The potential of stem cell therapy for stroke: is PISCES the sign?

Helen K. Smith and Felicity N. E. Gavins
Wolfson Neuroscience Laboratories, Department of Medicine, Imperial College London, London, UK

ABSTRACT    Substantial developments in the field of stem cell research point toward novel therapies for the treatment of diseases such as stroke. This review covers the establishment of tissue damage in stroke and the status of current therapies. We evaluate stem cell therapy with respect to other treatments, including clinical, preclinical, and failed, and provide a comprehensive account of stem cell clinical trials for stroke therapy currently underway. Finally, we describe mechanisms through which stem cells improve outcome in experimental stroke as well as potential pitfalls this basic research has identified.—Smith, H. K., and Gavins, F. N. E. The potential of stem cell therapy for stroke: is PISCES the sign? FASEB J. 26, 2239–2252 (2012). www.fasebj.org

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In November 2010, a man in his 60s was the first to receive a novel therapy to treat his severe and unyielding neurological deficits caused by a stroke 18 mo previously. He was the first patient to be operated on in the Pilot Investigation of Stem Cells in Stroke (PISCES). PISCES is a phase I clinical trial involving the injection of fetal stem cells into infarcted brain regions of 12 patients, 6-24 mo after ischemic stroke. Although primarily concerned with assessing the safety of ReNeuron’s ReN001 neural stem cell line for clinical use, the trial is based on accumulated evidence, which allows the tentative proposition that this treatment may be successful. This review will cover evidence that supports this and other promising trials, the mechanisms through which stem cells appear to provide benefit, and the practical advantages and disadvantages of stem cell treatment for stroke in the clinic.

ISCHEMIC STROKE: ETIOLOGY AND PREVALENCE

Stroke is the third leading cause of death in the United Kingdom, behind heart disease and cancer, and claims 15 million lives worldwide each year, 70,000 of those in the United Kingdom (1, 2). Despite this high mortality, 76% of those having strokes survive, often with moderate or severe disabilities. The subsequent cost is felt widely: the direct annual expenditure on stroke in the United Kingdom is estimated to be £4 billion (5.5% of the total UK expenditure on health care), and the cumulative costs (which include “indirect” care: specialist care and income support) to be close to £9 billion (3). Treatment with “clot-busting” tissue plasminogen activator (tPA) administered within 3 h of the onset of ischemia is currently the only accepted treatment for ischemic stroke. The demand for alternative, more flexible, and clinically relevant therapies is clear from these figures and is reflected in the enormous body of work targeting the zenith of stroke research—a salvaged penumbra.

The vast majority of strokes (85%) are ischemic in nature and are characterized by the obstruction of major vessels or arteriole ends, preventing or reducing blood flow to the brain (4). The causes of the obstructions are most commonly a thrombus or embolus from the heart, myocardial infarction, or trauma. Each of these will produce an ischemic core that will become the focus of (currently) irreversible damage (Fig. 1). The core region is encapsulated by cells whose survival is dependent on multiple factors; this region is termed the penumbra, and the mechanisms influencing its condition are discussed in the next section.

MECHANISMS OF TISSUE DAMAGE IN ISCHEMIC STROKE

Pathological changes develop in stroke as reduced oxygen and variably reduced glucose supplies (with respect to proximity to the ischemic core) result in

1 Correspondence: Wolfson Neuroscience Laboratories, Imperial College Faculty of Medicine, Hammersmith Hospital Campus, Burlington Danes Bldg., Du Cane Road, London W12 0NN, UK. E-mail: f.gavins@imperial.ac.uk doi: 10.1096/fj.11-195719
necrotic and/or apoptotic cell death by a range of mechanisms (5). In summary, disruption of neuronal ion channels produces unmediated release of excitatory glutamate (5, 6), leading to immense calcium influx through N-methyl-D-aspartate (NMDA) receptors and voltage-dependent calcium channels. In addition, single-strand DNA breaks may cause rapid activation of the nuclear protein poly(ADP-ribose) polymerase, reducing intracellular concentrations of its substrate \( \text{NAD}^{+} \), thereby slowing cellular mechanisms of energy production (7, 8).

Early restoration of blood flow is crucial to save as much

Figure 1. Proposed sources and routes of administration for stem cell therapy in ischemic stroke. Also shown is the common position of human ischemic stroke (MCA) and the location of the penumbra with respect to the ischemic core.
cerebral tissue as possible. Despite this, reperfusion itself is thought to compound tissue damage and worsen patient recovery, as demonstrated in several animal models (9–11). Ischemia/reperfusion (I/R) injury is characterized by inflammatory processes: blood returning to an infarcted brain region carries a flurry of polymorphonuclear (PMN) and mononuclear (MN) leukocytes set to respond to gradients of chemotactic agents, such as interleukin (IL)-8 and monocyte chemotactic protein-1 (MCP-1). When they reach the microvasculature of an ischemic region, these leukocytes extravasate into the brain parenchyma in response to cell adhesion molecule (CAM) expression on vessel endothelia (the mechanisms of the inflammatory response are to be considered carefully; CAMs will be referred to later in conjunction with stem cell navigation). Here leukocytes typically release reactive oxygen species, matrix metalloproteinases (MMPs), and further inflammatory mediators. The deployment of such highly reactive components is primarily designed to tackle pathogens, and their presence in excess during a sterile immune response destroys tissue. Inflammation can eventually be resolved through endogenous anti-inflammatory mediators, such as glucocorticoid-induced calcium and phospholipid-binding protein, annexin A1 (AnxA1), and eicosanoid-derived lipoxin A4 (LXA₄). These promote nonphlogistic apoptosis and phagocytosis of leukocytes already within the tissue and the release of anti-inflammatory cytokines, such as IL-6 and IL-10. Endogenous resolution of inflammation is now accepted as an active rather than passive process, modulation of which may itself have therapeutic value (12, 13).

Throughout subsequent weeks and months, necrotic cells are cleared and scar tissue forms. The peri-infarct cortex appears to provide a neurovascular recess into which new neurons and vasculature are encouraged to grow (14). Endogenous neurogenesis (see Proposed Mechanisms of Functional Recovery after Stem Cell Therapy below) is increased as newborn cells migrate toward the infarct, differentiate into a requisite phenotype, and associate with remodeling vasculature [neurogenesis and angiogenesis are inextricably linked through production of stromal-derived factor 1 (SDF1) and angiopoietin 1 (Ang1) by the new blood vessels] (15). There is no evidence that this neurogenesis occurring at this stage provides any functional improvement; many neurons in the neurovascular recess either die or remain undifferentiated. The implication from cell transplantation studies is that the benefit of these cells is their neuro/immuno-modulatory activity through paracrine mechanisms.

This dynamic stream of pathological events through stroke suggests possible opportunities for intervening therapeutically, increasing cell survival, and aiding recovery from stroke. It is therefore reasonable to ask why effective therapeutic strategies actually in existence are not correspondingly abundant.

**CURRENT STROKE THERAPIES**

More accurately, this heading should read “current stroke therapy,” because success with the translation of potential stroke therapies from laboratory to clinic has so far been nonexistent, with the single exception of tPA. The good news is that the transforming pathological processes throughout stroke and during subsequent weeks, months, and years present several opportunities for intervention as the disease progresses and also suggest that the most effective treatment may be a combined therapeutic approach. Research to date has observed and tried to compensate for several of the pathological processes described in the previous section, and more than a thousand basic studies have reached clinical trial stage (16). A few key examples are trials with NMDA antagonists (17) and GABA potentiators (18), designed to block initial excitotoxic cell death; thrombin inhibitors, with the aim of secondary prevention (19); and ICAM-1 inhibitors (20), targeting the recruitment of leukocytes to endothelial walls during inflammation. As alluded to above, not one was successful. The reasons for these failures have been covered elsewhere in detail (21, 22), but the crux of the problem seems to be that results obtained using animal models are not sufficiently representative of results in the clinic, the necessary temporal restrictions on treatment administration in basic research do not practically lend themselves to the clinic, and there is poor observation of inclusion criteria outlined after animal studies, once a drug has reached trial stage. The resounding message from each of these concerns is that new studies and therapies should be designed with flexibility in mind to provide the best outcomes for the greatest number of patients.

On these bases, how do studies show that stem cells might be effective in clinical trials? Do the data from animal experiments give a true indication of what can be expected from trials? How does the prospect of stem cell therapy compare with previous failed attempts at a treatment for stroke—will it be more inclusive than the current gold standard, tPA? The following sections contain evidence from the last decade, which addresses these questions and suggests how stem cells might figure in future stroke therapies. In addition, Table 1 covers clinical trials currently putting this evidence into practice.

**EVIDENCE FOR EFFECTIVE STEM CELL THERAPY IN STROKE AND CARDIOVASCULAR DISEASES**

Funding for research involving stem cells from the NIH is expected to reach $2.4 billion (of approximately $31 billion available) in 2011 (23, 24). The California Institute for Regenerative Medicine has recently awarded 14 research groups based in California $230 million in the hope that each will submit a new drug for phase I clinical trial within 4 yr (25). In the United Kingdom, funding for stem cell therapy over the last 4 yr has quadrupled, with projected spending on stem cell research in 2011 at £72–88 million (26). There is confidence, therefore, that fetal, embryonic, adult (including induced pluripotent), and nonhuman stem cells have great potential for use in
<table>
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<th>Title of trial</th>
<th>Inclusion criteria</th>
<th>Intervention</th>
<th>Outcome/status</th>
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<tbody>
<tr>
<td>Intravenous stem cells after ischemic stroke (ISIS)</td>
<td>Acute carotid ischemic stroke (&lt;14 d); NIHSS 2–24; 18–85 yr; M + F</td>
<td>Intravenous injection of autologous MSCs &lt;6 wk after stroke</td>
<td>Recruiting (phase IIa)</td>
</tr>
<tr>
<td>Efficacy study of CD34 stem cell in chronic stroke</td>
<td>Ischemic stroke in MCA territory (6–60 mo); NIHSS 9–20; 35–70 yr; M + F</td>
<td>Intracerebral implantation of 2–8 × 10^6/patient autologous CD34^+ cells (hematopoietic progenitors), plus conventional stroke therapy^+</td>
<td>Completed (phase II)</td>
</tr>
<tr>
<td>Autologous bone marrow stem cells in middle cerebral artery acute stroke treatment</td>
<td>Acute ischemic stroke in MCA territory (5–9 d); NIHSS &gt;8; 18–80 yr; M + F</td>
<td>Intra-arterial implantation of autologous CD34^+ cells via MCA</td>
<td>Completed</td>
</tr>
<tr>
<td>A study of allogeneic mesenchymal bone marrow cells in subjects with ischemic stroke</td>
<td>Ischemic stroke (&gt;6 mo); NIHSS 6–20; &gt;18 yr; M + F</td>
<td>Intravenous injection of 0.5–1.5 × 10^6/kg autologous MSCs &lt;6 wk after stroke</td>
<td>Recruiting (phase I/II)</td>
</tr>
<tr>
<td>Autologous bone marrow stem cells in ischemic stroke</td>
<td>Acute total anterior circulation ischemic stroke (&lt;7 d); NIHSS severe; 30–80 yr; M + F</td>
<td>Intra-arterial implantation of autologous CD34^+ cells via MCA</td>
<td>Unknown (phase I/II)</td>
</tr>
<tr>
<td>The variation of movement related cortical potential, cortico-cortical inhibition, and motor evoked potential in intracerebral implantation of autologous peripheral blood stem cells (CD34) in old ischemic stroke</td>
<td>Ischemic stroke in MCA territory (0.5–5 yr); NIHSS 9–20; &gt;30 yr; M + F</td>
<td>Stem cell therapy plus granulocyte colony-stimulating factor (no further details available)</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Pilot investigation of stem cells in stroke</td>
<td>Unilateral ischemic stroke (0.5–5 yr); NIHSS &gt;6; 60–85 yr; M only</td>
<td></td>
<td>Recruiting (phase I)</td>
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<tr>
<td>A study of modified stem cells in sable ischemic stroke</td>
<td>Ischemic stroke in subcortical region of MCA or lenticulostriate artery (with or without cortical involvement; 6–24 mo); NIHSS &gt;7; 18–75 yr; M + F</td>
<td></td>
<td>Recruiting (phase I/IIa)</td>
</tr>
<tr>
<td>fMRI in monitoring intracerebral stem cell implantation for chronic stroke patients</td>
<td>Ischemic stroke in MCA territory (0.5–3 yr); NIHSS 9–20; 35–75 yr; M + F</td>
<td>Intracerebral implantation of 2/5/10/20 × 10^6/patient ReNeuron CTX0E03 neural stem cells</td>
<td>Recruiting (phase I)</td>
</tr>
<tr>
<td>Intravenous autologous bone marrow-derived stem cells therapy for patients with acute ischemic stroke</td>
<td>Ischemic stroke (7–30 d); NIHSS &gt;7; 18–80 yr; M + F</td>
<td>Intracerebral implantation of hematopoietic stem cells; remyelination, increased perfusion, and cortical activity in penumbra observed using MRI</td>
<td>Active, not recruiting</td>
</tr>
<tr>
<td>Study of purified umbilical cord blood CD34^+ stem cell on chronic ischemic stroke</td>
<td>Ischemic stroke (6–60 mo); NIHSS 5–15; 35–70 yr; M + F</td>
<td>Intercerebral implantation of allogenic CD34^+ stem cells produced from StemCyte umbilical cord blood</td>
<td>Not yet recruiting</td>
</tr>
<tr>
<td>The clinical trial research of stem cell transplantation treats cerebral ischemia</td>
<td>Hemorrhagic or ischemic stroke (&lt;24 h); apoplexy and “obvious clinical symptoms”; 40–65 yr; M + F</td>
<td>Intravenous injection of umbilical cord mesenchymal stem cells (d 10–21 after hemorrhage and d 7–14 after ischemia); second transplant through lumbar puncture 7 d after initial transplant</td>
<td>Recruiting (phase II)</td>
</tr>
</tbody>
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developing new therapies. The pluripotency and proliferative nature of stem cells (reviewed extensively elsewhere; ref. 27) mean that much of this funding is focused on the development of techniques to tackle cardiovascular disease and stroke, both conditions requiring compensation for necrotic tissue. The potential in stem cell therapy is not only large, but broad; several possible applications are backed by accumulating evidence of their benefit in humans. These include stimulation of endogenous stem cells, exogenous production of implantable structures from host tissue, cell therapy through introduction of new stem cells into the host, and the provision of tissue for drug development (which one presumes will provide a superior alternative to the animal tissue it would replace). Evidence for the former three potential therapies is discussed below.

TABLE 1. (continued)

<table>
<thead>
<tr>
<th>Title of trial</th>
<th>Inclusion criteria</th>
<th>Intervention</th>
<th>Outcome/status</th>
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<tbody>
<tr>
<td>Autologous bone marrow stromal cell and endothelial progenitor cell transplantation in ischemic stroke</td>
<td>Ischemic stroke in MCA territory (7 d); NIHSS ≥7; 18–80 yr; M + F</td>
<td>2 times intravenous transplantation of 2.5 × 10^6 bone marrow stromal cells or endothelial progenitor cells/kg</td>
<td>Recruiting (phase I/II)</td>
</tr>
<tr>
<td>Intravenous autologous mesenchymal stem cells transplantation to treat middle cerebral artery infarct</td>
<td>Ischemic stroke in MCA territory; NIHSS 10–30; 30–70 yr; M + F</td>
<td>Intravenous autologous bone marrow-derived mesenchymal stem cells</td>
<td>Recruiting (phase I)</td>
</tr>
<tr>
<td>Study to assess the safety and effects of autologous adipose-derived stromal cells in stroke patients</td>
<td>Hemorrhagic or ischemic stroke; NIHSS &gt;8; 18–80 yr; M + F</td>
<td>Intracarotid or intravenous administration of autologous adipose-derived stromal cells (cells isolated and delivered within 1 h)</td>
<td>Recruiting (phase I/II)</td>
</tr>
<tr>
<td>Study to examine the effects of MultiStem in ischemic stroke</td>
<td>Cortical ischemic stroke (1–2 d); “moderate to moderately severe”; 18–79 yr; M + F</td>
<td>Infusion of MultiStem</td>
<td>Recruiting (phase II)</td>
</tr>
<tr>
<td>Safety/feasibility of autologous mononuclear bone marrow cells in stroke patients</td>
<td>Ischemic stroke (24–72 h); NIHSS 6–15(R)/18(L); 18–80 yr; M + F</td>
<td>Intravenous injection of autologous mononuclear bone marrow cells</td>
<td>Recruiting (phase I)</td>
</tr>
<tr>
<td>Ex vivo cultured adult allogenic MSCs in ischemic cerebral stroke</td>
<td>Ischemic stroke (10 d); modified Rankin scale ≤4; 20–80 yr; M + F</td>
<td>Intravenous injection of 2 ml/kg allogenic MSCs</td>
<td>Not yet recruiting (phase I/II)</td>
</tr>
<tr>
<td>Implantation of olfactory ensheathing cells (OECs)</td>
<td>Ischemic stroke in MCA territory (6–60 mo); NIHSS 5–15; 35–70 yr; M + F</td>
<td>Autologous OECs cultured before treatment; intracerebral implantation of 2–8 × 10^6/patient OECs</td>
<td>Recruiting (phase I)</td>
</tr>
<tr>
<td>Study of autologous stem cell transplantation for patients with ischemic stroke</td>
<td>Ischemic stroke in MCA territory (3–90 d); NIHSS 4–20; 18–75 yr; M + F</td>
<td>Intra-arterial or intravenous injection of 500^6/patient autologous bone marrow stem cells</td>
<td>Completed</td>
</tr>
<tr>
<td>Study of ALD-401 via intracarotid infusion in ischemic stroke subjects</td>
<td>Ischemic stroke in MCA territory (11–17 d); NIHSS ≥4; 30–75 yr; M + F</td>
<td>Intracarotid injection of ALD-401 autologous bone marrow stem cells</td>
<td>Recruiting (phase II)</td>
</tr>
<tr>
<td>Study of human placenta-derived cells (PDA001) to evaluate the safety and effectiveness for patients with ischemic stroke</td>
<td>Ischemic stroke in MCA or posterior communicating artery territory (no info for recency); NIHSS 6–20; 18–80 yr; M + F</td>
<td>Intravenous injection of 2 lots of 2 or 8 × 10^6/patient, or 1 lot of 2 × 10^6/patient PDA001 cells</td>
<td>Not yet recruiting (phase IIa)</td>
</tr>
</tbody>
</table>

All information collated from stem cell therapy for stroke trials registered on ClinicalTrials.gov. L, left; R, right; fMRI, functional MRI; M, male; F, female. Inclusion criteria were type of stroke (and days since occurrence of stroke), National Institutes of Health Stroke Score (NIHSS), age of participants, and sex of participants. aConventional therapy: antiplatelet treatment and management in a stroke facility.
Many groups focus on applying appropriate growth/migration stimulants to cells already present in the host through gene therapy. This is a reasonable approach that is reviewed elsewhere in detail (28, 29), although a promising example is embryonic morphogen sonic hedgehog (Shh), having been shown to increase vasculo genesis in a mouse model of peripheral limb ischemia, improving function (30). This is supported by additional work suggesting a beneficial role for Shh after ischemia (31–36).

The use of biological scaffolds and stem cells for organ or tissue generation for implants is incrementally becoming closer to reality (mesmerizing advances range from de-/recellularized livers to suitably ethereal “ghost hearts”; refs. 37, 38). On the assertion that there is little chance that these technologies will apply to brain regeneration en masse, at least for the time being, the focus in this section will be on cerebrovascular cell therapy.

The accumulation of evidence for the potential of cell therapy in stroke has relied on the use of animal models of the disease. Animal models have provided huge insights into the possibilities of stem cell therapy, although they are not without limitations. Fewer than 10 models of focal stroke exist in animals (39), the most commonly used being middle cerebral artery occlusion (MCAO), because of its reproducibility and similarity to the human condition (40, 41). Rodent studies frequently (although not always) use rats in preference to mice; the narrow apertures of the cerebrovasculature in mice render the rat model technically more amenable, but mice are used in genetic studies requiring, for example, knockout animals. MCAO involves passing a suture up the internal carotid artery until the origin of the middle cerebral artery (MCA) is met (42). The circulation supplying cerebral tissue not supplied by the MCA is fed by the circle of Willis throughout an ischemic period (often 1 h in duration), while the suture remains inserted. An infarct is produced, consistently and unilaterally, in the region of cortex supplied by the occluded MCA. Predominantly using this model, researchers have investigated cell therapy in animals with some key variables: the use of cells of exogenous vs. endogenous origin, the route of administration and cell type used (if an exogenous cell source; see Table 2 for cell types), and the growth factors used in association (if an endogenous cell source).

Fetal neural stem cells are able to form new neurons and improve behavioral outcomes in humans (43) and in animal models (44) after brain injury. Despite some positive results, ethical issues shrouding work involving fetal and embryonic stem cells (45), as well as regulatory considerations for potentially tumorigenic embryonic stem cells, have shifted the bulk of work toward the use of alternatives (or finding them; see Fig. 1 for current sources used for the generation of stem cells). The method of inducing pluripotency in human fibroblasts (46, 47), the use of adult stem cells and the manipulation of endogenous stem cells are popular alternatives (summarized in Table 2).

It has recently been discovered that by reprogramming fibroblasts with a combination of 4 transcription factors [either OCT4, SOX2, c-MYC, and KLF4 (46) or OCT4, SOX2, NANOG, and LIN28 (48)], it is possible to induce pluripotency in cells with no requirement for fetal or embryonic tissue. Induced pluripotent stem cells (iPSCs) have recently been used in animal studies as stroke treatments. Jiang et al. (49) transplanted human fibroblasts with the latter combination of transcription factors and injected them into the into the contralateral and ipsilateral peri-infarct regions of female rats after MCAO. The group discovered that these cells migrated to injured areas of the brain and showed some differentiation into neuron-like cells. The lesion volumes were reduced significantly, and, crucially, recovery of sensorimotor function was twice as successful in iPSC treatment groups vs. controls. These results were reflected by earlier work from Chen et al. (50), who found that implants promoted neural differentiation as well as immunomodulation. Kawai et al. (51) found that implantation of iPSCs after MCAO in mice resulted in tumorigenesis; the observed simultaneous increase in dividing neuroblasts led the researchers to conclude that the therapy still has potential, should the tumorigenesis be controlled. However, to date there have been no studies using neural precursors originating from iPSCs that definitively overcome such tumorigenic potential, which limits their therapeutic application.

The ethical and immunological implications of the use of autologous, adult stem cells has made suitable sources highly sought after. Adult bone marrow stromal cells contain mesenchymal stem cells (MSCs), which may be able to differentiate into neural cells (52, 53). It has been observed that beyond characteristic differentiation into bone cartilage and smooth and skeletal muscle, MSCs are able to transdifferentiate into skin, liver, and brain cells (including neurons and glia), as, conversely, neural cells may transdifferentiate into blood cells (54). The potential of hematopoietic bone marrow-derived cells is broad enough that they may be of additional therapeutic use in the treatment of vascular diseases, which would be of benefit in stroke prevention; ref. 55). MSCs grafted via stereotactic intracerebral injections into brains after MCAO reduced infarct size and improved somatosensory function in rats (56, 57). Transdifferentiation (also referred to as “plasticity”) of MSCs and hematopoietic stem cells to neural precursors as a mechanism for observed recovery after MSC transplantation in experimental stroke is regarded as a possibility by some authors. The concept is, however, largely contentious: it seems more likely that the “bystander effect” (see below), through which stem cells exert various neuroprotective functions, is the mechanism through which MSCs provide benefit. In this rat model, recovery was extended to improve motor function when the stem cells were preincubated with nerve growth factor or previously transfected with the brain-derived neurotrophic factor (BDNF) gene. Other groups have also shown that cytokines, including
<table>
<thead>
<tr>
<th>Cell type/source</th>
<th>Potential for differentiation</th>
<th>Relative benefits/problems with use</th>
<th>Use in experimental stroke</th>
<th>Use in human trials</th>
</tr>
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<tbody>
<tr>
<td>Embryonic/blastocysts (4–5 d after fertilization)</td>
<td>Pluripotent (able to produce neuronal progenitors); most undifferentiated stem cells available for potential clinical use</td>
<td>Ethically controversial; potentially tumorigenic—can predifferentiate in vitro to reduce tumor risk; others find predifferentiation makes no difference with homologous transplantation (vs. xenotransplantation; ref. 101)</td>
<td>Hemorrhagic (102) and ischemic (103); both showed improved functional outcome</td>
<td>No use to date in stroke trials; one phase I trial in spinal cord injury</td>
</tr>
<tr>
<td>Fetal</td>
<td>Pluripotent (able to produce neuronal progenitors)</td>
<td>Safety profile superior to embryonic cells; must be obtained from human fetal tissue therefore limited supply; cell lines overcome the latter (e.g., ReNeuron ReN001)</td>
<td>Hemorrhagic (104) and ischemic (44); both showed improved functional outcome</td>
<td>One phase I clinical trial (so far successfully completed); ischemic stroke</td>
</tr>
<tr>
<td>Mesenchymal (stromal cells)/bone marrow; autologous</td>
<td>Multipotent; osteoblasts, adipocytes, chondrocytes, and myocytes; also produce neuron-like cells (possibly nonfunctional therapeutically)</td>
<td>Autologous therefore free of immunological and ethical issues; mechanism of action ambiguous: are likely to provide support through neurotrophic support, immunomodulation and angiogenesis rather than direct replacement of neurons; retrieval from bone marrow (stromal)/adipose tissue (through liposuction and readministered in just 1 h (MultiStem trial)/extracted from teeth (several sources) during dental therapies/from deciduous teeth; dental cells very easily retrieved; adipose tissue may provide more favorable source than bone marrow as safer and easier access (110)</td>
<td>Hemorrhagic (105) and ischemic (106, 107); both showed improved functional outcome</td>
<td>Several phase I/II safety trials (in progress or completed; see Table 1); ischemic stroke</td>
</tr>
<tr>
<td>Mesenchymal-like/ adipose tissue; autologous</td>
<td>Multipotent; osteoblasts, adipocytes, chondrocytes and myocytes, endothelium, hematopoietic cells, hepatocytes and neuronal cells</td>
<td></td>
<td>Hemorrhagic (108) and ischemic (109); both showed improved functional outcome</td>
<td>One phase I/II (recruiting); hemorrhagic and ischemic stroke</td>
</tr>
<tr>
<td>Mesenchymal-like/ dental tissue (111); autologous</td>
<td>Multipotent; osteoblasts, adipocytes, chondrocytes; also produce neural-like cells (possibly non-functional)</td>
<td></td>
<td>Ischemic (112); showed improved functional outcome</td>
<td>None</td>
</tr>
<tr>
<td>Mesenchymal-like/ hematopoietic/ bone marrow/ umbilical cord blood; autologous</td>
<td>Multipotent; umbilical cord blood most undifferentiated MSCs available for clinical use</td>
<td>Autologous therefore free of immunological and ethical issues; may be retrieved non-invasively from umbilical; cord blood</td>
<td>Hemorrhagic (113) and ischemic (114); both showed improved functional outcome</td>
<td>One phase I/II (recruiting), cells from bone marrow; one phase I (recruiting); cells from umbilical cord blood transplanted in ischemic stroke</td>
</tr>
<tr>
<td>Induced pluripotent/ skin (predominantly fibroblasts)</td>
<td>Pluripotent</td>
<td></td>
<td>Three rodent studies of using MCAO (ischemic stroke; refs. 49–51); all improved outcome; one demonstrated teratoma formation (51)</td>
<td>None; current iPSC trials involve their production from human tissue only</td>
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<tr>
<td>Induced pluripotent/ oral gingiva</td>
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BDNF (58) and glial cell line-derived neurotrophic factor (GDNF; ref. 59), and peptide survivin (60), improve functional recovery when transfected into MSCs before implantation. Having established that these cells are able to cross the blood-brain barrier (61), studies were conducted using intravenous administration of MSCs (62, 63). Improved motor function was comparable to that observed after intracerebral administration, but recovery in somatosensory function was >3 times less effective (~90% improvement in intracerebral groups vs. 25% in intravenous groups). In a study comparing intravenous and intracarotid administration of MSCs, increased cell proliferation and number of blood vessels and decreased cell death and improved behavioral outcomes were all recorded, whereas there was no significant reduction in infarct volume (64). In terms of translation of research into clinical applications, close attention should be paid to studies comparing intravenous/intracarotid entry routes for stem cells rather than a direct injection into the brain. Although such studies frequently favor arterial administration (65), any benefit of cell therapy in human stroke is dependent on the practical viability of the route of delivery vs. efficacy (Fig. 1).

Although reduced infarct size seems to be indicative of improved recovery from experimentally imposed ischemic episodes, the mechanisms through which stem cell therapy aids this improvement are not entirely clear. These are discussed in the next section.

**PROPOSED MECHANISMS OF FUNCTIONAL RECOVERY AFTER STEM CELL THERAPY**

Subsequent to an injury, the body is inclined to scar, have the phagocytes pick up the pieces, and move on. This is the best method for avoiding infection, but not for instances in which rescuing tissue is vital to the proper functioning of the body. After the emergency clear-up after cerebral ischemia (and in apparent recognition of the brain as a vital organ), the body initiates its own repair strategy: neurogenesis in the adult brain is regulated by pathological as well as physiological events. The subgranular zone (SGZ) of the dentate gyrus and the rostral subventricular zone (SVZ) of the lateral ventricles each contain progenitor cells that are a perpetual source of new neurons. Neural progenitors from the SVZ are destined for the olfactory bulb, whereas those of the SGZ migrate into the neighboring granule layer of the dentate gyrus. Several studies have shown that in cerebral infarcts there is increased cellular proliferation (66, 67) and that increasing this proliferation [using surprising pharmacological approaches, including antidepressants (68), estrogen (69), and sildenafil (Viagra; ref. 70)] aids recovery. In addition to neurogenesis, additional means through which the brain may be able to repair itself include synaptogenesis and axonal branching (71), adapting the use of existing neuronal circuits (without the generation of new neurons or the repair of those injured; ref. 72), and “protective inflammation” (protective function of MN cells; ref. 73).

Working to enhance endogenous activity of the body to mend it is not a new concept. Nevertheless, on the basis that the body is frequently so adept at repairing itself, it is probably a sensible one. (For instance, in countering the excessive immune response in I/R injury after stroke, promising work aims to boost endogenous anti-inflammatory mechanisms rather than directly antagonize inflammation; refs. 9, 21). The route of neural progenitors from the SVZ is meticulously planned, so the question is, can we promote movement of these cells or injected cells to regions they are needed in after cell death? If not, or in addition to this, what are the properties of stem cells that have produced such promising preclinical results?

The premise behind the initial studies into the therapeutic potential of stem cells was their supposed ability to replace lost neuronal circuits. Further work has shown that although this is partly the case, the functional recovery associated with stem cell implantation does not correlate with the relatively minimal rewiring that evolves from exogenously introduced cells. In fact, the majority of recovery seen experimentally with stem cells is attributed to angiogenesis and growth factor secretion, and the benefit this affords to endogenous cells (Fig. 2). This was neatly demonstrated by Loffredo et al. (74) in work that strongly suggests this is true of the infarcted heart: the group used female mice engineered to express green fluorescent protein (GFP) in cardiomyocytes after a pulse of 4-hydroxytamoxifen. After receiving a pulse, mice were subjected to myocardial infarction (MI) and then were transplanted with c-kit expressing bone marrow-derived progenitors (or control cells) from male mouse donors. After MI, the population of GFP-positive cells was diluted by endogenously derived cardiomyocytes and to a much greater extent in groups receiving c-kit-positive cells. Although the generation of new neural networks in this way would be ideal, this is far less likely to be practical in the brain, where the intricacies of specific intercellular connections are more complex than those of the heart. The bystander effect is a mechanism through which transplanted cells aid tissue recovery, without the direct replacement of neurons; cells promote “therapeutic plasticity” (75), the functional compensation for a lesion, which is widely substantiated experimentally in several neurological diseases, including stroke (76). The bystander effect provides various means through which transplanted cells are able to deliver neuroprotective functions, including neurotrophic support, immunomodulation, and angiogenesis. The effect was initially described as a feature of neural stem cells but has since been used to explain the therapeutic effect exerted in brain disorders by other stem cells (those with a very low capacity for neural transdifferentiation; refs. 77, 78). The bystander effect of MSCs in stroke formed the basis for a phase I clinical trial in which a single intravenous infusion of autologous MSCs was administered to a cohort of 12 patients during the
subacute or chronic phase of stroke (36–133 d after stroke onset; ref. 79). Although this is a small sample, modest amounts of efficacy were observed in one-third of patients; this result, notably, correlated inversely with the time interval between stroke onset and treatment.

Fundamental to healthy brain function is a sufficient blood supply through extensive vascularization. The up-regulation of vascular growth factors seen in stroke models (particularly once applied with a range of potential stem cell therapies) therefore provides one of the best explanations for improved outcomes. More specifically, a neurogenic/vasculogenic region forms close to the infarct (15). Formation of this region along with mediation is both an endogenous artifact and a significant means through which stem cells promote recovery.

For this to occur, cells have to arrive at the correct location. Chemokine-driven migration enables stem cells to gather in the peri-infarct region (prevented from entering the ischemic core, possibly as a result of anoxia-induced tissue changes, such as extracellular acidosis; ref. 80), where they contribute to a regenerative environment (Fig. 2). Evidence indicates that local inflammation attracts stem cells to the infarct (81, 82). Paradoxically, immunosuppression promotes migration and survival of endogenous stem cells (83, 84). The neurogenic/vasculogenic region holds an assortment of cytokines, including BDNF, GDNF, SDF1, Ang1, and vascular endothelial growth factor (VEGF) (34–36, 58). Other factors implicated are basic fibroblast growth factor (bFGF; ref. 62), transforming growth factor β (TGF-β; ref. 44), insulin-like growth factor (IGF), and epidermal growth factor (EGF; ref. 63). In the studies in which up-regulation of these factors has been observed, transplanted cells have improved, provided, or induced the means for these factors to occur; interestingly, one group showed that after human MSC transplantation into a postischemic rat, IGF-I was the only human-derived cytokine detected in the core and ischemic border zone 3 d after MCAO. The subsequent up-regulation of growth factors of rat origin (which were increased compared with controls) suggests an inductive role for IGF in this model (63). It is also likely that transplanted cells improve the inflammatory status of the infarct, that is, enhance neuroprotection, again by changing the nature of the ischemic region. I/R injury in the acute stages of stroke (see Mechanisms of Tissue Damage in Ischemic Stroke above) develops through a combination of processes that stem cells appear to mediate to some extent. Lui et al. (85) showed that transplanted MSCs after MCAO in rats caused increased expression of anti-inflammatory cytokine IL-10 and decreased expression of (generally) proinflammatory tumor necrosis factor α (TNF-α). Another group show increased microglia/macrophage activation after MSC transplantation in the same model (44).

If the therapeutic benefit of stem cells relies on their purported ability to induce growth factors, mediate inflammation, and possibly proliferate, what are potential difficulties with their use in humans? The risk of oncogenesis where any dividing cells (or promotion of cell division) are concerned is well established, but this is not the only factor to be considered.

PITFALLS AND PROVISOS

Concerns with stem cells include ethical difficulties when dealing with embryonic or fetal tissue, potential oncogenesis or teratogenesis, and cytokine-related complications. How they will be traced in humans and (rather importantly) whether they will work are additional concerns.

The increasing use of autologous MSCs and the potential of iPSCs alleviate ethical pressure to reduce or
prevent the use of embryonic or fetal stem cells. This is a desirable transition, and there may be even more advantage in it than first appears; adult stem cells seem to have less oncogenic potential than fetal or embryonic stem cells (86). Possible links between oncogenesis and stem cell therapy have been reviewed elsewhere in detail (87); therefore, only two examples reflecting concerns for malignancy of stem cells in humans will be given here as caveats. Each has been mentioned in previous sections for positive preclinical results. The first refers to the reported ability of Shh to increase vasculogenesis and functional outcome in various models of ischemia (30–33). Zhao et al. (88) indicated that the hedgehog pathway is responsible for maintenance and proliferation of stem cells of chronic myeloid leukemia; notably, they demonstrated that ablating Smoothened (a gene from the pathway), increases susceptibility to the disease. Second, signaling through TGF-β (one of the cytokines responsible for maintaining the neurogenic/vasculogenic environment suitable for tissue renewal in the brain after injury) has a key role in the epithelial-mesenchymal-like differentiation involved in the development of breast cancer (89).

There are possible conflicting interests between stem cell and anti-inflammatory stroke therapies. MCP-1 and macrophage inflammatory protein 1α attract both (potentially therapeutic) stem cells (82) and (potentially damaging) leukocytes (90) to areas of damage. Resolvens are eicosanoid-derived mediators of anti-inflammation, which is partly effective through down-regulation of endothelial VCAM-1 (91); yet neural precursor cells demonstrate spontaneous adhesion enabled through expression the α4 subunit of VLA-4 (65, 92), the ligand for VCAM-1. The partial reliance of stem cells on inflammatory markers for successful migration must be considered by groups pursuing anti-inflammatory and cell-based stroke therapies: might the optimal conditions for one form of therapy oppose those of the other?

Irrespective of stem cell origin or mode of entry, tracking cells in vivo through clinical trials will be necessary. Tracking a relatively small number of cells, each essentially transplanted as a separate entity, is going to be difficult. In basic research, it is possible to observe cell locations effectively using bioluminescence (93), which requires bone marrow from a transgenic, luciferase-expressing mouse; stumbling blocks for this exact method to be applied in the clinic are rather obvious. In addition, although in theory donor cells could be genetically modified ex vivo to express luciferase, the enzymes generate only visible light (400–700 nm); this has very high absorption/scatter in vivo, which is highly impractical for visualizing deep cell penetration in humans.

More viable options involve the use of magnetic resonance imaging (MRI; refs. 94, 95) and (less commonly) positron emission tomography (PET; ref. 96). Contrast agents must be nontoxic and allow for the detection of a single cell; they must not alter the genetics of the stem cell or become too dilute as cells divide, and they would preferably enable repeated viewing of a patient without repeated administration (97). The scanners themselves pose other technical difficulties; e.g., with PET, there may be uptake of the tracer by nonspecific tissue, and patients with pacemakers and implantable devices cannot undergo MRI (undoubtedly a problem considering the cohort of patients likely to be in receipt of stem cell therapy for stroke). Tamaki et al. (98) have used a different approach. The group marked human CNS stem cells with reporter genes (enhanced GFP), introduced by lentiviral vectors. Using fluorescence and antibodies for GFP, they were able to identify migration and differentiation of the stem cells in a mouse model.

Finally, the success with cell therapy is not universal. Parr et al. (99) showed that MSCs and neural stem/progenitor cells (NSPCs) implanted in injured rat spinal cord caused no recovery of function and no neural differentiation (although about half of the transplanted NSPCs develop into astrocytes). The researchers suggest this could be the result of standard lesion sizes, which were impossibly large for the therapy to be of benefit. In addition, it has been shown elsewhere that stem cells fail to differentiate into neurons when transplanted into spinal cord, which is, of course, not the brain. Regardless, the situation is a reminder that negative results provide important information to be considered when clinical trials are designed.

**CONCLUSIONS**

The pathophysiologic process of stroke involves temporally executed interactions between numerous cell types, including neurons themselves, glia, endothelia, endogenous progenitors and cells of the immune system. A combination of therapies is ultimately likely to be of greatest benefit in stroke patients: clot dispersion, followed by mediation of a subsequent inflammatory response and neurogenesis/vasculogenesis, backed by solid imaging.

Before a cell-based therapy is set for translation, it is essential to have information on each aspect of the pathogenesis, in particular how the endogenous CAMs and cytokines expressed during inflammation may be involved in stem cells homing in on and dividing the appropriate region. It is important to identify molecular pathways responsible for directing stem cells to areas of ischemia and to learn how the cells, once in the brain parenchyma, may release factors that interfere with the immune response. The preferred and most feasible source of the cells, whether or not cells should be genetically modified, and best route of administration into the patient must all be considered, as well as the optimal time (should one exist) to administer cell therapy.

The use of stem cells as a treatment has been widespread since the early 1970s, when bone marrow grafts containing hematopoietic stem cells began to be used for management of acute myeloid leukemia (100).
Their use as treatment for brain damage after stroke to accommodate for the losses in neuronal tissue is new, and efficacy has yet to be proven in humans. Despite the minimal increased development of new neurons directly arising from exogenous pluripotent cells, vasculogenesis and the production of a tissue-regenerative microenvironment around an infarct suggest a positive future for stem cells as a therapy for stroke. The possibility that this treatment may not be so temporally restrictive as tPA is highly encouraging and could to some extent provoke a shift in the dogma surrounding infarcted neurons after stroke: what’s gone is not necessarily gone.

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