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Developing Cellular Therapies for Stroke

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Stem Cells; Stroke; Translation

Developing therapies using cells rather than drugs

Cell therapies refer to the use of cells or cellular material that exert a treatment effect in animal models of disease. Over the past 15 years, a range of different types of cell therapies have emerged as potential new treatments that are under development for stroke and other neurological disorders. The past quarter century has witnessed the success of thrombolytic therapy and the failure of neuroprotective agents for acute ischemic stroke. These therapeutic strategies often target a single pathway (tissue plasminogen to dissolve clots in the former case and specific biochemical pathways of injury in the latter case). Cell therapies represent an entirely different biopharmaceutical approach. In stroke, there are two broad goals for the therapeutic application of cell-based therapies. The first is to transplant cells, usually by direct injection to the brain, in order to graft and create new tissue that may restore neuronal connections damaged or lost in a stroke. In the 1990s, several groups of investigators initially pursued this goal to transplant grafted cells in patients with chronic stroke^{1, 2}. However, cell transplantation with the intention of engraftment is likely going to require extensive pre-clinical work to address several biological challenges that many investigators are actively pursuing. Those challenges to use cells and recreate lost circuitry after a stroke are well-described by Dihne³. The second goal, which is the focus of this review, has more immediate clinical applications in which exogenous cells administered to the body cause a range of paracrine and immunomodulatory effects, the end result of which leads to a reduction in secondary injury processes and stimulation of brain repair after stroke (Figure 1).

Different cell types and Definitions

The types of cell therapies under development range from embryonic/fetal sources (embryonic stem cells; neural stem cells) to birth related tissues (umbilical cord, placenta) to adult sources (bone marrow, blood, adipose, dental, skin). In this review, various cell types will be discussed. Some cell types are actual multipotent stem cells (e.g., mesenchymal stem cells, multipotent adult progenitor cells) that can give rise to various cells of different lineages. Other cell types are more tissue specific stem cells such as neural stem cells that

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differentiate specifically into the different cell types of the brain. Still other cellular preparations undergoing testing for stroke are mixed cell types that contain stem cells but also contain more mature cell types (e.g., the mononuclear fraction of bone marrow or mononuclear fraction of umbilical cord blood). It is therefore preferable to use the term “cell therapy” or “cellular therapy” rather than “stem cell therapy” to avoid confusion. The first cell therapies for systemic delivery that have moved forward to clinical trials in stroke are bone marrow cells derived directly from patients (autologous) such as bone marrow derived mononuclear cells or marrow stromal cells⁴ Other investigators are pursuing the autologous application of such cell types derived from adipose tissue or peripheral blood. The rest of the cell types that are in clinical trials are more purified cell populations, isolated from various tissues and then manufactured by passage in culture, for allogeneic administration (e.g., marrow stromal cells, adipose stromal cells, etc.). Some allogeneic cell types (e.g., marrow stromal cells - MSCs) can be given without the need for immunosuppressive agents because they may not elicit a clinically significant immune attack; interestingly, because of their immunomodulatory effects. MSCs are an approved treatment for graft vs host disease.

Mechanisms

Understanding how various types of cell therapies improve stroke outcome has been the subject of intense investigation for over a decade. Overall, it is important to emphasize that the majority of cell therapies under active investigation for clinical applications in stroke do not involve transformation of exogenous cells to new functional neurons leading to cell replacement. The majority of studies in the past few years on cell therapies for stroke are converging around the hypothesis that cells release biological factors impacting the inflammatory response, secondary degeneration, and brain repair processes. Whether the cell types are mixed cell populations or more homogenous populations, whether the cell types are neural stem cells or from bone marrow, and whether the injection route of delivery is direct intracranial or intravenous, it is likely that the biological factors released from exogenous cells and/or their cell to cell contacts with host cells are the key mediators of action on the brain and other organs. However, we know little about which specific secreted factors and cell to cell interactions are pivotal to the underlying mechanisms involved in recovery.

Inflammation

Stroke leads to a brisk inflammatory response involving both peripheral and central mechanisms.⁵ Inflammatory cells and cytokines from peripheral organs circulate to the brain and contribute to secondary injury. Within the brain, a series of orchestrated events involving the neurovascular unit exacerbate damage and threaten surviving neurons in the periphery of an infarct. However, some aspects of neuroinflammation are beneficial to the brain and are critical to recovery processes.⁵ A range of cell therapies have been shown to modify different aspects of the inflammatory response after stroke in such a way that pro-inflammatory detrimental pathways are downregulated and anti-inflammatory pathways are upregulated. Microglia/macrophages appear to be one of the principal cell types in neuroinflammation that are modulated by cell therapies.^{6–8}

Biological repair/plasticity

Brain repair after stroke involves a number of different inter-connected processes. The most common aspects of brain repair that many types of cell therapies have consistently been shown to enhance are the endogenous formation of new neurons (neurogenesis), blood vessels (angiogenesis), and synapses (synaptogenesis) in the areas around the infarct⁹. In addition, there are studies showing that intra-parenchymal transplantation of some types of neural progenitor cells differentiate into new mature neurons that elaborate new axonal connections¹⁰. Overall, it is likely that cell therapies upregulate a combination of repair mechanisms, rendering the isolation of individual pathways difficult to decipher. It also remains unclear which factors are the critical ones cell therapies release that enhance repair processes.

Intravenous Administration

One of the most intriguing areas in the field is understanding how intravenously administered cell therapies impact brain inflammation and repair. Over 10 years of research consistently has shown that various cell types by IV injection improve neurological outcome in stroke, upregulate brain repair, and/or reduce neuroinflammation. However, it is unlikely that cultured cell types such as MSCs enter the brain in sufficient quantities because MSCs have cell sizes that are larger than the sizes of pulmonary capillaries and, as a result, it is not surprising that intravenous delivery leads to trapping of MSCs in the lungs¹¹. Nevertheless, MSCs, similar to many other cell therapies given IV, exert profound effects on the CNS in various models of neurological disease.¹² It has even been shown that umbilical cord cells do not need to enter the brain to exert a therapeutic effect¹³ and an intravenous delivery of these cells leads to a greater effect on stroke recovery compared with intra-cranial delivery¹⁴.

The bioreactor hypothesis

The laboratories of Eva Mezey and Darwin Prockop provide some of the first findings that may elucidate a few of the mechanisms involved. Mezey's group found that MSCs IV increase survival and improve organ function in a mouse model of sepsis by migrating to the lungs and reprogramming endogenous macrophages to release the anti-inflammatory and neurotrophic cytokine, IL-10.¹⁵ Prockop's group then found that IV administration of MSCs in rodent models of myocardial infarction and corneal transplantation embolize to the lung where they then upregulate the anti-inflammatory protein TSG-6.¹⁶ TSG-6, among other effects, is known to inhibit neutrophil migration from the circulation. In a different line of investigations, other laboratories are showing that some types of cell therapies exert treatment effects in the brain by modulating the spleen, which contracts and contributes to the inflammatory response after acute brain injuries.¹⁷ Allison Willing's laboratory found that human umbilical cord cells migrate to the spleen, prevent splenic contraction, and alter the inflammatory behavior of the spleen, reducing its production of IFN- γ and increasing the production of IL-10.¹⁸ In our laboratory, we have also found that multipotent adult progenitor cells (Multistem manufactured by Athersys), an adherent bone marrow derived cell therapy, modulates the inflammatory response of the spleen after stroke and that the spleen is a pivotal target of action for Multistem to enhance stroke recovery.¹⁹ Even neural

stem cells administered IV reduce splenic inflammatory processes leading to an overall reduction in brain injury and cerebral edema in a model of intracerebral hemorrhage.²⁰ Collectively, these studies and others, confirming the same effects of cell therapies in traumatic brain injury,²¹ suggest that cell therapies administered IV may modify host cells within the lungs, spleen, and other lymphoid tissues to promote an anti-inflammatory, pro-regenerative environment for the brain. Further studies are needed to understand whether the changes within the spleen are observational or pivotal to the mechanisms underlying how certain types of cell therapies enhance stroke recovery.

Microvessicle Hypothesis

Emerging research on microRNAs has recently provided a new insights how some types of cell therapies exert beneficial effects by intravenous delivery. MSCs release microvessicles such as exosomes that contain genetic material including microRNAs. Exosomes can enter other cells where they release microRNAs which then can direct gene expression of the host cell. Exosomes therefore can facilitate inter-cell communications. Their submicrometer size allows them to pass through the lungs and even the blood brain barrier into the brain parenchyma. New studies now even suggest that exosomes derived from intravenously delivered MSCs can enter the brain and promote repair after stroke²² and by themselves exosomes administered IV to rodents enhance stroke recovery²³.

In reviewing these mechanistic studies, several thematic questions arise: (1) which of the multiple and broad mechanisms associated with different types of cell therapies are critical to their treatment effects in animals? This issue has raised concern among some scientists that the field is hampered by a lack of definitive studies identifying what are the critical, causative effects of cell therapies that lead to stroke recovery. Studies that include experiments that block specific mechanisms are emerging and highly encouraged²⁴; (2) do most cell therapies under current development for stroke cause a broad range of similar effects regardless of cell type or source and regardless whether the cellular therapy is composed of various cell types or is derived from a more purified homogenous cell culture, i.e., are there any unique mechanisms per cell type?; (3) if biological factors are the principal mediators underlying the effects of most cell therapies, are the factors the more important area to pursue, rather than the cells. If we can identify the critical factors, they would conceivably be easier and safer to administer than cells but which ones and in what proportions?

Other Delivery Routes: Intra-arterial and Intracranial

While the foregoing discussion has concentrated on intravenous delivery, there is also intense investigation to more selectively deliver cells to the brain by intra-arterial injection mostly involving carotid injections²⁵. Intra-arterial delivery carries the potential advantage of selectively targeting cells to specific brain regions using a lower number of cells in a more concentrated volume but may pose risk for microvascular plugging and subsequent ischemic injury²⁶. Designing safe protocols to prevent embolic complications will require addressing several variables including propensity to form aggregates, cell size, infusion rate, needle technique, and the extent of pre-existing arterial disease in prospective patients.^{27, 28} The most direct delivery routes involving stereotactic injection to the brain have been taken

forward to clinical trials for a smaller, select group of cell therapies in patients with chronic stroke discussed below.

STEPS in translation

Many investigators in academia and industry who develop new treatments for acute stroke follow a blueprint provided by the STAIR guidelines²⁹ which calls for testing in different stroke models, both genders, young and aged animals, and animals with co-morbidities. In addition, the guidelines call for defining an optimal dose and therapeutic window. Different therapeutic approaches are taken forward to clinical trial based upon fulfillment of some or all of these recommendations. Following the same STAIR format, a specific set of guidelines have been developed for Stem Cells as an Emerging Paradigm in Stroke (STEPS)³⁰. In addition to embracing the same general guidelines as STAIR, the STEPS recommendations also suggest for any cell therapy platform: (1) a full characterization of the cell type(s), (2) biodistribution studies in stroke models to monitor the migration and fate of injected cells; and (3) studies to identify the optimal delivery routes. Both STAIR and STEPS call for rigorously designed studies that involve randomization of treatments, blinding of outcome assessments, and sample size calculations. It is unknown if fulfillment of these guidelines will lead to positive results in clinical trials but the recommendations serve as guidance to those who wish to pursue or participate in this area of investigational stroke therapies.

Early stage clinical trials

As we learn more about the basic science of cell therapies, several small clinical trials have proceeded since the 1990s when Kondziolka et al.,³¹ implanted the first human cells stroke in a Phase I study. Most of the cell therapies that have been taken forward to clinical trials have been supported by at least some pre-clinical animal experiments. The most studied cell therapy for stroke taken forward to clinical trials supported by published preclinical data is autologous bone marrow mononuclear cells. The translational development of MNCs as an investigational treatment for ischemic stroke was recently reviewed in *Stroke*.³² Our group completed a feasibility/safety study on the IV administration of MNCs in patients with acute stroke.³³ Other investigators have reported the safety of autologous MNCs administered intra-arterially in subacute and chronic stroke.^{34,35} More selected populations of cells within MNCs have also been tested in small studies including CD34 cells³⁶ and aldehyde dehydrogenase bright cells. There are several challenges to conduct these trials: (1) patients have to undergo a bone marrow harvest; (2) for randomized controlled studies, there is no consensus on the appropriate sham procedure, and (3) intra-arterial delivery poses additional safety concern given that some types of cells (e.g., MSCs) administered IA can cause microvascular obstruction and ischemic injury. Another cell type that has been extensively studied in pre-clinical models is marrow stromal cells (MSCs). Autologous MSCs, prepared from the bone marrow harvest of individual patients, do not pose serious adverse events in various small stroke studies when the cells are administered IV.^{4, 37, 38} Larger two arm randomized trials are underway but the timing of administration is at least several weeks after stroke onset because their manufacture requires several passages in cell culture. This potential concern may be offset by using allogeneic MSCs in a shorter time frame after

stroke. In fact, the first phase II randomized placebo controlled trial testing an allogeneic bone marrow adherent stem cell – multipotent adult progenitor cells APCs (Multistem) in patients with acute stroke has just completed.³⁹ Lastly, there are two major active cell therapy platforms involving the intracranial delivery of neural stem cells (ReNeuron) and allogeneic MSCs (SanBio) in patients with chronic stroke.

Challenges of conducting cell therapy trials

Translating cell therapies to stroke patient populations involves a number of logistical challenges depending on the cell type.⁴⁰ Testing autologous bone marrow cells requires extensive infrastructure: a GMP facility to manufacture the cells, a monitored setting to conduct the bone marrow harvest under sedation and then to oversee the infusion, and processes to ensure purity and sterility of the cells. Multidisciplinary teams often include a hematologist and a critical care specialist. If preclinical studies support the superiority of an intra-arterial vs intravenous delivery or an intra-cranial delivery is preferable, these procedures require further resources. The use of allogeneic cells IV is far less complicated since these cell types are often frozen “off the shelf” products immediately available for testing. However, even allogeneic cell types may have detailed SOPs for thawing and re-animation prior to infusion. In addition, there are several manufacturing challenges to produce a stable, reproducible cell product in sufficient quantities for long term supply of allogeneic products. Furthermore, for allogeneic intracranial studies, immunosuppressive agents are often used which does pose some risk for adverse events.

Pivotal issues that will facilitate successful translation

While a wealth of pre-clinical data supports the clinical testing of various cell therapies for stroke, the field faces several translational barriers. We need studies to begin verifying in patients the mechanisms discovered in animals. Therefore, as clinical trials advance to two arm randomized studies, it is important to measure intended biological effects.

Blood Biomarkers

Serial blood studies, for example, would hopefully be useful to measure growth factors, cytokines, and perhaps microvessels as the very mediators underlying the treatment effects of intravenously administered cellular therapies. Similarly, immunophenotyping specific immune cells would be appropriate for certain cell types where immunomodulation plays a critical role in pre-clinical models. Measuring spleen size may be another surrogate marker to consider for certain cell types as we have shown that the spleen of patients likely reduces in size after stroke⁴¹ and certain cell types can restore or preserve splenic mass.^{18, 19} All of these approaches will need further testing to determine if any of them could serve as surrogate markers of the biological effects of cell therapies.

Neuroimaging Markers

Advances in neuroimaging should be harnessed more to study potential brain targets of cell therapies. For example, in animal models of cerebral ischemia, MNCs can reduce white matter injury⁴² while MSCs have been shown to promote white matter remodeling.⁴³ Diffusion tensor imaging, which can provide matrices to assess the integrity of specific

white matter tracts, could be applied to validate these findings in patients. While it is not possible yet to visualize neurogenesis or angiogenesis, it is possible to measure neuroinflammation at the level of microglia and a variety of cell therapies have been shown to reduce microglia. Activated microglia express a cholesterol transporter protein (TSPO) on the outer mitochondrial membrane. TSPO labeled with positron emitting isotopes can be used to image activated microglia in the brains of patients with various neurological disorders. In patients with stroke, Thiel et al, using serial PET found activated microglia around the infarct and in areas remote from the infarct in the brainstem.⁴⁴ Microglial imaging could become one of the first imaging modalities to explore the hypothesis that impacting microglia is an important target for certain types of cellular therapies.

Cell Labeling

Another major translational barrier is developing safe and reliable cell labeling techniques for cell tracking. In animals, various techniques are used to label injected cells to track and monitor in the body. In patients, there are very few studies. SPECT imaging has been used in stroke patients to monitor bone marrow MNCs labeled with technetium-99. Labeled cells accumulate in the cerebral hemisphere of the infarct, and in the liver and in the spleen.⁴⁵ Other strategies under development include 18-fluorodeoxyglucose-PET, 111-indium-SPECT, and superparamagnetic iron oxide (SPIO)-MRI. There are extensive regulatory issues from animal studies to clinical trials that are needed to develop labeling techniques. For each approach, it is important to assess if the label affects cell viability, functionality, and migration/motility. There is also the concern for transfer of the label from dying cells to host cells and long term stability vs degradation of the label. Some labels may even be extruded from cells by exocytosis.⁴⁶

Conclusion

The field of cell therapy for stroke is advancing slowly as we gain more valuable insights about their effects on both peripheral tissues and the brain in animal models. As more studies support the safety of various types of cell therapies, phase II trials need to include biomarkers to investigate and validate biological effects before proceeding to pivotal efficacy trials.

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Figure 1.

Temporal Windows and mechanistic targets for Cell therapies in stroke

Various temporal windows can be envisioned that provide different mechanistic targets for cell therapies in stroke.