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Biotechnology-based therapies for stroke treatment: review

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ABSTRACT: Various therapeutic agents have been used to treat stroke. However, currently there is extensive exploration of new potential therapies for stroke involving novel signaling pathways and development of therapeutic agents through biotechnological approaches. This article examines the recent advances in stroke therapy using biotechnology-based drugs. We conducted a comprehensive search using specific keywords relating to Ischemic Stroke, ATMP, Peptide, Antibody, Stem Cells, and connected topics in the databases of Medline, Scopus, Web of Science, and Pubmed. The main focus of the selection criteria was on English-language literature that explored the relationship between Ischemic Stroke, ATMP, Peptide, Antibody, Stem Cells, and related factors. This article exhibits that numerous studies are being conducted and have demonstrated the use of biotechnology-based therapeutic agents for stroke, including tissue plasminogen activators, therapeutic peptides, microRNA, monoclonal antibodies, as well as stem cells. These therapeutic agents have not only been tested on test animals but have also been commenced to be tested in clinical studies or have obtained marketing approval for use in ischemic stroke patients. In conclusion, despite the limited number of approved drugs, advancements in biotechnology are poised to make them common adjunct treatments for stroke patients, not just for managing the disease but also for its cure and regenerative effects in survivors.

KEYWORDS: antibody; ATMP; ischemic stroke; peptide stem cells.

INTRODUCTION

A Stroke is defined as a sudden focal neurologic dysfunction that lasts for at least 24 hours and can be caused by cerebral infarct, spinal, or retinal. Based on its etiology, stroke can be classified as ischemic and hemorrhagic stroke [1]. Between the two types of stroke, ischemic stroke occurs more frequently than hemorrhagic stroke [2]. Stroke is the second leading cause of mortality in the world and can give rise to chronic disability for half of its survivors, which makes it a disease with serious economic and social impacts with enormous public health importance [3].

A stroke attack can be manifested with various symptoms, such as feeling weak in half of the body, inability to speak, loss of vision, vertigo, or falling. Headaches are also experienced by ischemic stroke patients and can be more severe in hemorrhagic stroke patients [1]. Within 5 years after a stroke attack, most stroke patients will die or become functionally dependent, with this level of dependency being higher in hemorrhagic stroke patients. Although recent advances have been made in long-term stroke care and post-stroke rehabilitation, improvements are still needed in stroke therapy management, especially in the management of hemorrhagic stroke which still has few therapeutic options [4].

Biotechnology can be broadly defined as the process of manipulating organisms for the development and manufacture of products that are useful for everyday life. Biological products, including protein drugs and synthetic peptides, are biotechnology-based drug products and have become one of the most important sources of new therapies and therapeutic molecules. These drugs can be made by chemical synthesis process, expressed in host cells using the DNA recombinant technology, or directly isolated from the source tissues [5]. In addition to biological products, biotechnology methods also make it possible to create therapeutic agents in the form of gene therapy, somatic cell therapy, and tissue engineering products. This therapy is a new class of biopharmaceuticals, also known as Advanced Therapy Medicinal Products (ATMPs). Currently, ATMP

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continues to be developed for use as a therapeutic agent at the clinical level [5]. This article examines the recent advances in stroke therapy using biotechnology-based drugs.

Stroke pathophysiology

Stroke can be caused by several risk factors that are modifiable and non-modifiable. Some unmodified risk factors are age, race, sex, transient ischemic attack, and genetic predisposition. Meanwhile, some risk factors that can be modified are hypertension, smoking habits, alcohol consumption, drug abuse, obesity, dyslipidemia, physical inactivity, atrial fibrillation, and cardiovascular diseases [6], [7]. Other factors, such as inflammation diseases, infection, and air pollution can also cause stroke attacks [8].

Stroke attacks can cause secondary reactions that lead to the death of neuron cells. In ischemic stroke, cerebral blood flow disturbance will limit the supply of glucose and oxygen which can lead to excitotoxicity that is caused by excessive glutamate release, mitochondrial dysfunction, and eventually lead to oxidative stress that is induced by reactive oxygen species production. Simultaneously, several cell-death signaling pathways such as apoptosis, necroptosis, autophagy, ferroptosis, parthanatos, phagoptosis, and pyroptosis, as well as the recruitment of immune cells such as chemokines and cytokines, are also involved in the cascade of reactions leading to neuronal cell death and destruction of the blood-brain barrier (BBB) [9], [10]. In hemorrhagic stroke, hematoma mass expansion brought on by blood vessel leakage can result in hernias and subsequent brain injury, which in turn triggers inflammation and microglia activation. This may potentially result in brain damage [11].

Stroke pharmacology therapy

Pharmacological therapies are the treatment process of disease or medical conditions using drugs to alter the biochemical process in the body. When a stroke attack occurs, intravenous alteplase injection is considered a safe and effective therapy when given within 4.5 hours after stroke attacks. Other treatments, such as mechanical thrombectomy to dissolve the clot in blood vessels can be given within 16 to 24 hours after stroke attacks. The patients who will receive the therapy have to meet the eligibility criteria for the treatment obtained from an evaluation by a professional health worker [12], [13].

Antihypertensive medications can be used to lower hematoma expansion in hemorrhagic stroke patients by bringing their blood pressure under control. This can improve the effectiveness of therapy. Anticoagulant-treated individuals may experience increased rates of morbidity and mortality. Therefore, it is necessary to counteract the anticoagulant impact, such as by administering vitamin K, an antagonist of the warfarin anticoagulant. Another therapy that can be given is a prophylactic corticosteroid, meanwhile, hyperosmolar therapy seems to have no benefit in the clinical outcome of patients [14].

Patients who have had a stroke must take certain pharmaceutical treatments to avoid further stroke attacks. To keep the systolic blood pressure at 140 mmHg, antihypertensive medications belonging to the ACE-inhibitor, calcium channel blocker, and diuretic groups are frequently utilized. Statin medications are also administered to control blood cholesterol levels, as are antiplatelet medications like aspirin, clopidogrel, or aspirin plus dipyridamole. Anticoagulants must be administered to individuals who have a history of atrial fibrillation. However, their combination with antiplatelets should be avoided if at all feasible [15]. In addition to drug therapies, patients also need to change their lifestyle, such as adopting a balanced nutritional diet and maintaining physical activity, as well as maintaining other vascular risk factors such as diabetes, stopping smoking, maintaining blood fat levels and hypertension [16].

Stroke maintenance therapy can also be done by giving phytochemical agents from plants. Some potential phytochemicals that can be used to treat stroke are vitexin, eriodictyol, carveol, ferulic acid, rosmarinic acid, allicin, curcumin, ginkgolide K, forsythiaside A, isoquercetin, trilobatein, genistein, and tocotrienols [17]. Various interests in plant compounds for stroke therapy are due to their antioxidant, anti-inflammatory, and anti-apoptotic properties. However, although these effects have been shown in studies using test animal models, no phytochemical compounds have been successfully translated into clinical settings, so further studies are still needed to obtain the exact role of phytochemical compounds in stroke therapy [18]. As research into stroke treatments continue to advance, attention has recently shifted towards the use of biotechnology-derived drugs for more targeted and effective therapies. This class of drugs have many differences with other conventional small molecule drugs, especially in the nature of the products, the source of active agents and the manufacturing methods. However, this class of drugs can be used with more targeted action as well as rarely causing side effects that are frequently seen in small-molecule drugs [19], making it an attractive option in the development of new stroke therapeutic agents.

Biotechnololgy-based therapıes for stroke treatment

In the health field, biotechnology has helped in the discovery of more than 200 novel therapeutic agents and vaccines to treat various diseases. Nowadays, there are more than 400 drug products and vaccines that are made by biotechnology methods, that are currently undergoing clinical trials and targeting more than 200 diseases [20], including for the treatment of stroke. The application of modern biotechnology techniques has resulted in many different classes of drugs, primarily drugs based on protein biomolecules, such as blood clotting factors and monoclonal antibodies [21]. In the context of stroke therapies, pharmaceuticals obtained through biotechnological processes operate through various mechanisms. Protein-based drugs, such as recombinant tissue plasminogen activators (rtPAs), therapeutic peptides, and antibodies work by dissolving blood clot or disrupting detrimental protein signaling to attenuate stroke pathophysiology. Another therapy, a microRNA-based therapy, works by disrupting detrimental gene expression that occurs after a stroke attack. Stem cells, which can be considered as ATMPs, work by providing neuroregenerative effects to restore neural functions in stroke survivors (**Figure 1**).

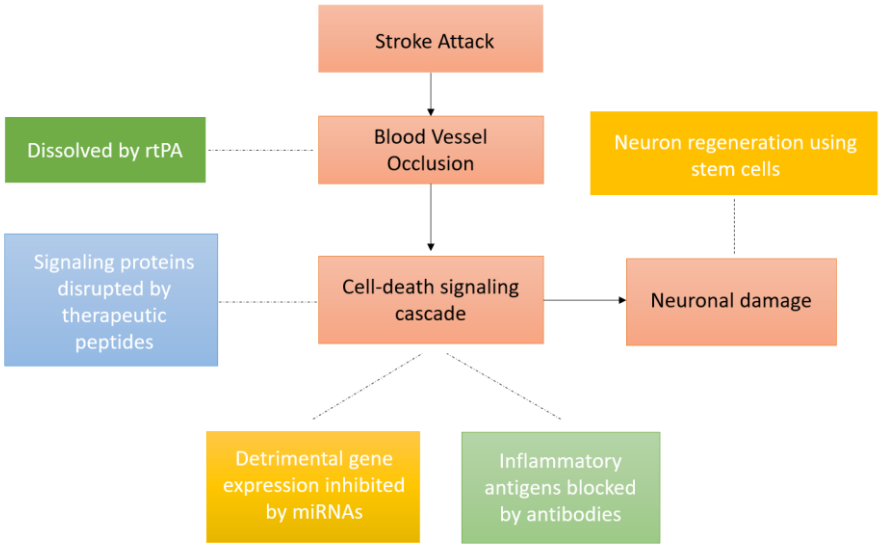

Figure 1. Mechanism of action of various biotechnology-based drugs for stroke therapy.

Recombinant tissue plasminogen activator

Tissue-type Plasminogen Activator (tPA) is a serine protease enzyme that can convert plasminogen to plasmin which can degrade fibrin [22]. Insoluble fibrin protein can clot and block blood arteries during a stroke attack. The clot will dissolve when plasmin, which has been activated by plasminogen with the aid of tPA, breaks down the fibrin that has formed. Because of this, t-PA-type medications are frequently used to speed up the clearance of hematomas in hemorrhagic stroke and recanalize blocked arteries in ischemic stroke [23], [24].

Alteplase is one of the commercial tPA that is produced by using the recombinant DNA method, where its manufacturing process is done by inserting an alteplase coding gene into a mammalian Chinese Hamster Ovary (CHO). This insertion will cause the cells to produce alteplase and secrete it outside the cells [25]. Alteplase administration to stroke patients within the 4.5 golden hours is proven to be beneficial for brain improvement and survival in stroke survivors [26]. However, alteplase also has some drawbacks such as low plasma half-live and tends to be metabolized in a short time, so it must be given as an infusion over 1 hour [1], [25].

To overcome the weaknesses of alteplase, new thrombolytic agents such as reteplase and tenecteplase were developed. Reteplase is a derivative of tPA that has been engineered by the removal of some of its domains, such as finger, EGF-like, and kringle-1 domains. Reteplase activity is also not affected by the presence of carbohydrate side chains at the N-glycosylation site, so the glycosylation process can be bypassed and allows it to be produced recombinantly in *E. coli* cells [27], [28]. In addition to the overexpression strategy

in E. coli, reteplase can also be produced using other expression systems such as *B. subtilis*, *T. subcordiformis*, and *N. benthamiana* [29], [30], [31], or by integrating reteplase gene to the genome of *P. pastoris* [32].

Another new generation of a thrombolytic agent, tenecteplase, is an engineered thrombolytic agent that has tetra-alanine residue substitutions at Lys296-His297-Arg298-Arg299 residues in addition to Thr103Asn and Asn117Gln residues. These substitutions prevent the interaction between tenecteplase and metabolic receptors, thus providing a longer half-life and allowing for intravenous bolus administration [27], [33]. In addition to its practical convenience, other advantages of tenecteplase compared to alteplase are better fibrin specificity, less systemic depletion of fibrinogen, and greater resistance to plasminogen activator inhibitors [34]. Both reteplase and tenecteplase have received marketing authorization from the FDA, in 1998 and 2000, respectively, for use as intravenous thrombolytic agents. However, several ongoing clinical trials are currently investigating their effectiveness specifically for the treatment of ischemic stroke [35], [36].

Therapeutic peptide

Peptides are a unique class of drug compounds that are molecularly intermediate between small molecules and proteins, but biochemically and therapeutically distinct from both. Peptides are able to mimic natural molecular signaling pathways, making them useful as replacement therapy or given as an adjunct to endogenous peptide hormone deficiency conditions [37]. Therapeutic peptides can be developed from various natural sources and then undergo some structural modifications to produce a peptide analog that has differences in their stability profiles and pharmacodynamic–pharmacokinetic properties [38].

Disruption of protein-protein interactions (PPIs) is known to play a role in many diseases. To overcome this, peptides can be used as an alternative to small molecule drug therapy, because peptides are flexible enough to target binding interfaces with better binding affinity and specificity [38], [39]. In addition to targeting protein-protein interactions, therapeutic peptides can also directly target other molecular targets such as GPCR receptors, catalytic receptors, ion channels, antimicrobial targets, and other intracellular and extracellular targets, making them useful for cardiovascular, oncology, and metabolic disease therapies [37].

In a stroke event, one of the most studied cell-death signaling pathways is excitotoxicity GluN2B-PSD95-nNOS. In this mechanism, PSD95 scaffold protein that connects NMDAR receptor with nitric oxide synthase (nNOS) forms a GluN2B-PSD95-nNOS complex. This complex causes calcium ion influx from the NMDAR receptor, thus causing nNOS overactivation. This overactivation leads to the production of nitric oxide (NO) that is reactive and can damage DNA as well as activate the pro-death p38-MAPK signaling pathway [40], [41].

To block this signaling, a peptide consisting of 20 amino acid residues, namely NA-1/Tat-NR2B9c, was designed to be able to permeate through the cell membrane and disrupt the interaction between NMDAR and PSD95, thereby disrupting downstream neurotoxic signaling. This peptide can work inside the cell and has been shown to protect neuron cells in some in vivo experiments [42]. The NA-1 peptide has undergone a phase 2 clinical trial. The trial showed that patients who received NA-1 peptide experienced fewer number of lesions and an improvement in radiological and clinical outcomes, indicating neuroprotective effect on this peptide. Currently, this peptide is undergoing a phase 3 clinical trial on a wider scale in more than 25 cities around the world [43].

Another complex involved in cell death is GluN2B-NMDAR-PTEN. In this pathway, NMDAR stimulation can induce nuclear translocation of PTEN and increase PTEN-induced cell-death activity, thereby mediating excitotoxicity-induced neuronal cell death [40], [41]. Tat-K13/SY-007 peptide is an interfering peptide that consists of 24 amino acid residues. This peptide can target PTEN translocation that causes cell death, where this translocation occurs over a longer period of time. The pre-clinical study of this peptide showed that animals who received Tat-K13 experienced a significant reduction in lesion size compared to controls. Therefore, this peptide can be an effective alternative stroke therapy in acute stroke patients who have passed the therapeutic window for more than 6 hours [44]. The Tat-K13/SY-007 peptide has reportedly undergone phase 1 clinical trials, where infusion at doses of 1–60 mg appeared to provide safe effects in participants and the effective dose for ischemic stroke therapy was estimated to be in the range of 10–30 mg [45].

Striatal-enriched protein tyrosine phosphatase (STEP) is a tyrosine phosphatase enzyme that functions in regulating the duration of ERK activity and downstream signaling pathways, thus enabling it to modulate the transcription process and function of neuronal cells [46]. The important role of STEP as a neuroprotective agent is seen in animal studies, where deletion of the STEP gene in animals can cause rapid and prolonged

release of p38-MAPK, where p38-MAPK itself is a signaling molecule involved in the production of pro-inflammatory mediators and induces cyclooxygenase-2 expression mediated by nuclear factor-kB induction. Prolonged release of p38-MAPK can cause severe brain damage and neurological deficits in test animals [47], [48].

A therapeutic peptide, TAT-STEP-myc, was developed to mimic the function of STEP. This peptide was constructed by using cDNA from amino acids 173–279 of the STEP61 isoform and fused with the TAT peptide and mutagenesis of residues S221A, T231E, and S244E was performed to maintain the activity and stability of STEP [49]. This peptide is shown to be able to hinder the activation of delayed p38-MAPK as well as reduce ischemic damage in the brain of animals [47], [48]. In addition, this peptide can also reduce the mortality rate of test animals when administered within 6 hours after the ischemic event, so that it can mimic real conditions in a clinical setting and become the basis for further development of this peptide in clinical trials [50]. For now, there is no peptide-based therapy that has been approved by the FDA to be used for stroke therapy. However, as clinical trials progress, it is not possible for therapeutic peptides to be used in stroke treatment in the future.

Table 1. Stroke treatment therapies: in vitro, in vivo, and clinical research.

No	Therapeutic agents	Stage	Results	References
1.	Reteplase	Phase 2 clinical trial	Reteplase is well-tolerated in stroke patients as well as exhibiting comparable effectiveness to alteplase, with further assessments to be done in this study	[36]
2.	Tenecteplase	Phase 2 clinical trial	Tenecteplase does not cause an increase in intracranial hemorrhage and mortality as well as better recanalization and non-inferiority in disability-free 3-month outcome	[35]
3.	NA-1 peptide	Phase 2 clinical trial	Patients administered the NA-1 peptide experienced a reduction in the number of lesions and showed enhanced radiological and clinical outcomes	[42]
4.	TAT-K13/SY-007	Phase 1 clinical trial	The SY-007 peptide is well-tolerated by patients across doses ranging from 1 to 60 mg, exhibiting non-linear pharmacokinetic properties and expected effective dose within 10 to 30 mg	[45]
5.	TAT-STEP-myc	Pre-clinical trial	TAT-STEP-myc is able to inhibit delayed-activation of p38-MAPK pathway, reduce ischemic damage, and mortality rate of subjects	[47], [48], [50]
6.	miRNA-31	Pre-clinical trial	miRNA-31 reduces cerebral infarct volume and signaling related to apoptosis process	[51]
7.	miRNA-130a	Pre-clinical trial	miRNA-130a exhibits neuroprotective effects by activation of PI3K/Akt pathway as well as enhanced cell survival and decreased apoptosis in OGDR cell model	[52]
8.	L13 antibody	Pre-clinical trial	L13 antibody reduces brain injury and improve neurological outcome by inhibition of MMP-9 activity	[53]
9.	PD-L1 antibody	Pre-clinical trial	PD-L1 is able to reduce infarction volume, partially restore splenic atrophy, improve neurological function as well as decrease proinflammatory cells	[54], [55], [56]
10.	Allogeneic stem cells	Phase 1/2 clinical trial	Allogeneic stem cells are well-tolerated in patients and provide functional improvements in stroke patients, but the study needs to be conducted over a longer period to comprehensively assess the clinical benefits of stem cells.	[57], [58], [59]

MicroRNA

Nucleic acids are universal building blocks of genetic material. In its use as a therapeutic agent, nucleic acids used for disease therapy are given in the form of antisense oligonucleotides (ASO), small interfering RNA (siRNA), micro RNA (miRNA), and messenger RNA (mRNA) and are used to perform gene knockdown

and induce the expression of certain proteins [60]. MicroRNA (miRNA) is a small non-coding RNA that can interact with the 5'UTR, 3'UTR, exon, and promoter regions of the genes. This feature makes miRNA have a role in regulating gene expression and communication between cells [61]. For use as therapeutic agents, miRNA-based drugs can be classified as miRNA-mimics and miRNA-inhibitor/antagomir. miRNA-mimics drugs are given to restore miRNA level to the baseline level, while antagomirs work by correcting miRNA expression patterns [62].

Various *in vitro* and *in vivo* stroke models have shown that miRNA can give protective effects after a stroke attack. In a study conducted by Lv *et al*. [51], miRNA-31 that is isolated from the extracellular vesicle adipose-derived stem cell (EV-ADSC) has been shown to increase functional reuptake after a stroke attack, reducing cerebral infarct volume and apoptosis, as well as hindering TRAF6 and IRF5 signaling that has a role in the apoptosis process in OGD cells and *in vivo* animals. Another miRNA, miRNA-130a is a new microRNA that is involved in the etiology of acute ischemic stroke, where miRNA-130a can give pro-angiogenic effect as well as anti-inflammatory effects. The levels of miRNA-130a in the plasma of acute ischemic stroke patients have been shown to correlate with lower disease risk, reduced severity, and reduced inflammatory effects after ischemic stroke attacks [63]. In addition, a study conducted by Zheng *et al*. [52] has shown that overexpression of miRNA-130a in the OGDR cell model was able to increase the survival of these cells and reduce apoptosis, as well as providing a protective effect on test animals against neurological deficits. This effect was given through inhibition of the PTEN pathway which causes PI3k/Akt activation, making miRNA-130a a promising target for stroke therapy.

Another microRNA, miRNA-128, in various studies has also been shown to have a protective role in the pathogenesis of acute ischemic stroke. In animal models, administration of miRNA-128 antagomir provides neurotoxic effects through reactivation of cell cycle input by promoting ERK and PTEN phosphorylation and increasing the expression of ERK, PTEN, and cyclinA [64]. The protective effect of miRNA-128 is also shown in an *in vitro* study. The study showed that miRNA-128 could lower the protein regulation of p38α-MAPK as well as reduce the infarct volume in animals with contrary effects found in the group that received miRNA-128 antagomir [65]. In addition, miRNA-128 has also been shown to play a role in regulating the proliferation and differentiation process of neural progenitor cells by targeting the 3'UTR position of the PCM1 gene, thereby suppressing PCM1 protein expression. This suppression inhibits cell proliferation while increasing the differentiation of NPC cells into neuronal cells [66]. These findings showed the potency of using miRNA-128 as an alternative in neuroregeneration therapy after ischemic stroke.

Although many preclinical models have demonstrated the therapeutic effects of miRNA, translation of miRNA use in clinical settings is still very limited. Currently, no miRNA-based therapy has received marketing authorization approval from the FDA, including for stroke indications. Several things, such as the large number of genes targeted by miRNA, its abundant expression, and its accumulation in tissues that can cause increased side effects are still obstacles to the use of miRNA in clinical settings [67]. In addition, miRNA is also susceptible to degradation by exoribonuclease enzymes if the miRNA is not in the miRNA-induced silencing complex (miRISC) [68], so various efforts are still needed to maintain the stability of exogenously administered miRNA. Various nanoparticle-based delivery systems have been developed to improve the stability and specificity of exogenous miRNA delivery. In addition to nanoparticles, other delivery systems such as virus-based, lipid-based, polymeric, and extracellular vesicle-based delivery have also been developed [69], [70].

Antibody

Antibody is a natural anti-infective agent that can react with various antigens. Structurally, the antibody is a heterodimer protein with two heavy chains and two identical light chains that is connected by disulfide bonds. As a therapeutic agent for humans, antibodies can be engineered to be their humanized version, conjugated with other drugs, or modified with other techniques for other purposes [71]. Moreover, the development of therapeutic antibodies also involves some extensive engineering processes, so that some fragments of antibody, such as conjugation fragments, fusion protein, or bispecific antibodies can be used and replace the traditional antibody as a therapeutic agent [72].

Antibodies work by some different mechanisms. In general, antibodies can provide a neutralizing effect by binding to ligands or receptors on the cell surface, thereby blocking the target signaling pathway and resulting in the loss of cell activity. The bond between the antibody and the antigen can also recruit immune cells to lyse target cells or form complexes that can attack cell membranes, making antibodies widely developed for cancer therapy [73]. In the case of ischemic stroke, there are several alternatives for determining

the target of an antibody. For example, monoclonal antibodies can be designed to target inflammatory mediators, inhibitors of neuronal regeneration factors, acid-sensing ion channels (ASICs), and NMDA receptors that may contribute to the severity of ischemic stroke [74].

α2-plasmin inhibitor (α2AP) is a plasmin inhibitor that can form a covalent complex with plasmin, causing resistance to plasmin fibrinolytic activity against thrombus. In various studies on test animals, α2AP has been shown to worsen infarction that occurs after ischemic stroke attacks [75]. Therefore, one of the therapeutic strategies in ischemic stroke therapy is to create a therapeutic agent that can inhibit the activity of α2AP, so that it can accelerate the dissolution process of the blockage that forms after a stroke attack [75], [76]. One of the therapeutic agents that can inhibit α2AP activity is monoclonal antibodies. Research conducted by Reed *et al*. [77] has successfully developed RWR antibodies that are able to inhibit cross-linking between fibrin and α2AP, so that they can spontaneously break down blood clots and have synergistic activity with plasminogen activator agents such as urokinase and streptokinase. Furthermore, Reed [78] also identified 3 new monoclonal antibodies that also have α2AP inhibitory activity that can work on different epitope parts than RWR antibodies. These antibodies are currently being tested for their effects in a clinical setting, where clinical trials are currently being conducted in phase 2 [79]. However, the outcomes obtained from trials have not yet been available.

Other antibodies are currently being developed to treat ischemic stroke by targeting other targets. The L13 Antibody, developed by Ji *et al*. [53], shown to improve clinical outcomes in animal models that have been induced to experience ischemic stroke through a specific MMP-9 activity inhibition mechanism, thereby providing a protective effect on the integrity of the blood-brain barrier. Furthermore, the antibody also showed similar activity in *ex vivo* tests in humans. The L13 antibody itself was developed using a high-throughput method, the selection of which was carried out based on its functional ability to inhibit the activity of 4 different classes of proteases, where MMP-9 is included as one of the targets inhibited by this antibody [80]. Another antibody, the Anti-programmed Death (PD)-L1 antibody, in various preclinical tests has also been shown to improve clinical outcomes in animal models. These improvements are manifested in decreased infarct volume, partial reversal of splenic atrophy, improved neurological outcomes, and increased CD8+ and CD122+ levels as well as improvements in inflammatory markers characterized by decreased proinflammatory cell counts and increased interleukin-10 levels [54], [55], [56].

Although several antibodies have been tested in clinical settings, no antibody-based therapy has received FDA approval for use in stroke therapy to date. Several factors, such as poor uptake of antibodies into the central nervous system across the blood-brain barrier and delayed administration of antibodies, have contributed to the lack of successful clinical translation of antibody-based therapies. Several strategies have been developed to address this, such as improving antibody delivery to the central nervous system, either by using specialized delivery systems or by using antibodies that can interact with receptors on the blood-brain barrier, thereby increasing antibody penetration into the brain [81].

Stem cell

A stem cell is a unique cell type that has the capability to self-renew and differentiate into different cell lineages [82]. Based on their potency, stem cells can be classified as totipotent stem cells that can give rise to extraembryonic tissues and differentiate into all types of cells, pluripotent stem cells that can differentiate into the three germ layers, multipotent stem cells that can only differentiate to one type of germ layer tissue, and unipotent stem cell that can only differentiate to one type of specific cell [82], [83]. In the adult body, multipotent, oligopotent, and unipotent stem cells are distributed in the whole body and function in maintaining homeostasis and tissue regeneration [84].

A stem cell-based therapy is a treatment of medical conditions that involves the use of human stem cells [85]. Due to ethical reasons, the use of stem cells for clinical needs is only restricted to adult stem cells, in which various types of stem cells, such as mesenchymal, hematopoietic, neural, and dental pulp stem cells are included [84]. In its use for therapy, stem cells can come from autologous or allogeneic sources, where autologous stem cells use stem cells that come from the patient themselves, while allogeneic stem cells are stem cells that come from healthy donors which are then processed before being used for therapy [86].

The pathophysiological process of ischemic stroke involves various molecular mechanisms that ultimately cause cell death, so patients will experience nerve cell loss and brain damage [87]. Therefore, stem cell therapy can be given as an adjuvant stroke therapy agent because it can provide a regenerative effect to replace brain cells damaged by stroke and provide a paracrine effect that is useful in the cell regeneration

process. Stem cells can also provide the effect of changing synaptic plasticity and reorganizing neural circuits [88], [89].

In recent years, as stem cell therapy has become more popular and affordable, many clinical trials have been conducted to test the safety and efficacy of stem cells. Autologous stem cell therapy, which can be derived from adult multipotent stem cells or from somatic cells that are then reprogrammed into pluripotent stem cells, has the advantage of immune system compatibility between donor and patient. This compatibility also eliminates the need for recipients to take immunosuppressive agents, which are often a problem in the transplant process [90]. A meta-analysis conducted by Hassani *et al*. [91] showed that the administration of autologous stem cells to ischemic stroke patients did not have a damaging impact, so the safety profile was good enough to be given to stroke patients. However, in terms of efficacy, the improvement in the clinical outcomes of the clinical trial subjects was not significant when compared to the clinical outcomes shown by the control group.

Allogeneic stem cells, on the other hand, are obtained from the person who donated the cells and then transplanted to other recipients. This type of stem cell has also begun to be widely tested as a therapeutic agent for ischemic stroke in clinical settings. As is the case with autologous stem cell therapy, in the phase 1 and 2 clinical trials that are conducted by Levy *et al*. [57] and Steinberg *et al*. [58], they showed that the allogeneic stem cells that are given to ischemic stroke patients did not give detrimental effect for the clinical trial subjects. The administration of stem cells also caused functional improvements in stroke patients who were in the chronic phase, which is usually characterized by neurological deficits. However, the clinical trial needs to be continued in the next stage with a more stringent study design involving a control group, so that the assessment of the efficacy of stem cell administration can be more reliable. In another clinical trial, a phase 3 clinical trial conducted by Houkin *et al*. [59], it has been shown that allogeneic stem cell administration has not been able to provide significant improvements in clinical outcomes in the short term. A longer period is needed to be able to conduct a more comprehensive assessment of the clinical benefits of stem cells for stroke indications. To date, there is no allogeneic stem cell product that has received marketing approval from the FDA for stroke therapy. Thus, it will take more time to assess the efficacy of stem cell therapies for stroke treatment.

▪ CONCLUSION

Stroke therapy has entered a new era, where some biotechnology-based therapeutic agents such as novel tissue plasminogen activators, therapeutic peptides, microRNA, antibodies, and stem cells have been widely tested in animal models as well as clinical trials. Despite only very few of the drugs that have received approval from regulators, the development and advances in the field of biotechnology will eventually drive those therapies to be widely used as adjuvant therapy for stroke patients, which is not only given to control the disease progression but also to cure and give the regeneration effect for stroke survivors.

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