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Stroke

Research is a dynamic activity that creates new ideas. It provides a forum for generating observations and testing why they occur. Because people and their diseases are so diverse, clinical trials are the ONLY WAY it is possible to test whether new ideas about how to diagnose or treat human disease will work. But the process of taking research from bench to bedside is a lengthy one and demands not only vision but also years of teamwork and dedication on the part of scientists, physicians and patients. This document presents basic information about stroke and frames the context for the discussion that follows about how lessons learned from stem cell research may help fight this disease. Readers may also wish to peruse additional web resources or speak with their physicians for more information about this disease.

About Stroke

Stroke afflicts one Canadian every 10 minutes. It is a leading cause of adult disability worldwide and the second highest cause of death in the world. Stroke currently afflicts 15 million people annually and the numbers are expected to rise. This trend is due primarily to the aging population but the numbers are also increasing in the middle-aged population at an alarming pace. The communal burden of stroke is enormous because one in five stroke victims die and another third are left with permanent disabilities that inevitably limit their independence.

Stroke is a vascular syndrome of the brain. It is called a cerebrovascular disease, meaning that it involves both the brain (cerebro-) tissue and the blood vessels (vascular) within the body. There are two broad types of stroke – ischemic stroke (85% of all strokes) and hemorrhagic stroke (15% of all strokes). Ischemia means a lack of blood flow to the tissues, and in strokes this occurs when a blood clot interrupts the flow of blood to the brain for more than a few seconds. When this happens, multiple cell types in the brain – neurons, glia, and endothelia – are starved of essential nutrients and oxygen. Sometimes the situation may resolve itself very quickly and the blood flow to the brain is restored. In these cases, individuals suffer only very brief periods of neurological deficit and the stroke is termed a transient ischemic attack (TIA). When reduced blood flow lasts longer – for minutes – brain cells supplied by the blocked vessel begins to die. The core zone of dying tissue is called an 'infarct" where neurons are dying at a rate of 1.9 million per minute. The most severe from of ischemic stroke is a fatal malignant brain infarction.

Hemorrhagic stroke is of two types. Intracerebral hemorrhage (~7-8% of all strokes) is primary bleeding into the brain tissue. This occurs due to rupture of a brain artery. Sub-arachnoid hemorrhage (~7-8% of all strokes) is caused by a ruptured brain aneurysm (a weakening of the artery wall that causes it to balloon out). Bleeding in sub-arachnoid hemorrhage occurs in the space between the brain and the meninges (layers of connective tissue covering the brain and spinal cord), called the sub-arachnoid space. Hemorrhagic forms of stroke have approximately double the mortality of ischemic stroke.

Regardless of the type of stroke a person experiences, immediate medical attention is required to stem the tide of damage to the brain. A computerized tomography (CT) scan is routinely used to determine the type of stroke a person has suffered – ischemic or hemorrhagic – and helps physicians evaluate if anything can be done to prevent or mitigate chronic functional impairment.

Stroke survivors suffer from various degrees of disability depending on which part of the brain is affected and the degree of damage that ensues. The aftermath for patients may result in long term hospitalization, extended nursing care, rehabilitation, lost income, lost independence, and protracted responsibilities for caregivers. These demands not only take a huge toll on the individuals who have suffered the stroke but also place a heavy burden on their families

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and the health care system.

Causes

When blood flow to the brain is interrupted, a cascade of events triggers the death of the brain tissue normally fed by the blocked blood vessel. Shortly after a stroke occurs, neurons become over-stimulated, their calcium levels skyrocket and they become stressed from oxygen deprivation. These events lead to the death of brain tissue located at the infarction. As time progresses, the blood-brain barrier is compromised, inflammation sets in and the tissue surrounding the infarct, called the penumbra, also begins to die.

The risk factors for stroke are well known. High blood pressure is the strongest risk factor for both ischemic and hemorrhagic strokes. High blood pressure, atrial fibrillation (irregular heartbeat), diabetes mellitus, smoking, and carotid artery narrowing are other important risk factors. High blood cholesterol is a weak risk factor for stroke; in contrast, elevated cholesterol is a strong risk factor for heart attack. Lifestyle factors – exercise, diet (sodium and fat intake), alcohol intake – are critical components of stroke risk. Although strokes can happen at any age the risk tends to increase as we age. An individual's risk also escalates if either of the parents developed stroke by the age of 65. In terms of preventive measures, recommendations include a diet low in fat and sodium, controlling blood pressure, maintaining a healthy weight, and regular exercise.

Symptoms and Treatment

Stroke is a medical emergency. The symptoms are not difficult to recognize however, and with prompt treatment disability can sometimes be avoided or minimized. The key warning signs are sudden weakness, sudden trouble speaking or understanding speech, and sudden loss of vision. However, other symptoms may occur depending upon what part of the brain is affected; these can include imbalance, incoordination, double vision, and severe, sudden onset headache.

The most effective treatment for ischemic stroke is one that can restore the flow of blood to the brain. Drugs that can achieve this are called thrombolytic because they lyse or break apart the blockages in blood vessels. Sadly, death and disability are common because current treatments are not curative, and the window of opportunity to use thrombolytic interventions to restore the flow of nutrients and minimize downstream damage is very short. For a minority of patients, the clot-busting enzyme tPA (tissue-type plasminogen activator) may help but only if it is administered within the first 4.5 hours of stroke. The lack of comprehensive systems to get patients rapidly – within the 4.5h window - to the right medical care limits the widespread use of tPA. Unwanted side effects may also occur from thrombolytic drugs, and in the case of treatment with tPA there is an increased risk that patients will develop a hemorrhage or bleed in the brain.

Beyond acute interventions such as tPA, other treatments may also be employed in an attempt to improve the outcome for stroke patients. These include surgical techniques to open blocked arteries, blood-thinning anti-platelet agents (e.g., aspirin), and anticoagulants to stop clotting (e.g., heparin). Neuroprotective drugs (e.g., NA-1) are also being designed with a view to protect neurons by sopping up tissue-destroying free radicals that accumulate during stroke. Clinical trials have thus far not demonstrated a great benefit of any of these drugs, and therapies combining neuroprotective agents with thromobolytic drugs are currently being explored.

For stroke survivors, the road to recovery is a long one. Depending upon which of the two sides of the brain is damaged, all sorts of brain functions may be affected – the ability to remember, make decisions, speak, move muscles, reason, do simple calculations, control bodily functions and emotions, understand directions, take in new information, and read and write. One of the most common outcomes of stroke is weakness or paralysis on one side of the body. Because the damage is localized to particular areas of the brain, rehabilitation strategies are also quite specific for recovering certain functions. Physical, occupational and speech therapy have helped many people restore some level of functioning by teaching the brain to compensate for the neurons lost in the damaged areas. It is clear from the absence of universally effective treatments for stroke, that newer therapies that repair damaged tissue and promote recovery after stroke are urgently needed.

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How can stem cells help?

Two main strategies underpin the exploration of stem cells and the cells they make, also known as progeny, as potential therapies for stroke. The first is endogenous (meaning inside the body) repair. The idea behind endogenous repair is to stimulate stem cells that are already present in the brain to heal damaged tissue. The second strategy is exogenous (meaning outside the body) transplantation where stem cells are harvested, purified and then partially or completely differentiated prior to being transplanted into patients.

Using stem cells to actually replace brain cells lost during stroke is a very long-term goal and will be no simple feat given that multiple cell types in the brain are destroyed, and transplanted cells will need to integrate and re-establish neural pathways to restore function in the damaged parts of the brain. Scientists predict that it will take at least 5-10 years of hard work to determine if stem cell therapies can significantly improve the outcome for stroke victims. In the more immediate future, it is hoped that stem cell therapy may be used to extend the window of opportunity for using neuroprotective drugs which salvage ischemic tissue, limit infarct size, prolong the window for therapeutic thrombolysis and minimize inflammation or reperfusion (damage to brain tissue that sometimes occurs once blood flow to the area is restored) .

Research directions

Endogenous repair

Until recently, it was believed that once brain functions were lost they could never be regained. It was not until researchers and clinicians began to document that the brain could reorganize itself and compensate for disease, surgery and injury that there was a significant change in thinking. Exercise, hormones and an enriched environment are thought to facilitate recovery, possibly via the brain establishing new pathways and retraining the surviving brain cells to compensate for the damage.

In 1992, Canadian researcher Samuel Weiss discovered that the brain has its own store of stem cells. These are “hidden,” as if in reserve, and much current research since then has been devoted to figuring out how to coax these cells into repairing the brain after stroke. High on the list of candidates for study are growth factors and their downstream signaling pathways. Studies have shown that neural precursor cells (stem cells and their progeny) will proliferate and differentiate into more mature cells in response to growth factors, many of which continue to be tested in rodent models of stroke. Although the results thus far have been positive in that neural function in test animals improved, scientists are still trying to understand if the benefit resulted from the growth of new cells or from some other neuroprotective effect of the growth factors themselves.

Scientists are also looking to mobilize other endogenous stem cells to come out of their hiding places in the body and help with the aftermath of stroke. They have found that the growth factor G-CSF can mobilize hematopoietic stem cells from the bone marrow to enter the bloodstream. From the blood, the cells track to the areas of brain damaged by stroke. In rodent models, this process leads to functional recovery and the formation of new neurons and blood vessels. The precise reason for this is still not totally understood, but it is clear that hematopoietic stem cells are not making new neurons. Instead, the positive effects of the growth factors secreted by hematopoietic stem cells are thought to sustain neurons that would otherwise have died and also stimulate the production of new blood vessels. Another possibility yet to be proven is that the secreted growth factors can “switch on” endogenous neural precursors in the brain to make new neurons. Clinical trials testing the ability of G-CSF to promote recovery in stroke are underway.

Canadian researchers are tackling the problem of drug delivery for stimulating endogenous repair of damaged brain tissue. Current delivery methods – via the blood or nasal cavity – are less than satisfactory. Less than one per cent of the drugs administered via these routes are able to cross the blood-brain barrier, which means that the high doses of drugs required become toxic to the body. Other techniques for delivering drugs directly into brain are quite invasive and so researchers set about devising a minimally invasive technique to deliver drugs such as erythropoietin, which can stimulate endogenous neural precursors to contribute to tissue repair after stroke. Their solution is a hydrogel delivery system called HAMC (hyaluronan and methylcellulose). It can be injected onto the surface of the brain and is biodegradable once its job is done. Preliminary experiments have shown that this way of delivering EPO leads to neuroprotection and neurogenesis in mouse models of stroke and further, helps to control local inflammation. This technology may also prove to be a very useful tool for delivering drugs to other diseases of the central nervous system.

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Exogenous repair

The exogenous repair strategy harnesses the power of stem cells from a different angle than the endogenous strategy. Rather than stimulating resident stem cells into action, stem cells are first harvested from patients or donors, and then manipulated in the lab prior to being transplanted into a patient. The harvested stem cells can be purified and expanded in number and partially or completely differentiated into progeny of the type required to repair damaged tissue. Examples of the types of stem cells currently being investigated are neural stem cells (from adult or embryonic sources), mesenchymal stem cells, olfactory ensheathing cells, and endothelial progenitor cells (EPCs). One of the inherent challenges with the exogenous transplantation approach is deciding on the best method – intracerebral, intravenous, or intraarterial – to deliver the different types of stem cells. Once transplanted into the body, ensuring stem cell survival becomes the next challenge. One novel way to improve the outcome is to first grow the stem cells in the laboratory on 3-dimensional scaffolds that mimic the natural microenvironment in the body and then transplant them into the stroke injury site.

Neural stem cells

Nearly a decade ago, Canadian researcher Samuel Weiss at the University of Calgary kindled great excitement in the field when he discovered neural stem cells in the adult mammalian brain. His work showed that adult neural stem cells in mice are active throughout life and can generate all three kinds of brain cells in vivo: neurons, astrocytes and oligodendrocytes in response to injury. This discovery opened the door for the possibility of repairing neurological damage by transplanting neural stem cells or their progeny.

Neural stem can be readily isolated and grown into colonies of neural precursor cells and tested for their ability to promote healing in animal models of stroke. In the studies done to date, it is still difficult to equate the functional recovery observed in the animals with new neurons derived from the transplanted neural precursors or with other effects such as cell protection, control of inflammation or formation of new blood vessels.

Industry is helping to advance the application of neural stem cells to stroke. In 2010,The UK company ReNeuron began recruiting patients into their PISCES trial (Pilot Investigation of Stem Cells in Stroke). This European trial is the world's first fully regulated clinical trial for a stem cell therapy to treat stroke patients and operates on the premise or exogenous repair of damaged neurons. The trial is testing the ReN001 stem cell therapy which is a genetically engineered neural stem cell line called CTX derived from 12 week old fetal tissue. One of four different doses will be injected intracranially into 12 male patients over 60 years of age who have suffered stroke, but who are physically stable. The trial will follow patients for a minimum of two years and will take the lessons learned into subsequent studies. As of January 2013, no adverse side effects have been reported in the first five patients treated.

Stem Cell Therapeutics is a Canadian biotechnology company devoted to using the patient's own stem cells to treat central nervous system diseases. Their patented drug, Regenesis (NTX-265) combines human chorionic gonadotrophin with erythropoietin to increase the number of neural stem cells (NSCs) in the brain. This drug proceeded to a 96-patient phase IIb clinical trial, which in the end, was terminated. Although the company was encouraged by the substantial improvements in patients based on the National Institute of Health Stroke Scale, they were confounded by the greater improvement observed in the placebo group. Despite this setback, Stem Cell Therapeutics has not given up, and is re-evaluating the placebo group and measuring other secondary endpoints in the hopes of better understanding how to take the results forward.

Mesenchymal stem cells

Researchers are excited about the early results that use mesenchymal stem cells (MSCs) in animal models of stroke. These stem cells are located in many different organs and tissues and can be found lining the blood vessels throughout the body. When transplanted in animal stroke models, MSCs are able to migrate to damaged areas, and improve neurological recovery. Scientists believe that the neuroprotective benefits from MSCs may originate from them releasing growth factors that promote neurogenesis, regulate the immune system and stimulate the formation of new blood vessels.

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In an effort to boost the beneficial effects of transplanted stem cells, researchers have also tried genetically modifying the cells to pump out factors known to enhance neural repair. Genetically modified stem cells, including neural stem cells, MSCs and olfacatory ensheathing cells (stem-like cells from the nasal mucosa) have demonstrated the ability to improve neurological function and also reduce infarct size in a number of different mouse models of stroke.

Encouraging results were recently published in a 5-year follow-up a randomized study that evaluated the benefit of mesenchymal stem cells in 52 patients with severe stroke. In the study, 16 patients received autologous (their own) mesenchymal stem cells and 36 patients were in the control group. The success of the trial lies in the fact that the treatment was safe and that there was a higher level of recovery in the treated group than in the control group. Although only conjecture, the research group hypothesizes that the transplanted MSCs may work by stimulating endogenous neurogenesis in stroke patients. They suggest that further pre-clinical and clinical studies will help to optimize the benefits of MSC transplantation, and that it would be a good idea to consider transplanting MSCs sooner after the initial stroke attack. Manipulating the blood-brain barrier, genetically modifying MSCs, and conditioning patients prior to the transplants may also help to boost the positive effects of MSC transplants. Although the number of patients treated in this trial was very small, one very interesting result is that the level of clinical improvement correlated with the amount of stromal cell-derived factor-1 (SDF-1) circulating in the brain. SDF-1 could perhaps become a useful predictor of which stroke patients may be most likely to benefit from MSC transplant therapy.

Endothelial progenitor cells (EPCs)

Another intriguing avenue of exploration involves endothelial progenitor cells (EPCs). These cells mature into endothelial cells that line all the blood vessels in the body. EPCs are important moderators of the process called angiogenesis where new blood vessels are formed. Preclinical studies are encouraging in that transplanted EPCs from umbilical cord blood have been found in newly formed blood vessels in animal models of ischemic stroke. The hope is that EPCs will be able to contribute to the formation of new blood vessels that supply ischemic tissue, and also minimize inflammation in the brain by restoring the endothelial lining of the blood-brain barrier that is compromised during stroke. There are a few clinical trials underway to test these theories but much more work needs to be done to test the safety and usefulness of EPCs for stroke victims. Future studies may also combine EPCs with small molecules that control the cell death that inevitably accompanies ischemic stroke.

Looking to the future

Clinical trials using stem cells to treat stroke are at a very early stage. The few trials that have been performed to date have enrolled only very small numbers of patients in a cautious effort to ensure patient safety. Early trials took place from 2000 - 2005 and involved the transplantation of human neural cells derived from embryonic stem cell lines. The absence of adverse effects in the patients treated and the presence of some motor improvement provided preliminary proof of the safety and potential efficacy of the treatment. Although subsequent studies using mesenchymal stem cells and autologous bone marrow stem cells showed no adverse effects, they failed to demonstrate any significant clinical changes for the patients enrolled.

Currently, there are many ongoing academic clinical trials from all over the world testing stem cell transplants for treating ischemic stroke. The majority use autologous bone marrow stem cells taken directly from the patient, or neural or mesenchymal stem cells.

It is clear from early trials that many variables will need to be optimized in order to translate the benefits observed from preclinical studies into safe and successful clinical trials. A few of the key parameters being investigated include the route of delivery of cells into the brain, the post-stroke timing after which the cells are transplanted, and the types of endpoints used to measure whether the therapies are working. One of the most challenging issues remains the incredible heterogeneity among stroke patients and how that may obscure the measurement of clinical efficacy observed in trials.

Going forward, clinicians and scientists may look to the STEPS program (stem cell therapeutics as an emerging paradigm for stroke), for recommendations on using stem cells and other cell therapies for stroke. STEPS is the cellular counterpart to STAIR (stroke treatment academic industry roundtable) which recommends how to translate drugs from the laboratory to the clinic. In the 2011 update of the STEPS recommendations, three areas that could advance

the field were highlighted: developing ways to label transplanted cells so that they may be safely tracked in human subjects; identifying markers that can reliably signal patient recovery status; and developing better imaging endpoints for phase II trials. These and other recommendations from the STEPS II update are intended to spur on the successful translation from bench to the bedside of stem cell therapies for stroke patients.

Web Resources

- Heart and Stroke Foundation: http://www.heartandstroke.com/
- National Institute of Neurological Disorders and Stroke: http://www.ninds.nih.gov/
- American Stroke Association: http://www.strokeassociation.org/
- World Health Organization: http://www.who.int/cardiovascular_diseases/resources/atlas/en/

Selected Reading List

- Glover L, et al. A Step-up Approach for Cell Therapy in Stroke: Translational Hurdles of Bone Marrow-Derived Stem Cells. Transl Stroke Res. 2012;3(1):90-98.
- Kalladka D. & Muir. K. Stem cell therapy in stroke: Designing clinical trials. Neurochemistry International. 2011;59:367-370.
- Mezey E. Bone marrow-derived stem cells in neurological diseases: stones or masons? Regen Med 2007;2(1):37-39.
- Muthuaian R, Minhas G, Anand, A. Pathophysiology of Stroke and Stroke-Induce Retinal Ischemia: Emerging Role of Stem Cells. J. Cell. Physiol. 2012;227:1269-1279.
- Savitz SI. Stem cells and stroke: are we further away than anyone is willing to admit? International Journal of Stroke. 2012;7:34-35.
- Savitz SI et al. Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) II. Stroke. 2011;42:825-829.
- Sutherland BA et al. Neuroprotection for ischaemic stroke: Translation from the bench to the bedside. International Journal of Stroke. 2012.
- The Editors. Circulation. 2012;125:e947-e955.
- Weschler LR. Intravenous Thrombolytic Therapy for Acute Ischemic Stroke N Engl J Med. 2011;364:2138-2146.
- Yu F. & Morshead CM. Adult Stem Cells and Bioengineering Strategies for the Treatment of Cerebral Ischemic Stroke. 2011;6:190-207.
- Yuanfei W et al. Hydrogel delivery of erythropoietin to the brain for endogenous stem cell stimulation after stroke injury. Biomaterials. 2012;2681e2692.