Abstract

Stroke is a leading cause of death and disability. Globally, 15 million people suffer a stroke each year, of whom more than 5 million die, and a further 5 million are left permanently disabled. Current treatment options offer modest benefits, and there is a pressing need for new and effective treatments. Stem cell therapy is a well-established treatment modality for various haematological diseases, with its use now being explored in different disease processes, including various neurological diseases, as well as vascular conditions such as ischaemic heart disease and peripheral vascular disease. Promising results have been seen in animal models of stroke, with evidence of significant functional benefits. Translation to the bedside, however, is in its early stages. This review will discuss the scientific background to stem cell therapy in ischaemic stroke, including evidence from current clinical trials.

Keywords: stroke, stem cells, clinical trials, elderly

Introduction

Stroke is the second most common single cause of death worldwide (after ischaemic heart disease) and is the commonest cause of adult disability. In the UK, there is a stroke every 5 min, and on a global basis, it causes over five million deaths in the world each year. Moreover, in Europe, a quarter of a million people will become disabled after their first stroke each year. Even if age-specific stroke incidence remains stable or falls slightly, as more people live into old age, the number of new cases of acute stroke per year will rise.

Although recent advances have substantially improved stroke management, current acute treatment options offer only modest effects. Aspirin provides only a 1% absolute reduction in death and recurrent ischaemic stroke. Thrombolysis with tissue plasminogen activator is only applicable within a narrow time window and until recently fewer than 1% of stroke patients in England received this treatment annually [1].

Most patients show some spontaneous recovery after a stroke, which can be further improved by interventions such as neurorehabilitation. However, this recovery is often incomplete. Stroke therefore remains a major source of adult disability and there continues to be a compelling need for effective treatments for severe, disabling stroke.

Regenerative potential of the brain

Cells of the brain and central nervous system were, until recently, thought to be incapable of regeneration. However, in the last decade, evidence of neurogenesis in the human adult brain has been demonstrated in the dentate nucleus of the hippocampus and the sub-ventricular zone [2, 3]. This has formed the basis for experimental work investigating the potential role of cell transplantation therapy in the treatment of various neurological diseases, in particular ischaemic stroke.

Ischaemic strokes make up the majority of all strokes, accounting for 80% of the total. A recent study of patients with ischaemic stroke was able to demonstrate evidence of neurogenesis in the ischaemic penumbra, where cells were...
found to preferentially localise to the vicinity of blood vessels [4]. The findings are suggestive of stroke-induced compensatory neurogenesis, where it may contribute to post-ischaemic recovery, and therefore represent a potential target for stroke therapy.

**Approaches to stem cell therapy in ischaemic stroke**

Human stem cell transplantation therapy is now a well-established treatment for various malignant and non-malignant haematological diseases and some autoimmune disorders. Pre-clinical studies over the last decade have demonstrated significant benefits of stem cell therapy in rodent models of ischaemic stroke. Translation to the bedside, however, is in its early stages.

Clinical approaches to stem cell therapy in stroke can be broadly divided into endogenous and exogenous approaches. The endogenous approach aims to stimulate mobilisation of stem cells already present within the individual. Examples of this approach include the use of granulocyte-colony stimulating factor (G-CSF) which is routinely used to mobilise stem cells for transplantation in haematological malignancies. G-CSF has been shown to be beneficial in rodent models of stroke (exhibiting neuroprotective and neuroregenerative activities) [5, 6] and furthermore has been shown to be safe in phase I clinical trials of human stroke when used within 7 days [7, 8] or 7–30 days post-stroke [9]. Of note, G-CSF appears to have direct benefits, beyond simply mobilisation of stem cells. A number of phase II trials are currently underway to investigate its efficacy in patients with ischaemic stroke.

The exogenous approach involves the transplantation of the patient with stem cells delivered locally (e.g. direct intracerebral implantation) or systemically (e.g. intravenous) and may involve *in vitro* culture of cells for the expansion of cell numbers prior to administration.

This review will discuss the exogenous approaches to stem cell therapy in ischaemic stroke, in particular the various types of human stem cells being considered for therapy, and results of the early clinical studies.

**Stem cells overview**

Stem cells can be defined as clonogenic cells that have the capacity to self-renew and differentiate into multiple cell lineages [10]. Stem cells are present throughout life, though differences in differentiation potential (potency) allow for further sub-classification (Figure 1).

**Figure 1.** Potency of different stages of stem cells [11].
potential, with the former capable of producing more than one type of cell (e.g. clonal common myeloid progenitor), and the latter capable of producing only one mature cell type.

Progenitor cells are those cells generated by stem cells, which go on to differentiate into mature cells (e.g. endothelial progenitor cells). Unlike stem cells which can replicate indefinitely, they can only divide a limited number of times, and therefore lie at an intermediate position between stem cells and mature fully differentiated cells.

Source of stem cells
Stem cells can be classified according to their source, with the two major types being embryonic stem (ES) cells and adult stem cells. The relative merits of each are presented in Table 1.

Human ES cells are pluripotent and are isolated from 5-day-old human blastocysts. The major limiting factor to their widespread use includes ethical concerns regarding the use of unwanted embryos. Furthermore, ES cells can be characterised by their ability to form teratomas [12], and therefore there is concern regarding their tumorigenicity.

Adult stem cells are multipotent stem cells found in developed organisms, which are used to replace cells that have died or lost function. They can be obtained from adults as well as children, including from umbilical cord blood. They have been identified within many different organ systems, including bone marrow, brain, heart, skin and bone. Adult stem cells make up 1–2% of the total cell population within a particular tissue type. They are usually quiescent and held in an undifferentiated state until they receive a stimulus to differentiate.

Recent advances in stem cell research have seen the ability to transform adult stem cells from human skin fibroblasts into pluripotent stem cells (ES cell-like), in a phenomenon known as induced pluripotency [13–15]. This is in its very early stages of research, but has the potential advantage of obtaining pluripotent stem cells from individuals, without the ethical dilemmas associated with the use of ES cells.

Table 1. Relative merits of different sources of stem cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES cells</td>
<td>Pluripotent</td>
<td>Ethical issues</td>
</tr>
<tr>
<td></td>
<td>Immortal in vitro</td>
<td>Insufficient availability</td>
</tr>
<tr>
<td>Adult stem cells</td>
<td>Multipotent</td>
<td>Immunosuppression required for allografting</td>
</tr>
<tr>
<td></td>
<td>Potential for induced pluripotency</td>
<td>Certain types difficult to obtain, e.g. NSCs</td>
</tr>
<tr>
<td></td>
<td>Suitable for autologous and allogeneic use</td>
<td>Expansion issues</td>
</tr>
</tbody>
</table>

Stem cell transplantation for ischaemic stroke
Following a stroke, all brain tissue elements are lost, unlike Parkinson’s disease, for example, where a specific neuronal type is damaged. After an episode of ischaemia, a characteristic pathophysiological change ensues in affected brain tissue. At the core of the infarct cavity, the affected cells die rapidly by necrosis, and an area of surrounding tissue, the ‘penumbra’, contains tissue in which neurones and glia variably survive or die by a mixture of ischaemic degeneration and apoptosis over an extended time course of several hours to days and even weeks [16].

Stem cell therapy for ischaemic stroke, therefore, focuses on a regenerative strategy required to restore not only neural elements, but also supporting structures such as blood vessels. A variety of stem cell types from humans have been tested in stroke, both in experimental and in clinical studies. These are discussed below.

Neural stem/progenitor cells
It is now generally accepted that active neurogenesis, originating from neural stem cells (NSCs), occurs in discrete areas of the mammalian adult brain throughout life, giving rise to new populations of neurones and glia [17]. Neurogenesis can be physiological or can be subject to external modulation by certain conditions, e.g. CNS damage, such as ischaemia.

Preclinical experiments
NSCs can be isolated from foetal as well as adult mammals. Several studies have documented the isolation of NSCs from the adult rodent brain [18, 19], and more recent work has extended to the adult human brain [20, 21]. Human NSCs delivered intravenously or stereotactically surrounding the lesion have been shown to survive, migrate towards the lesion and differentiate (mainly neurones and astrocytes), while improving functional recovery in rodent models of stroke [22–24].

Clinical studies
The practicability of routine brain biopsies for isolation of adult human NSCs and autologous transplantation post-stroke remains to be seen, and no clinical trials utilising adult NSCs have been undertaken.

Immortalised NSC lines can be developed, however, from ES or foetal stem cells by the addition of specific growth factors. There is currently a phase I trial planned using a commercially developed (foetally derived) NSC line designated CTX, to be delivered by stereotactic injection in patients with ischaemic stroke (ReNeuron, UK) [25].
NT2 cell line

Immortalised cell lines are attractive for use in cellular therapy, as they form an abundant source of cells for transplantation, in addition to the relative ease of preparation and long-term maintenance. The immortalised cell line NTera-2 (NT2) was derived from a human teratocarcinoma over 20 years ago [21]. After several weeks of exposure to retinoic acid and mitotic inhibitors, these cells differentiate into post-mitotic neurone-like cells, named NT2N cells which display a variety of neuronal features [26]. These cells maintain their neuronal phenotype both in vitro and in vivo for over a year, without reverting to a neoplastic state [27, 28].

Preclinical experiments

The transplantation of NT2N cells into the ipsilateral striatum of ischaemic rat brains improved functional recovery, with some parameters of behavioural improvement persisting for up to 6 months post-transplantation [29, 30, 31]. Moreover, no evidence of tumorigenicity was found following post-mortem examination of the rats, an important consideration given the derivation of this cell line [32].

Clinical studies

NT2N cells were the first human cells to be tested in stroke clinical trials. A phase I trial examined the safety of stereotactic transplantation of cells into patients with basal ganglia stroke which had occurred 6 months to 6 years previously [33]. Twelve patients with stable motor deficits received the treatment, along with an immunosuppressive regimen of cyclosporine-A for 8 weeks post-transplantation. The small study population size limited any meaningful interpretation of efficacy, though there was a trend towards improved functional outcome in four patients. Autopsy on one patient, who died of myocardial infarction 27 months post-transplantation, showed apparent survival of NT2N cells in the brain [34]. Furthermore, positron emission tomography scanning at 6 months post-transplantation showed increased metabolic activity at the site of infarct in six patients [35], although this may have indicated an inflammatory response rather than graft survival; however, no signs of inflammation were seen on MRI.

This initial safety trial led to a Phase II study assessing the effect of NT2N cell transplantation in 18 patients with substantial fixed motor deficits (between 1 and 6 years post-stroke) associated with a basal ganglia infarct [36]. The authors compared stereotactic cellular transplantation plus two months of rehabilitation \( (n = 14) \) against control patients who received rehabilitation alone \( (n = 4) \). Again, the study was not powered to demonstrate efficacy, although 6 of the 14 patients who received a transplant appeared to show improvement on a standardised stroke scale, but this was not statistically significant against control patients. Of note, this trial included ischaemic \( (n = 9) \) and haemorrhagic strokes \( (n = 9) \).

Despite the initial promise of using NT2N cells for cellular transplantation, much remains to be seen about their utility, particularly in a clinical setting. Following an ischaemic stroke, damage is rarely restricted to the striatum, with many patients having cortical damage. Furthermore, the feasibility of NT2N cellular transplantation in the acute stroke setting has yet to be examined.

Bone-marrow-derived stem cells

BROADLY speak, bone-marrow-derived stem cells consist of both haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). HSCs are the precursors of all blood and lymphoid lineages. MSCs give rise to the stromal cells of the bone marrow, including the formation of osteoblasts, chondrocytes and adipocytes. Bone marrow is an attractive source of stem cells, being easily accessible and suitable for autologous (obviating the need for immunosuppression) as well as allogeneic use.

Bone marrow cells have been shown to be able to migrate to the brain and differentiate into cells that express neuron-specific markers [37]. Both populations of bone marrow cells have been studied extensively with regard to their potential for neuronal differentiation and possible use in neuroregenerative therapy.

Mesenchymal stem cells

These cells are probably the most widely studied in experimental models of neurotransplantation post-stroke.

Preclinical experiments. Several studies have demonstrated that MSCs can improve functional recovery after middle cerebral artery occlusion in rats. This recovery occurs when the MSCs are delivered by a variety of routes, intravenous, intracarotid and intrastrial, starting 1 day post-ischaemia [38-40]. In addition, delayed delivery of MSCs, even up until one month post-infarct, can also improve long-term functional outcome [41]. Following transplantation, however, very few cells are actually found in the brain, and even fewer of these cells express neural markers [42]. Despite this, animals which received MSCs did have some amelioration of functional recovery, strongly suggesting a mechanism of action other than integration into the host’s neural circuitry.

Clinical studies. The extensive pre-clinical work on MSCs led to a phase I/II clinical trial using autologous culture-expanded MSCs in patients with ischaemic stroke. Five patients with established stroke (>1 month post-infarct) received MSCs by the intravenous route following ex vivo culture expansion of the cells [43]. Cells were extracted by bone marrow aspiration and culture-expanded. There was no cell-related toxicity related to the MSCs administration, and the authors suggested that functional improvement may have been better in the transplanted group compared with the control group, although this was not statistically significant. There were no significant differences between the two groups in relation to changes in infarct volume at 1-year follow-up.
Further to the above trial, a phase II trial of intravenous, autologous MSC therapy in patients within 6 weeks of carotid territory ischaemic stroke is due to commence recruiting shortly.

**Haematopoietic stem cells**

The use of HSCs for bone marrow transplantation is well established, and the therapeutic potential of stem cells in bone marrow in the regeneration of other non-haematopoietic tissue such as heart and skeletal muscle has also been explored [44–46].

**Preclinical experiments.** Much of the preclinical work examining the use of HSCs for stroke therapy has focused on the potential mechanisms of functional recovery. Two studies in particular have demonstrated increased angiogenesis in penumbral tissue following CD34+ cell transplantation, whether given systemically or by the intracerebral route [47, 48]. CD34+ bone marrow-derived cells include populations of haematopoietic and endothelial stem and progenitor cells. Both the aforementioned studies showed evidence of functional recovery, as well as reduced infarct size. The authors of one of these studies also used an anti-angiogenic compound, endostatin, administered 7 days after CD34+ cell transplantation and demonstrated that endogenous neurogenesis was suppressed by diminishing angiogenesis, thus suggesting a possible role for CD34+ cells in angiogenesis-mediated neural plasticity post-stroke.

**Clinical studies.** A number of clinical trials are currently underway investigating the role of bone-marrow-derived stem cell therapy at different stages of ischaemic stroke, utilising different methods of delivery (Table 2). Three trials utilising autologous bone marrow mononuclear cells are ongoing. Further three trials are underway utilising autologous CD34+ cells in acute as well as chronic ischaemic stroke patients. Results from these trials are awaited.

**Potential mechanisms of recovery**

The functional recovery seen in many experimental models following stem cell transplantation is likely to be mediated by several overlapping mechanisms, and understanding them is crucial in order to determine the best approach for translation into the clinical setting.

The integration of transplanted cells into the ischaemic brain, with the replacement of lost cells, is an unlikely mechanism of repair. Most studies have shown the survival of very few transplanted cells following neurotransplantation, despite evidence of significant functional recovery. More likely is the stimulation of a trophic effect on the ischaemic brain (e.g. by secretion of various growth factors), including the recruitment of endogenous repair processes. Anti-inflammatory effects may also play a role [49].

**Unresolved issues**

As is evident from the diversity among the various completed and ongoing trials (Table 2), there are a number of unresolved issues in the translation of stem cell therapy. These include:

1. choice of cell type,
2. cell numbers to be given,
3. optimum timing of treatment,
4. optimum route of delivery.

Table 2. Overview of published and ongoing clinical trials of human stem cell therapy in ischaemic stroke (exogenous approaches)

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Study design</th>
<th>Number of patients</th>
<th>Timing of delivery post-stroke</th>
<th>Route of delivery</th>
<th>Trial outcome</th>
<th>Trial status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT2N [28]</td>
<td>PI, NR-SB</td>
<td>12</td>
<td>6 months–6 years</td>
<td>Intracerebral</td>
<td>Safe and feasible; trend towards functional improvement with higher dose (6 × 10^6 versus 2 × 10^6 cells)</td>
<td>Complete</td>
</tr>
<tr>
<td>NT2N [31]</td>
<td>PII, R-SB</td>
<td>18</td>
<td>1–6 years</td>
<td>Intracerebral</td>
<td>Safe and feasible; no significant functional improvement in treated group (14 treated versus 4 controls); ischaemic and haemorrhagic strokes included</td>
<td>Complete</td>
</tr>
<tr>
<td>Bone marrow-derived cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSC [38]</td>
<td>PI/II, R-OL</td>
<td>30</td>
<td>&gt;1 month</td>
<td>Intravenous</td>
<td>Safe and feasible; non-significant trend towards functional improvement in treated group (5 treated versus 25 controls)</td>
<td>Complete</td>
</tr>
<tr>
<td>BMMNC (NCT00859014)</td>
<td>PI, NR-OL</td>
<td>10</td>
<td>24–72 h</td>
<td>Intravenous</td>
<td>Awaited</td>
<td>Ongoing</td>
</tr>
<tr>
<td>BMMNC (NCT00473057)</td>
<td>PI, NR-OL</td>
<td>10</td>
<td>3–90 days</td>
<td>Intra-arterial</td>
<td>Awaited</td>
<td>Ongoing</td>
</tr>
<tr>
<td>BMMNC (CTRI/2008/091/000046)</td>
<td>PI/II, R-SB</td>
<td>120</td>
<td>7–30 days</td>
<td>Intravenous</td>
<td>Awaited</td>
<td>Ongoing</td>
</tr>
<tr>
<td>CD34+ (NCT00535197)</td>
<td>PI/II, NR-OL</td>
<td>10</td>
<td>7 days</td>
<td>Intra-arterial</td>
<td>Awaited</td>
<td>Ongoing</td>
</tr>
<tr>
<td>CD34+ (NCT00761982)</td>
<td>PI/II, NR-SB</td>
<td>20</td>
<td>5–9 days</td>
<td>Intra-arterial</td>
<td>Awaited</td>
<td>Ongoing</td>
</tr>
<tr>
<td>CD34+ (NCT00950521)</td>
<td>PII, R-OL</td>
<td>30</td>
<td>6–60 months</td>
<td>Intracerebral</td>
<td>Awaited</td>
<td>Ongoing</td>
</tr>
</tbody>
</table>

Abbreviations: BMMNC, bone-marrow mononuclear cells; NR, non-randomised; NT2N, NTera-2 immortalised neurone-like cell line; OL, open label; PI and II, phase I and II trials; R, randomised; SB, single blind.
These issues are discussed in more detail in Supplementary data available in Age and Ageing online.

Tracking of stem cells is another area of interest, which is essential to our further understanding of distribution and mechanism of action of cells. Potential approaches to this include labelling of cells with a magnetic label (e.g. superparamagnetic iron oxide particles) allowing MRI tracking of the cells. This has yet to enter the clinical arena in stroke studies, though, it has shown promise in rodent models of stroke [51]. The ideal method of tracking delivered stem cells is yet to be established, though it will probably involve a combination of different imaging modalities, and is likely to evolve with emerging imaging technologies.

Another important consideration regarding cellular therapy for stroke is safety, in terms of both the cell type used and the mechanism for extracting, preparing and administering the cells. Long-term biosafety studies are essential in this respect, particularly with regard to the potential for tumorigenicity—this will become increasingly important if prolonged secretion of trophic factors by genetically modified cells enters the clinical arena. In addition, the potential for tumour formation in other organs must be considered, particularly with systemic delivery of cells. Furthermore, appropriate quality assurance and control standards must be in place to allow the standardisation of cell preparations.

Conclusions

Stem cell therapy for the treatment ischaemic stroke is an exciting emerging area of research in the clinical arena of stroke therapy. Animal studies have shown promising results in induced models of stroke, though translation to the bedside is still in its infancy. A number of issues remain unanswered, and further investigation of these will be imperative in the ongoing quest for an effective, feasible and safe cell-based therapy.

Key points

• Stem cell therapy has been explored in pre-clinical models of acute and chronic ischaemic stroke, with evidence of significant functional benefits in animal studies.
• A number of clinical trials of stem cell treatment in ischaemic stroke are now underway, though translation to clinical practice remains a long way off.
• A number of unanswered questions remain, including questions regarding type and quantity of stem cells to be given, ideal mode of delivery and tracking of cells.

Supplementary data

Supplementary data mentioned in the text is available to subscribers in Age and Ageing online.

Conflicts of interest

All authors are involved in an ongoing clinical trial of autologous CD34+ stem cell therapy in ischaemic stroke. N.H. and M.G. have shares in OmniCyte Ltd.

References

The long list of references supporting this review has meant that only the most important are listed here and are represented by bold text throughout the paper. The full list of references is available at Age and Ageing online.


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