well as a vascular endothelial growth factor, resulting in the formation of new vascular branches. EXOSOMES from multipotent stem cells (MSCs) are lipid particles of 30–100 nm in diameter by a double membrane shape that contain micro-and mRNAs, DNAs, and bioactive compounds such as protein and fatty acids. It is believed that the paracrine impact induced by their exterior secretion is crucial in the recovery from a stroke. Exosomes were found to be associated with increased angiogenesis and decreased post-ischemic immunosuppression (i.e., B cells, natural killer cells, and T-cell lymphocytosis) in some mouse models of stroke, indicating that they may provide an appropriate external environment for successful brain remodeling (Li et al., 2021).

5.3 Mitochondrial Transfer

It has been widely reported on mitochondrial transfer, which is a unique technique of stem cell treatment that has gotten a lot of interest. Through a number of mechanisms, MSCs may

transfer mitochondria to wounded cells with a mitochondrial malfunction in order to restore aerobic cell respiration and mitochondrial function, ultimately resulting in the recovery of injured cells (H. Han et al., 2016a; Islam et al., 2012a; Spees et al., 2006a). Ischemia/reperfusion damage has long been recognized as a symptom of ischemia/reperfusion damage in the complicated cell process; thus, mitochondrial transfer could be one of the methods through which MSC therapy is beneficial in the treatment of stroke (Han et al., 2020). Co-cultured BM-MSCs have the ability to transport intact mitochondria to injured cells via transient tunneling nanotubes (TNT), therefore restoring the mitochondrial activity of those cells (Han et al., 2016). Yang et al. revealed that iPSC-MSCs were able to protect injured PC12 cells by recovering mitochondrial function in their experiments. In addition to the paracrine impact of MSCs, this was linked to the mitochondrial transfer from MSCs to the wounded PC12 cells, which was seen in the laboratory (Yang et al., 2020a). A growing body of data suggests that mitochondrial transfer between MSCs and injured cells is mostly conducted by constricted nanotubes and micro-vesicles (Islam et al., 2012b; X. Li et al., 2014; Murray & Krasnodembskaya, 2019; Spees et al., 2006). As a result of these findings, (Babenko et al., 2015) demonstrated that BM-MSCs may donate mitochondria to damaged astrocytes and recover their mitochondrial functions, suggesting the protective effect of MSC on nerves. (Tseng et al., 2021) revealed in vivo and in vitro that the migration of mitochondria by the MSCs to injured neurons produced by oxidative stress resulted in metabolic improvements. The BM-MSCs were labeled, and the transplanted mitochondria were followed by the researchers. It was discovered that rats with cerebral Ischemia were able to transfer mitochondria and that this had a protective impact on the injured cerebral microvascular system (K. Liu et al., 2019; Yang et al., 2020). As a result, mitochondrial transfer from MSCs to injured cells may open up a new therapy option for those suffering from a stroke.

Chapter 6

Study Design

6.1 Control Group

To adhere to the concept of comparability, it is necessary to incorporate a control group in order to determine the treatment's weight in the outcome. Additionally, this permits the exclusion or explanation of effects seen throughout the experiment; however, not linked to the therapy and determines the trial's viability (Rascón-Ramírez et al., 2021b).

6.2 Multicenter

Heterogeneity of the sample boosts the data's external reliability, enabling it to extrapolate to additional populations. Almost all of the virtual research reported so far was undertaken at a single center except for three (Rascón-Ramírez et al., 2021b).

6.3 Randomization

Even with limited groups, group projects must be random to prevent project bias and adhere to the causality principle. Not all of the research described above fits the requirements for randomization (Rascón-Ramírez et al., 2021b).

6.4 Sample Size

Most of the research in this sector used small groups (n = 65), often due to recruiting issues. The heterogeneity of the test groups is reduced by the great diversity of baseline clinical states and stroke sites. Future protocols should take into account available strategies for maximizing population homogeneity.

Sample sizes were often smaller in studies administered through the IC route. However, research has demonstrated that IC treatment is safe in most instances and has no substantial risk compared to alternative choices (Rascón-Ramírez et al., 2021b).

6.5 Blindness

Investigators, doctors, and patients must all be unaware of the participant's allocation (triple-blind). It is recommended that a single research participant be responsible for giving the therapy and not be involved in hospital patient tasks or baseline or follow-up evaluations. The benefit of a triple-blind design over a blind design is that it eliminates observer bias rather than minimizes the placebo outcome (single-blind design) (Rascón-Ramírez et al., 2021b).

Chapter 7

Treatment Characteristics

7.1 Cell Origin

There is no agreement about the optimal cell phenotype for stroke treatment. Although the great majority of clinical studies have used BM-MNCs via various modes of delivery, other kinds of cells have shown comparable effects, including I neurons developed from a type of neural progenitors; (ii) MSCs grown in cellular cultures; and (iii) chosen CD34+ cells that impart an autologous character to the therapy. This brings up another point of contention: autologous vs. allogeneic transplantation. The "safety" of autologous vs. allogeneic transplants is greatly dependent on the cell origin because BM-MNCs from an HLA-incompatible donor are unsafe. However, MSCs from an HLA-incompatible donor seems to be reasonably safe. Despite the many benefits of autologous transplantation, allogeneic transplantation provides therapeutic uniformity when therapies are "made" following good manufacturing practices (Rascón-Ramírez et al., 2021b).

7.2 Dose

The ideal dosage and timing of cell therapy administration have not been determined. This may vary according to various circumstances, including the cell type delivered, the mode of delivery, the features of the patient, as well as the severity of the sickness. To achieve their goals, parenteral routes of dosing (IA, IV) need further SCs, which may exacerbate a few of the adverse effects. However, pre-clinical research has shown that one of the adverse events associated with these delivery methods (pulmonary embolism) may be managed by controlling the number of cells supplied and the infusion rate. Local administration via the IC route, on the other hand, needs fewer cells since the medicine is delivered directly to the brain parenchyma. Nevertheless, it is an intrusive procedure that may carry extra hazards.

As shown throughout this analysis, clinical trial dosages range considerably between 1 and 2 million cells/kg of body weight and 50 to 60 million cells/kg. Additionally, the best time for transplantation is dependent on how the brain tissue's microenvironment changes after stroke. Early cell implantation after a stroke is anticipated to have a neuroprotective impact due to its potential to reverse toxicity and inflammation. On the other hand, after 2–4 weeks past the stroke, cell transplantation may affect endogenous neuronal healing by boosting plasticity, angiogenesis, and neurogenesis, all of which are more active during that time interval (Rascón-Ramírez et al., 2021b).

7.3 Cell Survival

Numerous studies have shown that several variables may alter cell survival. The phase of the stroke during which we provide the medication may have a detrimental effect on cell survival owing to reduced blood flow, oxygen shortage, trophic factors, oxidative stress, or inflammation inside the afflicted area. Other research used genetic changes or overexpression of growth factors to increase MSC or NSC lifespan to mitigate these consequences. The encapsulation of cells in biomaterial scaffolds prior to implantation into the ischemia zone is one of the more recent uses (Rascón-Ramírez et al., 2021b).

Chapter 8

Efficacy Tests

Almost majority of the trials included safety as a primary outcome measure, with just a handful demonstrating therapeutic effectiveness. This issue partly stems from the variability of neurological sequelae in individuals, even though employing established measures results in more "universal" meaning for scores. In other words, several research reveals just "trends toward improvement" rather than statistically significant changes. As a result, future research should use measures with more substantial discrimination scores. More precisely, it may be beneficial to include more selective neurological scales. At least one research has previously considered such scales. Finally, anatomical neuroimaging methods may be utilized to assess safety and effectiveness. Combining anatomical and functional MRI sequences, including the BOLD and DTI signals, enables a direct assessment of the strength of interconnections, simulation patterns, and metabolism, all of which may be connected to the treatment's mid- and long-term success (Rascón-Ramírez et al., 2021b).

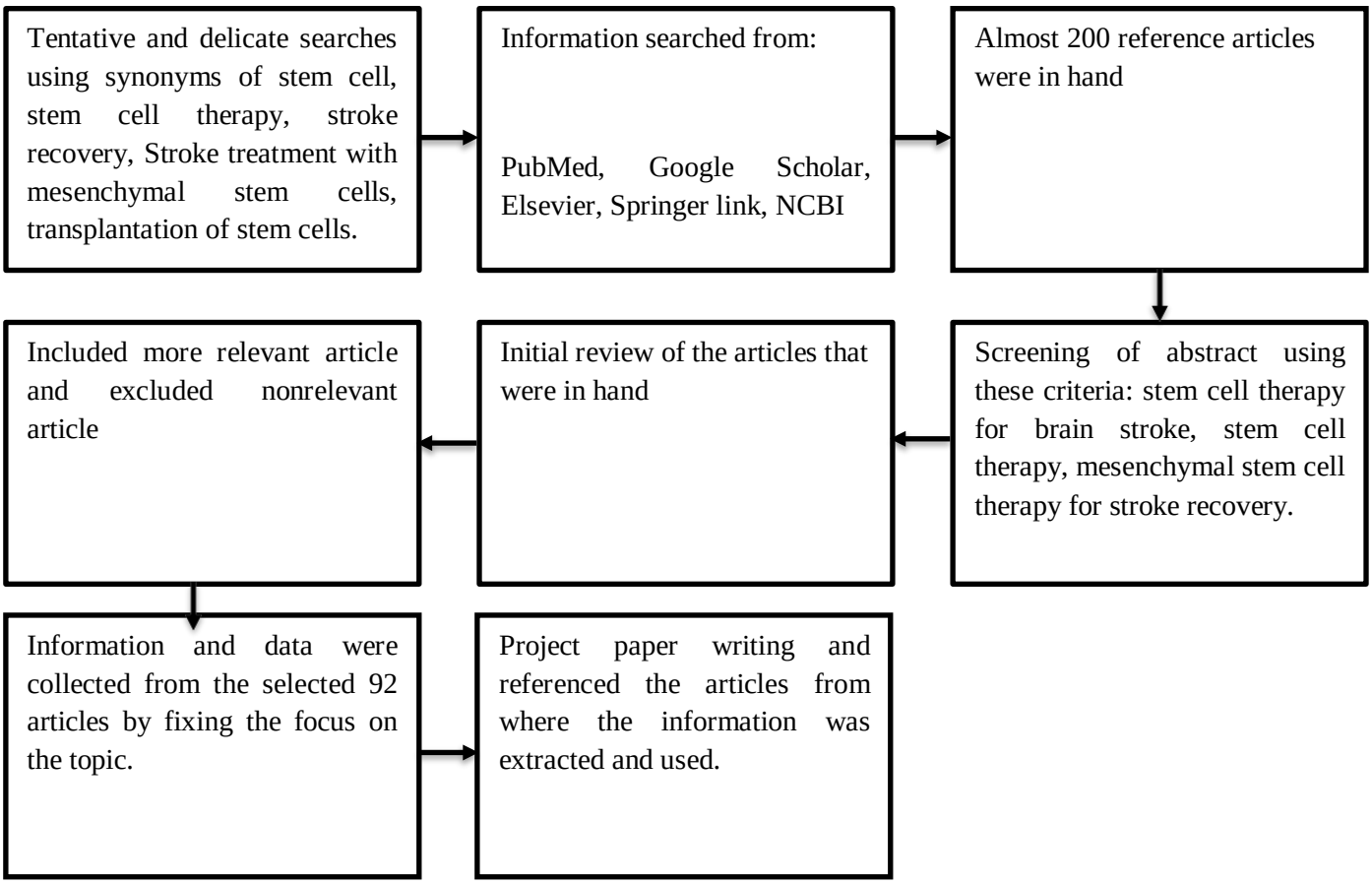
Chapter 9

Safety Consideration

Almost all published clinical studies suggest safety at the moment. Only a few people have had problems because of the surgery, not because of the treatment. The procedure's adverse effects vary from moderate to severe. Each one of them might be decreased depending on the route of delivery employed. Simultaneously, it is suggested to analyze side effects on a patient-by-patient basis to ascertain their cause and any association with concurrent conditions. Moll et al. emphasize the critical need to recognize coagulopathies in patients candidates for cell therapy and initiate thromboprophylaxis prior to treatment, therefore minimizing adverse consequences. Similarly, Caplan et al. investigate the impact of immune activation, both innate and adaptive, on the efficacy of stem cell therapy, integrating pre-clinical and clinical information. Despite these considerable gains, it is essential to remember that most clinical trials reported to date are pilot studies with tiny sample sizes. Clinical studies on a broad scale, including many centers and large sample size, are ongoing and will give critical clinical data on safety and associated concerns (Rascón-Ramírez et al., 2021b).

Chapter 10

Methodology



Chapter 11

Discussion

A stroke, or a cerebrovascular accident, is a medical emergency that occurs when the blood supply to a region of the brain gets cut off. Brain cells need a steady supply of oxygen and nutrients from the blood to operate correctly. When the blood supply to a portion of the brain is abruptly cut off, brain cells are injured or die, which may lead to brain damage also may cause death. Various sections of the brain regulate different portions of the body, each of which is supplied with blood by four major arteries in the neck, as well as smaller branches in the brain. The amount of brain damage produced by a stroke varies widely and is determined by the blood artery that has been damaged. If just a tiny blood vessel is injured, a stroke may go unrecognized. If a major vessel is disrupted, however, it might result in long-term impairment or death. Stroke is mainly classified into two: A blood clot causes an ischemic stroke or when fatty materials deposit in a vessel in the brain, restricting its blood flow. On the other hand, hemorrhagic stroke happens if the walls of a specific blood vessel in the brain become vulnerable and burst and bleed, resulting in brain damage. Thirdly, transient ischaemic stroke is comparable to a stroke in that it results in temporarily blocked blood supply in the brain, and it gets better within 24 hours. However, it does not cause any permanent damage. Therefore, it is very alarming for a future stroke.

Stroke is an enormous global issue that must be acknowledged, and effective treatment should be made available to stroke patients' to avoid high rates of mortality and morbidity worldwide. The use of tissue plasminogen activator or mechanical thrombectomy for the treatment of stroke patients is currently the only proven option available to people suffering from a stroke (Hacke et al., 2008) (Powers et al., 2015) (Saver et al., 2016). On the other hand, thrombolysis has a limited therapeutic frame, clinically efficacious within 4.5 hours after a stroke and

decreasing its efficacy when thrombus is massive, or if the stroke was severe, among other things (Bhaskar et al., 2018). This type of treatment is only given to about 5% of ischemic stroke victims, and those who do continue to experience neurological abnormalities after treatment have no therapeutic options accessible to aid in their rehabilitation (Lyden et al., 2019). The therapeutic value of mechanical thrombectomy has been established in treating acute ischemic stroke due to obstruction of the proximal intracranial artery. However, this approach has not yet reached its full potential, and the effectiveness, as well as safety for endovascular reperfusion beyond 6 hours, is still up in the air, according to the latest research (Berkhemer et al., 2015)(Smith, 2019). A small percentage of stroke patients may improve from such methods and experience excellent outcomes (Detante et al., 2017a).

Stem cell therapy has received considerable attention in recent decades as a developing treatment for stroke, with the expectation that it may be able to restore the damaged core neuronal networks in the affected brain regions (Wan Safwani et al., 2017) (Choi et al., 2018). For patients suffering from ischemic stroke, stem cell treatment has shown considerable benefits on functional recovery, providing faith for the survival of brain tissue in the critical stage and the restoration of damaged tissue in the regular phase (Wei et al., 2017).

Non-hematopoietic stem cells, which are Mesenchymal stem cells (MSCs), have the capacity to develop into a variety of cell origins such as osteoblasts, chondrocytes, as well as neuron-like cells (Uccelli et al., 2006) (B. A. Williams & Keating, 2008). Their isolation from practically all mammalian tissues, such as bone marrow (BM) and adipose tissue, as well as several other tissues, as well as from various different tissues (Pinho et al., 2020), makes them very straightforward to grow and expand. MSCs generated from bone marrow are the most prevalent. However, adipose-derived MSCs have grown more popular in recent years owing to their improvements in productivity and ease of availability (Faghih et al., 2017) (Perteghella

et al., 2017). MSCs derived from bone marrow and adipose tissue have full trilineage differentiation capacity (adipogenic, osteogenic, and chondrogenic) as well as excellent immunomodulatory properties when compared to other stem cell sources, and as a result, they are the best contributor for cell therapy (Heo et al., 2016). MSCs are appropriate for transplantation even in the acute period of a stroke and exhibit significant neurotrophic benefits even in this stage (Walczak et al., 2008). MSCs generated from adult tissues also offer no threat of cancer, and the minimal presence of major histocompatibility complex (MHC)-I as well as MHC-I II antigens in these cells reduce the requirement for immunosuppression after allogeneic delivery (Bhatia & Hare, 2005) (A. R. Williams & Hare, 2011). Anti-inflammatory, anti-apoptotic, angiogenesis, and neurogenesis are only a few of the methods through which MSCs exert their therapeutic benefits (W. Li et al., 2021a).

11.1 Pre-Clinical Results

In the majority of investigations, Sprague Dawley (SD) rats were used to generate a model of cerebral Ischemia, which was caused by blockage of the middle cerebral artery (MCAO). Several studies have demonstrated that MSC transplantation following ischemic stroke promotes the progress of neurologic function (Toyoshima et al., 2015) (Moisan et al., 2016a) (Hu et al., 2019), effectively protecting ischemic neurons, and restores brain damage (Son et al., 2019). In contrast, other research utilized young adults and healthy animals, failing to take into consideration the fact that a major number of ischemic stroke patients are older, as well as the existence of comorbid diseases such as hypertension as well as diabetes (Howells et al., 2010) (Laso-García et al., 2019). These animal models, in this case, serve to obstruct the translation of data from animal models to clinical trials. Therefore, we concentrated on research that included comorbidities in animal studies of stroke in this section. It has been estimated that around 75% of all strokes occur in the older population (Yousufuddin & Young, 2019). To develop an ischemic stroke model, (Shen et al., 2007) used 10–12 month-old female

retired breeder rats to validate the long-term neurological therapeutic potential of MSC on ischemic stroke. Additionally, (Saraf et al., 2019)found that a stroke caused CaN hyperactivation in neurons, which in turn triggered an apoptotic path in neurons, ultimately leading to neuronal cell death in middle-aged female rats. CaN expression was reduced in neurons after treatment with MSCs, resulting in improved neuronal survival (Saraf et al., 2019). Stroke of any form is greatly increased by hypertension, which is the most common risk factor. High-risk spontaneously hypertensive rats (SHRSP) are frequently used in hypertensive ischemic stroke models as recently as 2017 (Hong, 2017)(Cipolla et al., 2018) because they acquire 100 percent hypertension without genetic alteration, have cerebrovascular pathophysiology that is highly comparable to that of human hypertension, and are prone to spontaneous stroke at a rate of more than 60%. (Liao et al., 2013). Using SHRSP rats, (Calió et al., 2014) revealed that MSCs increased the expression of the anti-apoptotic gene Bcl-2 as well as preserved brain tissue via anti-apoptosis and antioxidation. This suggests that MSCs have a protective impact on neuronal cells. In another study, therapy with placental-derived MSCs significantly enhanced functional recovery and decreased infarct size in mice suffering from hypertensive ischemic stroke (Kranz et al., 2010). The presence of diabetes is proven to be one of the factors resulting in a stroke. Diabetic patients are more likely than non-diabetics to have an ischemic stroke, and hyperglycemia worsens the microvascular and macrovascular damage that occurs in ischemic strokes (Rehni et al., 2017) (Lau et al., 2019). Studying diabetic stroke models, as a result, has become more important. It has been found that lesions in the hyperglycemic group were more severe than lesions in the non-hyperglycemic sample six weeks after permanent MCAO was performed. Although therapy with human adipose tissue-derived MSCs for hyperglycemic stroke rats showed no reduction in lesion size, it did result in a substantial improvement in nerve function (Gómez-De Frutos et al., 2019). (C. Cui et al., 2016) found that BM-MSCs had therapeutic effects on type 1

diabetic mice with stroke through regulating the expression of miR-145. Treatment with exosomes derived from BM-MSCs dramatically enhanced the blood-brain barrier (BBB) function, boosted white matter remodeling, and facilitated neural healing in type 2 diabetic Wistar rats suffering from stroke (Venkat et al., 2020).

Moreover, the neuroprotective impact of MSCs-derived treatments in treating ischemic stroke and subarachnoid hemorrhage (SAH) has long been recognized. However, their therapeutic potential in SAH is just now being studied. It is widely known that MSCs have anti-inflammation and anti-death capabilities in animal models of SAH and the ability to reduce microglia polarization and prevent brain cell apoptosis in human patients with stroke. More research is required to determine if they impact SAH; however, pooled analyses have shown that they can alleviate neurological deficits and decrease brain edema in preliminary mouse models of the disease. Specifically, the SAH animal studies are well-suited for pre-clinical research of MSC transplantation. The mortality rate in the autologous blood injection form is less than that in the endovascular perforation model, which is a significant difference (He et al., 2022).

According to the reports, there was no difference in the impact size of SAH models in terms of either the neurobehavioral score or the amount of water in the brain. According to the findings, EVs produced from MSCs proved to be more effective than MSCs in the stimulation of neurological function recovery. However, to investigate these difficulties in detail, more extensive pre-clinical studies with sound design are required. There is currently insufficient proof to support the use of MSCs to treat EBI and late brain damage after SAH. According to our findings, IV injection was more beneficial than IN injection to improve neurobehavioral outcomes. The data's robustness was reduced due to the small sample sizes. These data suggest

that the EBI produced by SAH generation may react more favorably to MSC-derived treatments than other types of EBI (He et al., 2022).

Animal species	Stroke type	Comorbidity	Cell source	Cell number	Delivery route	Timing	Results	References
SD	MCAO		BM	1×10^5	IA (carotid artery)	10 days	Neuronal regeneration	Hu et al., 2019
SD	MCAO		BM	3×10^6	IV(tail vein)	Eight days	Angiogenesis	Moisan et al., 2016
Wistar	MCAO		BM	1×10^6	IA	1, 6, 24, and 48 h	Reduce infarction volume	Toyoshima et al., 2015
SD	MCAO		BM	2×10^5	IC (brain tissue)	One day	Protect ischemic neurons	Son et al., 2019
Wistar	MCAO	Aging	BM	2×10^6	IA (carotid artery)	One day	Long-term improvement in functional outcome	Shen et al., 2007a
SD	MCAO	Aging	BM	1×10^5	IA	Six h	Improve the functional outcome	Saraf et al., 2019
SHR	Stroke prone	Hypertension	BM	1×10^6	IC (atlanto-occipital membrane)		Neuroprotective and antioxidant potential	Calio et al., 2014
SHR	MCAO	Hypertension	Placenta	1×10^6	IV (tail vein)	8 and 24 h	Functional recovery	Kranz et al., 2010
SD	MCAO	Hyperglycemia	Adipose tissue	1×10^6	IV (tail vein)	48 h	Neurological recovery	Gomez-de Frutos et al., 2019
Wistar	MCAO	Diabetes	BM	5×10^6	IV (tail vein)	24 h	Neurorepair effects	Cui et al., 2016
Wistar	MCAO	Diabetes	BM	3×10^{11}	IV (tail vein)	Three days	Improve the functional outcome	Venkat et al., 2020

Table 1 A summary list of the pre-clinical research on the use of MSCs in the treatment of Stroke
Source: (Li et al., 2021)

11.2 Clinical Results

In spite of the fact that preclinical research has shown that MSCs have a favorable impact on stroke (Moisan et al., 2016) (Cunningham et al., 2018), clinical trials have revealed a number of safety concerns, including inflammation, tumor growth, and metastasis (Gazdic et al., 2015) (Dhere et al., 2016) (Volarevic et al., 2018). Over the last several decades, clinical studies have been conducted to determine the safety, feasibility, and efficacy of mesenchymal stem cells (MSCs) in the therapy of stroke (Table 2). Previous clinical studies have shown that MSCs derived from various tissues have high efficacy for the treatment of strokes in various settings (Chrostek et al., 2019; L. li Cui et al., 2019; Detante et al., 2017; Suda et al., 2020; Wechsler et al., 2018). Several delivery methods have been considered, namely intracerebral (IC), intra-

arterial (IA), and intravenous (IV) injections, among others (W. Li et al., 2021b). The intracerebral approach is the most successful and least invasive of all the options. On the other hand, the intravenous conduit, on the other hand, is the least intrusive, but the amount of MSC cells that can be delivered to the wounded brain is the highest restricted. The intra-arterial route is characterized by its relative neutrality. In 2005, autologous bone marrow stem cell transplantation was conducted intravenously in five patients suffering from acute ischemic stroke, with no adverse effects noted (Bang et al., 2005). Similar findings were obtained in a major long-term trial conducted in 2010 that assessed the safety and effectiveness of autologous intravenous BMSCs transplantation (Lee et al., 2010). In 2011, the delivery of serum-expanded autologous BMSCs into chronic stroke patients resulted in a decrease in infarct lesion volume and the restoration of neurological function (Honmou et al., 2011). Following that, a phase I/II study of intracerebral cell transplantation in people with chronic stroke discovered that intracerebral implantation of genetically modified MSCs resulted in substantial improvements in neurological function in these individuals (Steinberg et al., 2016, 2018). BMSCs were shown to be effective in improving motor function in a single-center, open-label Randomized Controlled Trial research. This suggests that MSCs therapy may be a viable therapeutic option for stroke patients in the future (Jaillard et al., 2020).

Phase	Patients number	Delivery route	Cell source	Cell number	Timing	Results	References
I	5	IV	Autologous BM-MSCs	1×10^8	Seven days	Improve in BI	Bang et al., 2005
II	16	IV	Autologous BM-MSCs	5×10^7	5-7 weeks	Improve in mRS	Lee et al., 2010
I	12	IV	Autologous BM-MSCs	1×10^8	36-133 days	Improve in NIHSS	Honmou et al., 2011
I	8	IV	Autologous BM-MSCs	$5 - 6 \times 10^7$	Three months-1 Year	Improve in Fugle-Meyer and mRS, increase in the number of cluster activation of Brodmann areas BA 4 and BA 6	Bhasin et al., 2011
II	20	IV	Allogeneic AD-MSCs	1×10^6 cells/kg	Two weeks	Safe and effective	Diez-Tejedor et al. 2014
I/IIa	18	IC	Modified MSCS (SB623)	Dose-escalation: 2.5×10^6, 5.0×10^6 Or 10×10^6	6-60 months	improve in ESS, NIHSS, Fugle-Meyer	Steinberg et al., 2016, 2018
II	48	IA	$BM - ALDH^{br}$cells	$0.5 \times 10^5 - 2.5 \times 10^7$	9-15 days	Safe	Savitz et al., 2019
I	10	IV	Allogeneic UC-MSCs	$5 \times 10^6 - 5 \times 10^7$ /kg	7-10 days	Safe and feasible	Laskowitz et al., 2018
II	16	IV	Autologous BM-MSCs	$10 \times 10^7 - 30 \times 10^7$	14 days	Improve in motor-NIHSS, Fugle-Meyer, task-related fMRI activity	Jaillard et al., 2020

Table 2 A summary list of the clinical research on the use of MSCs in the treatment of stroke

Source: (Li et al., 2021)

Chapter 12

Future Prospective

Although stem cells have been shown as a valuable resource in the treatment of stroke, there are a number of challenges that must be overcome in the future. Multiple stem cell types with various properties are being tested for the therapy of stroke in clinical studies. In terms of the several types of stem cells that are now being used, pluripotent (ESCs and iPSCs), adult (MSCs), and neural stem cells. With the use of pluripotent stem cells, there are ethical considerations to consider. Furthermore, NSCs have restrictions when it comes to in vitro growth. MSCs are effective in dealing with this issue. Another concern is both the host body and the transplanted stem cells have developed immunological tolerance. This problem may be remedied by utilizing the patient's own cells to generate induced pluripotent stem cells (iPSCs) from MSCs (as they lack HLA class II). Other considerations include if cell extraction, growth, and differentiation are effective enough for transplantation and whether the best type of injection and the right number of injections are employed. When there are numerous barriers to overcome in order to mainstream stem cell therapy as a therapeutic option for different illnesses, stem cell treatments have already been proven effective in the treatment of certain degenerative and other types of disorders. All of these issues of concern must be addressed in the future if stem cell therapy is to become a permanent therapeutic option for stroke patients (Singh et al., 2020b).

Chapter 13

Conclusion

Based on the results of clinical trials, cell therapy as a stroke treatment is safe. There is a trend for clinical improvement in the individuals' study sequelae, although statistical significance has yet to be shown. Efforts should be made in the future to include standardized criteria in clinical studies. Moreover, the factors identified in this research may be used to "design future clinical trial procedures" to generate more robust and trustworthy outcomes. In this regard, it is critical to balance the dangers and advantages of this new stroke therapy, which is why the additional study is needed.

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