Neural stem/precursor cells for the treatment of ischemic stroke

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Abstract

In ischemic stroke, the third most frequent cause of mortality in industrialized countries, therapeutic options have until now been limited to the first hours after disease onset. Cell transplantation has emerged in various neurological disorders, including experimental stroke, as a successful recovery-promoting approach also in the post-acute stroke phase. However, before envisaging any translation into humans of such promising cell-based approaches we still need to clarify: (i) the ideal cell source for transplantation, (ii) the most appropriate route of cell administration, and, last but not least, (iii) the best approach to achieve an appropriate and functional integration of transplanted cells into the host tissue. Here we discuss, with special emphasis on neural stem/precursor cells, potential mechanisms that may be involved in the action of cell-based therapies in stroke.

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1. Introduction

Ischemic stroke is the leading cause of long-term disability and the third most frequent cause of mortality in industrialized countries. With an annual incidence of approximately 250–400 in 100,000 inhabitants [1,2], about one million people in the EU suffer a stroke each year, many of them being struck by persistent long-term handicaps. Stroke is caused by the interruption of blood flow in a brain-supplying artery, in most cases by embolic vascular occlusion [3]. Despite considerable progress in recanalizing (i.e., thrombolytic) [4] and secondary stroke prevention [5] therapies in recent years there are still no neuroprotective therapies available that allow to reduce brain damage and to improve neurological recovery once a stroke has occurred [6].

Following the discovery in the late sixties that neural stem/precursor cells (NPCs) are continuously generated in the adult rodent brain [7], it was recently shown that endogenous NPCs are activated in response to ischemia, both in rodents [8] and humans [9]. Stem/precursor cells residing within the germinal niches of the brain have the ability to migrate towards the stroke lesion, where they may induce recovery of the surrounding non-lesioned tissue [8–11]. While this observation has raised hopes that stem cells may be used for therapeutic purposes, it is still a matter of debate which mechanisms underlie the therapeutic efficacy of such cells.

2. NPCs for stroke

Different types of stem/precursor cells were used in experimental stroke in the past, among which neural, hematopoietic, bone marrow, and umbilical cord cells should be mentioned (see [12,13]; for NPCs see also Table 1). Several different delivery techniques were tested, including intraperitoneal transplantations, intrathecal injections or intravenous infusions (Table 1). As a consequence of the first encouraging results from experimental studies, pre-clinical phase I and II trials, using different types of stem cells, were tested in patients suffering from stroke (Table 2).

Although some of these trials could demonstrate neurological improvements and cell transplantations appeared to be
a safe procedure, the precise mechanisms underlying the restorative effects of stem cells were still poorly known at the time of these early studies. Furthermore, the lack of reliable surrogate marker to test the mechanism of action in patients with stroke and the non-existence of long-term observations regarding the influence of stem cells in the patients’ brains, together with observations of tumor formation in mice with experimental stroke treated with local parenchymal implantations of embryonic stem (ES) cells [32], raised safety concerns, which slowed down further progress.

However, the scientific community has to intensify efforts in order to evaluate the efficacy of these strategies since in these days thousands of desperate patients are estimated to be on the waiting list to receive stem cell-based therapies in centers not rigorously following approved clinical trial protocols [33]. In the following, we shortly summarize (a) advantages and disadvantages of ES cells compared with adult NPCs, (b) concepts on the pathotropism of NPCs, as well as (c) prevailing ideas about precursor cell actions in the stroke brain.

### 3. Stem cell sources: ES-derived vs. adult NPCs

The ideal cell for transplantation should meet first of all the criteria of safety for the receiver as well as offer the highest therapeutic potential. Unlike hematopoietic bone marrow reconstitution, where a single cell may be sufficient to replenish the whole body’s stores, therapeutic preparations for stroke requires an adequate cell number, which raises the need to expand in vitro the putative precursor cell source.

Despite several groups reporting that ES cells have promising therapeutic potential [13,23,24], ethical and safety concerns (such as feeder-independent expansion and in vivo teratocarcinoma formation) still limit their translation to clinics. On the other hand, adult NPCs, which can be obtained

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### Table 1

Neural precursor cells in animal models of ischemic stroke

<table>
<thead>
<tr>
<th>Animal Stroke model</th>
<th>Cell type</th>
<th>Time of transplantation (after stroke)</th>
<th>Route of transplantation</th>
<th>Therapeutic action</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat MCAO</td>
<td>Human NT2N teratocarcinoma-derived NPCs</td>
<td>1 month</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Improved [14]</td>
</tr>
<tr>
<td>Mouse CCAO + Hypoxia</td>
<td>C17.2-CD NPCs</td>
<td>7 days</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>Rat MCAO</td>
<td>Immortalized murine neuroepithelial MHP36 cells</td>
<td>2–3 weeks</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Improved [16]</td>
</tr>
<tr>
<td>Rat MCAO</td>
<td>Human fetal NPCs</td>
<td>7 days</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>Rat MCAO</td>
<td>Human fetal HB1.F3 NPCs</td>
<td>24 h</td>
<td>Intravenous</td>
<td>+ + Not tested</td>
<td>Improved [18]</td>
</tr>
<tr>
<td>Gerbil CCAO</td>
<td>Human fetal NPCs</td>
<td>96 h</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Improved [19]</td>
</tr>
<tr>
<td>Rat MCAO</td>
<td>Rat NPCs</td>
<td>48 h</td>
<td>Intracisternal</td>
<td>Not tested +</td>
<td>Not tested</td>
</tr>
<tr>
<td>Rat MCAO</td>
<td>Human fetal NPCs</td>
<td>48 h</td>
<td>Intraarterial</td>
<td>Not tested +</td>
<td>Not tested</td>
</tr>
<tr>
<td>Rat MCAO</td>
<td>Immortalized human CTX0E03 NPCs</td>
<td>3–4 weeks</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Improved [22]</td>
</tr>
<tr>
<td>Rat MCAO</td>
<td>Murine ES cell-derived NPCs</td>
<td>7 days</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>Mouse MCAO</td>
<td>ES cell-derived NPCs</td>
<td>24 h</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>Mouse MCAO</td>
<td>Neonatal cerebellar</td>
<td>24 h, 72 h, 1, 2 and 5 weeks</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Not tested</td>
</tr>
<tr>
<td>Monkey MCAO</td>
<td>Human NPCs</td>
<td>7 days</td>
<td>Intraparenchymal</td>
<td>+ Not tested</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

Abbreviations: MCAO, middle cerebral artery occlusion; CCAO, common carotid artery occlusion. (+), favourable influence found.

### Table 2

Cell-based therapies tested in pre-clinical trials

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>No. of patients</th>
<th>Disease</th>
<th>Cell type</th>
<th>Time of transplantation (after stroke)</th>
<th>Route of transplantation</th>
<th>Therapeutic action</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>12</td>
<td>Basal ganglia infarcts</td>
<td>Human NT2/D1 teratocarcinoma-derived NPCs</td>
<td>Mean: 27 months (range: 7–55)</td>
<td>Intraparenchymal (+)</td>
<td>Some improvement</td>
<td>[27,28]</td>
</tr>
<tr>
<td>Phase II</td>
<td>18</td>
<td>Ischemic/hemorrhagic infarcts</td>
<td>Human NT2/D1 teratocarcinoma-derived NPCs</td>
<td>Mean: 3.5 years (range: 1–5)</td>
<td>Intraparenchymal</td>
<td>Not tested</td>
<td>Some improvement</td>
</tr>
<tr>
<td>Phase I/II</td>
<td>30</td>
<td>MCA infarcts</td>
<td>Autologous mesenchymal precursor cells</td>
<td>4–9 weeks</td>
<td>Intravenous</td>
<td>Not tested</td>
<td>Improved</td>
</tr>
<tr>
<td>Phase I</td>
<td>5</td>
<td>Basal ganglia infarcts</td>
<td>Fetal porcine cells</td>
<td>Mean 5 years</td>
<td>Intraparenchymal</td>
<td>Not tested</td>
<td>No improvement</td>
</tr>
</tbody>
</table>

Abbreviations: MCA, middle cerebral artery. (+), histological evidence in one single patient.
from different tissues and safely expanded in vitro, have shown promising therapeutic effects in several neurological disorders without causing serious side effects.

4. Pathotropism of NPCs

Whatever route of transplantation is chosen (local in-parachymal or systemic), NPCs, both embryonic and adult, have the capacity to migrate long distances along chemotactic gradients induced in sites of brain injury [24,34]. It has been, in fact, shown that transplanted stem/precursor cells are able to follow, via the blood stream or cerebrospinal fluid circulation, gradients of pro-inflammatory cytokines and chemokines that are released at the site of brain lesions. While promoting interaction between transplanted NPCs and activated endothelial/ependymal cells around inflamed CNS tissues, this chemotactic gradient leads to selective and specific homing of transplanted cells in inflamed CNS areas [35–37].

Although the specific homing of transplanted cells has been demonstrated in spinal cord injury, brain tumors, epilepsy as well as in stroke, the exact molecular mechanisms that sustain this phenomenon have been detailed in particular in experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. Tethering, rolling and firm adhesion to inflamed endothelial cells and extravasation into inflamed CNS areas are sequentially mediated by the constitutive expression of functional cell adhesion molecules, integrins and chemokine receptors on the surface of NPCs [38,39].

5. Differentiation of stem cells: ES cell-derived precursors as sources for cell replacement

In experimental stroke, it has been shown that stem/precursor cells exhibit the capacity to differentiate into mature neurons and glial cells once migrated into lesioned brain areas [8,23]. Differentiated neurons may reveal histochemical characteristics of host cells, producing neurotransmitters such as serotonin, GABA, acetyl choline or substance P, and forming dendritic branches that grow out into the cell environment [23]. Electrophysiological studies of these neurons may show characteristics of resting potentials, membrane currents and action potentials very similar to mature neurons [23].

However, only a small percentage of transplanted cells undergo terminal differentiation in the host tissue [12] and the number of differentiated transplanted cells remains very small compared with the large number of cells that are lost following a stroke, even when optimistic stereological estimations are used. Thus, doubts have been raised whether the improvement of function may indeed be a consequence of a significant cell replacement phenomenon. Since not only neurons, but also the tissue matrix, is injured by a stroke, hopes that three-dimensional tissue architectures may easily be reconstructed by transplantation of loose cells have largely been abandoned in the meantime [12].

6. Supportive effects: direct and indirect neuroprotective actions of NPCs

Experimental evidence does not strongly support the possibility that recovery from brain damage via cell replacement is easily achievable in stroke via transplantation of both ES and adult NPCs [8,23]. However, these cells may promote the survival and remodeling of the injured brain via the so-called bystander effect, which defines the capacity of NPCs to exert direct neuroprotection through neutralization of free radicals, inflammatory cytokines, excitotoxins, lipases peroxidases and other toxic metabolites that are released following an ischemic event [40]. In addition to these effects, it has also been shown that adult NPCs may exert an immunomodulatory action, while in an undifferentiated state, causing a profound downregulation of inflammatory T cells and macrophages within inflamed brain areas [15,38,39,41–43].

7. Supportive effects: plasticity-promoting actions of NPCs

Once ischemic injury has developed, the affected tissue activates recovery processes aiming at the restoration of function. Factors released in a paracrine and/or endocrine way by injured as well as spared nervous tissue (neurons, glia, inflammatory cells) induce axonal sprouting of surviving neurons, facilitating the formation of new synaptic contacts that take over lost functions [44,45]. The timing and coordination of such events is crucial for the success of the regenerative process [44].

Synaptic plasticity can be enhanced by physical activity, as well as by experimental manipulations aiming at the antagonization of growth-repulsive influences [45]. NPCs might have important roles in augmenting recovery processes and scavenging inhibitory molecules that might limit the reorganization of the injured brain [46]. That cell-based therapies may indeed enhance the reorganization of white matter tracts surrounding an ischemic infarct has recently been shown by magnetic resonance imaging using fractional anisotropy (FA) and diffusion tensor imaging (DTI) sequences [21].

8. Genetically-modified NPCs as tool for drug delivery

Studies in experimental models of ischemic stroke have shown that a major limitation for the efficacy of neuroprotective therapies is the inability of drugs to reach their target tissue [6,47]. In view of their pathotropism and their long-term persistence in target tissues, NPCs represent a promising vehicle for targeted drug delivery. The innate capacity of stem cells to release protective molecules can further be increased by genetically transfecting cells to secrete additional neuroprotective peptides [48] or molecules that modify the transplanted stem cell fate [49]. In Parkinson’s disease, NPC transplants secreting glial-derived neurotrophic factor (GDNF) and vascular-endothelial growth factor (VEGF) have shown beneficial results in experimental studies and are presently assessed in pre-clinical trials [50].
9. Recommendations for clinical trials

Recent evidence consistently challenges the sole and limited view that neural stem/precursor cells may protect the CNS from inflammatory damage leading to neurodegeneration exclusively throughout cell replacement. As a matter of fact, NPC transplantation may also promote CNS repair via intrinsic neuroprotective bystander capacities, mainly exerted by undifferentiated stem cells releasing, at the site of tissue damage, a milieu of survival promoting molecules whose in situ release is temporally and spatially orchestrated by environmental needs. Thus, the concept of ‘therapeutic plasticity’ is emerging since stem/precursor cells might adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions. The challenging ability of transplanted NPCs to protect the brain from several types of injuries using different and/or articulated bystander strategies is of pivotal importance for the future of stem cell-based therapeutic approaches in humans.

In preparation for such trials, molecular actions of NPCs should be evaluated more thoroughly in the stroke brain. Specifically, additional insight from animal experiments is urgently needed regarding the long-term effects of NPCs in the host organism, which should preferably be obtained also in primates, in order to test the safety of the cells used. In subsequent patient trials, control conditions should stringently be included in phase II studies, possibly in form of cross-over designs, in which a verum and placebo are delivered at two different time points. This is necessary, as control conditions provide the only possibility to provide the proof-of-concept of the efficacy of NPCs. With concerted efforts on an EU basis, it should be possible soon to obtain reliable data about the efficacy of NPCs in ischemic stroke.

References


