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Toward a More Effective Intravascular Cell Therapy in Stroke

Bhimashankar Mitkari[1], Erja Kerkelä[2], Johanna Nystedt[2], Matti Korhonen[2], Tuulia Huhtala[3] and Jukka Jolkkonen[1]

[1]*Institute of Clinical Medicine – Neurology, University of Eastern Finland, Kuopio,*
[2]*Finnish Red Cross Blood Service, Advanced Therapies and Product Development, Helsinki,*
[3]*A. I. Virtanen Institute, University of Eastern Finland, Kuopio, Finland*

1. Introduction

Cerebral ischemia remains the main cause of adult disability in Western countries. More than 50% of stroke survivors are left with a motor disability, causing a huge burden for patients, relatives and healthcare systems (Bonita et al., 1997). Cell-based therapies have emerged as some of the most promising experimental approaches to restore brain function after stroke (Bliss et al., 2010; Banerjee et al., 2011; Lindvall & Kokaia, 2011). A wide variety of cell types have been studied, such as neural progenitors from different sources, including bone marrow- and blood-derived stem cells. Preclinical data with cell therapies are promising (Bliss et al., 2007; Hicks & Jolkkonen, 2009; Hicks et al., 2009a; Janowski et al., 2010). The understanding of how transplanted cells exert their therapeutic effect is, however, not clear, but it is believed that the positive outcome is due to paracrine effects with an improved protective cellular environment (e.g., reduced inflammation, neuroprotection, reduced apoptosis, activation of endogenous repair) rather than as a consequence of neuronal differentiation and cell replacement (Zhang & Chopp, 2009).

The robust therapeutic effect shown in the majority of preclinical studies is somewhat surprising given that cell preparations, experimental models and outcome measures have varied greatly (Table 1). More work is definitely needed to establish standard treatment protocols, which in turn should be expected to lead to effective translation of experimental data. The recently published STEPS guidelines are one step forward to guide future cell-based research in stroke (STEPS Participant, 2009; Savitz et al., 2011). In addition to preclinical recommendations, guidelines on designing early-stage clinical trials are included.

The first patient studies have shown the safety and feasibility of systemic cell therapy, but only marginal therapeutic benefit has so far been observed (Bang et al., 2005; Battistella et al., 2011; Honmou et al., 2011). Whether this is related to the type of cells, study design or low engraftment of the delivered cells is not known. This review provides an update of the current progress in intravascular cell therapy in stroke with a particular emphasis on strategies of how to improve the therapeutic effects.

Stroke model	tMCAO, pMCAO, endothelin-1, cortical photothrombosis, hypoxia-ischemia
Species	rats, mice
Cell type	rat/mouse/human cells from BM, UCB or adipose tissue, neural cells, genetically modified cells
Delivery route	intravenous (tail vein, femoral vein), intra-arterial (common carotid artery, internal carotid artery, external carotid artery)
Delivery time	30 min - 1 month after the ischemic event
Outcome measures	histology (e.g., MAB1248), behavioral testing (e.g. sensorimotor, cognitive), imaging (e.g. MRI, SPECT, optical imaging)

BM – bone marrow; MRI – magnetic resonance imaging; UCB – umbilical cord blood; SPECT – single photon emission computed tomography; tMCAO – transient middle cerebral artery occlusion; pMCAO – permanent middle cerebral artery occlusion

Table 1. Variables with cell-based therapy in experimental stroke

2. Special challenges in intravascular cell therapy in stroke

Cell-based therapy after massive ischemic damage in stroke patients can be challenging compared to diabetes or Parkinson's disease, in which a restricted population of cells is lost. Not only neurons, but also glial cells and blood vessels need to be repaired. Severe odema and vascular compression associated with ischemic damage may limit the engraftment of cells, particularly in areas adjacent to infarct. Another distinction is that stroke is an acute injury with little or no degenerative process. Appropriate transplantable cells may not be immediately available for such an emergency. In addition, while early cell transplantation may provide neuroprotection, the hostile environment endangers the long-term survival of transplanted cells. Transplantation at later time points may be more realistic, targeting secondary neurodegeneration and promoting enhancement of the brain's own repair mechanisms (Zhang & Chopp, 2009). Although cell survival may be preferable, scar formation and a lack of functional vasculature may limit the therapeutic benefit. The advantage is, however, that cell transplantation can be combined with other rehabilitative treatments to ensure maximal therapeutic benefit (Hicks et al., 2009b).

Efficient cell delivery and an optimal delivery route are the keys to successful clinical outcomes, especially in all novel forms of cell therapy. Optimal cell delivery will be indication-dependent and local transplantation has until now been considered as the primary choice for regenerative tissue treatments. Systemic introduction should, however, be the ultimate goal for cell therapy, enabling rapid off-the-shelf therapy in any clinic and this would also allow less invasive treatments. Both stereotactic transplantation of cells into the brain and systemic delivery have been applied in experimental stroke (Guzman et al., 2008; Hicks & Jolkkonen, 2009). Given that stroke often produces large ischemic damage, it is not known whether a targeted approach can provide efficient and extensive cell engraftment, even with the aid of anatomical and functional imaging to explore the location of cell transplantation. Another concern is the invasive nature of intracerebral transplantation. In contrast, the systemic introduction is minimally invasive and thus perhaps more easily applied in the clinic.

There are, however, some obstacles in the intravenous delivery route for cellular therapeutics, one of the main ones being massive lung adhesion, which has been observed after intravenous injection (Allers et al., 2004; Barbash et al., 2003; Fischer et al., 2009; Gao et al., 2001; Hakkarainen et al., 2007; Kang et al., 2006; Mäkinen et al., 2006; Meyerrose et al., 2007; Nystedt et al., 2006; Schrepfer et al., 2007; Tolar et al., 2006; Vilalta et al., 2008). In addition to the negative impact this has on the possibility of reaching clinically relevant cell numbers in target organs, lung entrapment of mesenchymal stem cells (MSC) has also been observed causing severe lung damage in mouse models (Anjos-Afonso et al., 2004; Lee et al., 2009a). Importantly, pulmonary toxicity is reported as one of the most common non-hematological complications after autologous bone marrow transplantation in humans, a complication that is also detectable in a mouse model (Bhalla & Folz, 2002). Interestingly, and on the contrary, beneficial effects have been found after MSC lung entrapment, where embolized human MSCs improved myocardial infarction in mice through secreting the anti-inflammatory protein tumour factor-stimulated gene-6 (TSG-6) (Lee et al., 2009b).

3. Cell types used in stroke

Stem cells are defined as undifferentiated cells capable of self-renewal and differentiation. Truly totipotent stem cells can only be found in the embryo and these are capable of producing a new individual upon implantation. Depending on their origin, stem cells are classified as pluripotent (i.e., embryonic) or multipotent (i.e., fetal and adult) stem cells, referring also to their differentiation capacity. Intracerebral transplantation is the primary delivery route for embryonic stem cells (ESC), induced pluripotent stem cells and fetal stem cells in experimental stroke. Thus only intravascular delivery of adult stem/progenitor cells and genetically modified cells will be discussed in the following chapters.

3.1 Adult stem/progenitor cells

The majority of systemic transplantation studies in stroke have used non-neural cells; cells from bone marrow (BM), umbilical cord blood (UCB), adipose tissue, or peripheral blood. These are all typically defined as adult stem/progenitor cells and represent a group of heterogeneous cell types. Usually many cell types are present in the population, such as mesenchymal stem/stromal cells, hematopoietic progenitors and endothelial progenitors, as well as more mature cell types (Erices et al., 2000; Herzog et al., 2003; Harris et al., 2008). Typically either the whole cell population has been used or a subpopulation has been selected with, e.g., cell surface markers or culture conditions (like adherent MSCs). Adult stem cells lack the ethical controversies associated with embryonic or fetal cells and they are rather easily obtained from different clinical sources.

Different adult stem/progenitor cell populations have been reported to enhance functional recovery in experimental stroke models. When considering studies using human cells, mostly bone marrow stem/stromal cells (BM-MSC) (Li et al., 2002; Zhao et al., 2002; Chen et al., 2003; Zhang et al., 2004; Omori et al., 2008; Andrews et al., 2008; Mays et al., 2010; Yang et al., 2010; Bao et al., 2011) or UCBCs (Chen et al., 2001; Willing et al., 2003a; Borlongan et al., 2004; Vendrame et al., 2004; Xiao et al., 2005; Newcomb et al., 2006; Chen et al., 2006; Mäkinen et al., 2006; Zhang et al., 2011; Riegelsberger et al., 2011) have been used. Most studies have administered cells early (6-48 h) or at subacute phase (2-7 days) after stroke

and only few comparisons have been made. Omori et al. (2008) compared multiple time points and found that the greatest functional benefit was achieved when BM-MSCs were injected 6 h after stroke compared to later time points, which is supported by the finding of Yang et al. (2010) that cells delivered 1 day have greater effect than those at 7 days. Instead, Mays et al. (2010) reported time window from 1 to 7 days post-stroke to be equally beneficial. For UCBCs, time window up to 30 days post-stroke was found to be therapeutically beneficial (Zhang et al., 2011).

In addition to BM and UCB cells, peripheral blood progenitor cells (Willing et al., 2003b), endothelial progenitors (Fan et al., 2010; Moubarik et al., 2011), CD34-positive progenitors from UCB (Taguchi et al., 2004; Boltze et al., 2005; Nystedt et al., 2006), CD133-positive cells from BM (Borlongan et al., 2005; Bakondi et al., 2009), as well as MSCs from placenta (Kranz et al., 2010) have provided therapeutic benefit in stroke. Also in these studies mostly early administration has been employed. For CD133 cells, delayed administration (7 d) was shown to improve graft survival but behavioral improvement was only apparent in immediate intravenous delivery (Borlongan et al., 2005).

MSCs from BM or UCB (or other tissues) are a particularly promising candidate for cell therapy in stroke. MSCs are defined as multipotent stem cells that are adherent and express CD73, CD90 and CD105. They show the potential to differentiate into bone, cartilage and fat, and also exhibit additional differentiation capacity (Dominici et al., 2006). MSCs can be highly expanded in culture with a minimal loss of multipotency and they show very little immunogenic activity. This is a major advantage, allowing them to be potentially used as allogeneic "off-the-shelf" products. They have already been explored in many experimental models and clinical trials for their beneficial effects to, e.g., regenerate damaged tissue, treat adverse immune reactions, promote angiogenesis, and increase tissue protection, and MSCs are generally considered safe (Malgieri et al., 2010).

Adult stem cells are particularly well suited for non-invasive vascular delivery, since they have been shown to target injured tissue and exert their therapeutic effect through secreted factors (Karp & Teo, 2008; Hess & Hill, 2011). The targeting of cells to the brain and especially their survival *in situ* have proven challenging, as in most studies very few cells are actually found in the brain. Interestingly, however, this may not be crucial, as intravenously administered cells may have a therapeutic effect on the brain by acting from peripheral organs as well, such as the spleen and the lung (Hess & Hill, 2011).

As a summary, adult stem cells have been shown to exert their positive effect through soluble factors that reduce apoptosis and promote neuroprotection, angiogenesis, brain plasticity, and/or endogenous progenitor proliferation. Some studies have shown differentiation towards neuronal phenotype, but the significance of this remains unclear.

3.2 Genetically modified cells

In addition to stem cells, several neural cell lines have been reported to enhance functional recovery after experimental stroke by intravenous delivery of cells (Jeong et al., 2003; Chu et al., 2004; Lee et al., 2008; Narantuya et al., 2010). These cell lines are immortalised and thus have the advantage of unlimited expansion in culture. However, there is a potential risk of malignant transformation (Newman et al., 2005).

One approach has been to use immortalised human MSCs to, e.g., expand the limited life-span of MSCs or include a gene for efficient *in vivo* tracking of cells. These cells have also shown positive effects in experimental stroke models when delivered intravenously (Honma et al., 2006; Wakabayashi et al., 2010). A critical aspect with these cells is that they should not lose their MSC phenotype upon modification.

Human BM-MSCs have also been genetically modified to express neuroprotective/ angiogenic growth factors, such as brain derived neurotrophic factor (BDNF) (Kurozumi et al., 2004; Nomura et al., 2005), placental growth factor (PlGF) (Liu et al., 2006), glial cell line-derived neurotrophic factor (GDNF) (Horita et al., 2006), erythropoietin (Cho et al., 2010), and vascular endothelial growth factor (VEGF) combined with angiopoietin-1 (Toyama et al., 2009). All these modified MSCs have shown their ability to improve functional recovery in ischemic rats, compared to unmodified MSCs, when delivered intravenously. GDNF-modified human UCB CD34+ cells have also shown similar positive effects *in vivo* supporting the combined gene and stem cell therapy for the treatment of stroke (Ou et al., 2010).

4. Cell modifications that improve the efficiency of cell therapy

The major problem with intravenous delivery is cell trapping within organs that filter the bloodstream. Previous studies have explored different strategies to minimize lung adhesion and improve homing of systemically introduced cells: use of vasodilators (Schrepfer et al., 2007), pre-bolus injection of MSCs (Fischer et al., 2009), reducing the number of injected cells (Lee et al., 2009b), blockade of α6 and α4 integrins (Bonig et al., 2007; Qian et al., 2006; Bonig et al., 2009; Fischer et al., 2009), heparin saturation of MSCs (Deak et al., 2010) or preincubation of cells with white blood cells (Chute, 2006). Some beneficial effects on lung adhesion have been concluded, but the major mechanism behind this profound phenomenon is still unsolved. Interestingly, glycosylation engineering of stem cell surfaces by enzymatic *ex vivo* cell surface fucosylation has improved the homing and engraftment capacity of cord blood-derived cells (Xia et al., 2004) and, interestingly, the homing of BM-MSCs to the bone marrow (Sackstein et al., 2008). One feasible approach to alter cell surface structures and migratory behavior is also through culture conditions. A recent preclinical study has shown that low passage and low-density cultures of BM-MSCs impact cell structures that favour *in vivo* targeting to the infarcted heart (Lee et al., 2009a). Culturing MSCs in low oxygen increases the levels of relevant cell surface chemokine and growth factor receptors, subsequently increasing the *in vitro* migratory behavior and the therapeutic potential of MSCs (Hung et al., 2007; Rosova et al., 2008). Cells are normally maintained in a 20% O_2 tension in culture, but a lower oxygen tension in culture is more akin to the physiological niche for the MSC in the bone marrow or placenta (2-7% O_2) and would facilitate in maintaining the authentic *in vivo* identity of the MSCs. Culturing MSCs without animal-derived reagents can produce beneficial changes in expression levels of important adhesion receptors and the secretion potential of trophic mediators, which might have an important impact on cell migratory behavior and therapeutic potential. To support this, Bieback et al. (2009) recently showed differential expression of the fibronectin receptor CD29 between MSCs cultured in fetal bovine serum versus human blood components. The impact of MSC xenofree culture conditions have not yet been studied or reported in preclinical stroke models.

5. Effect of administration route

The most effective transplantation route to deliver cells into the brain following cerebral ischemia remains to be addressed. Noninvasive intravascular administration of cells has perhaps the most immediate access for clinical applications. It provides a broad distribution of cells in close proximity to ischemic tissue, although the entry of intravenously injected cells into the central nervous system may not be required for therapeutic effects (Borlongan et al. 2004). However, a reliable estimation of cell numbers in the brain in relation to other organs is lacking.

Modern imaging methods such as single photon emission computed tomography (SPECT), positron emission computed tomography (PET), magnetic resonance imaging (MRI) or optical imaging can be used for the *in vivo* tracking of cells. Excellent reviews on the different imaging modalities are available (Sykova & Jendelova, 2007; Gera et al., 2010). SPECT imaging with indium oxine (^{111}In-oxine) offers an efficient method to study the whole body biodistribution of cells in stroke models (Figure 1). Firstly, the labeling of the cells is straightforward and relatively simple without significant loss of cell viability. The most common labels are ^{111}In-oxine or technetium-hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO). Double labeling with ^{111}In and ^{131}I or ^{18}F- fluorodeoxyglucose (FDG) and ^{111}In is also possible (Blocklet et al., 2006; Stodilka et al., 2006). Secondly, the half-life of ^{111}In is optimal for several days follow-up after a single injection. Additional advantages of SPECT include high sensitivity, short scanning times (<5 min), and possible multimodal imaging (MRI, CT) with the same stereotaxic coordinates. More importantly, whole-body imaging provides an estimation of the proportion of injected cells that eventually enter the brain in relation to other organs, to help in the assessment of the functional value of transplantation. SPECT imaging is also truly translational and the same tracers can be used in human studies (Correa et al., 2007; Barbosa da Fonseca et al., 2010).

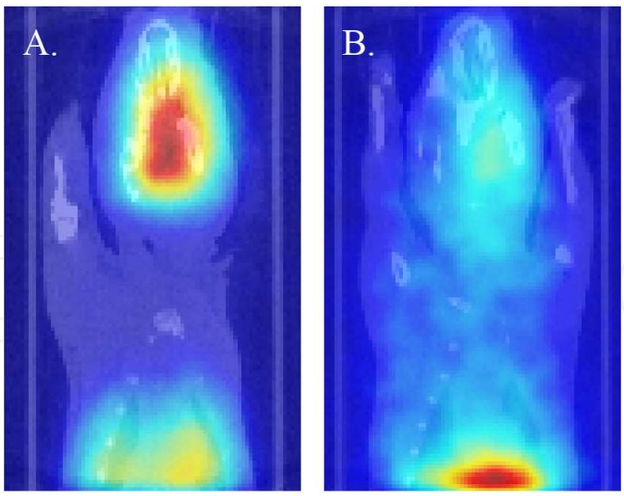

Fig. 1. Combined SPECT/CT images of ^{111}In-oxine labeled human bone marrow-derived mesenchymal stem cells (BM-MSCs) in a rat subjected to middle cerebral artery occlusion (MCAO). Images are taken 20 min (A) and 24 h (B) after intra-arterial administration of cells (4×10^5 cells; 3 MBq) 24 h after MCAO surgery. Please note the initial high signal in the brain followed by relocation of cells into the internal organs. A minor signal remains in the ischemic hemisphere.

Several studies have compared different administration routes. Willing et al. (2003a) concluded that intravenous administration of human UCBCs may be more effective that intracerebral transplantation. ^{111}In–oxine labeled human UCBCs have, however, been shown to localize primarily to the internal organs post intravenous injection in rats after middle cerebral artery occlusion (MCAO) (Mäkinen et al., 2006). Chen and co-workers also showed that after intravenous administration of human UCBCs in MCAO rats, only 1% of injected cells were detected in the brain (Chen et al., 2001). Undesirable biodistribution is most likely caused by the accumulation of cells in the trapping and filtering organs such as the lung, liver, and spleen, rather than due to the cell type injected or timing of administration. Thus, intra-arterial cell infusion may be a more efficient route to circumvent trapping in the internal organs and to target cells towards the ischemic brain (Lappalainen et al., 2008; Walczak et al., 2008; Li et al., 2010; Chua et al., 2011). Indeed, intra-arterial infusion resulted in minor engraftment of human ESCs into the ischemic hemisphere while no SPECT signal was detected after intravenous infusion (Lappalainen et al., 2008). Walczak et al. (2008) compared intravenous and intra-arterial delivery of MSCs in MCAO rats by using combined laser Doppler blood flow monitoring and MRI of iron labeled cells. The intra-arterial but not intravenous cell injection was shown to provide successful but variable cerebral engraftment, which was possibly due to microvascular occlusions. Engraftment was associated with high morbidity as also confirmed by Li et al. (2010). Later, it was shown that a modified injection technique with preserved flow in the carotid artery prevented decrease in cerebral blood flow and micro-occlusions (Chua et al., 2011). Intra-arterial over intravenous administration is also supported by the transplantation of mouse neural stem cells in a hypoxia-ischemia mouse model (Pendharkar et al., 2010). More importantly, a sustained presence (2 weeks) of transplanted cells in the brain was observed after intra-arterial administration. However, recently both intravenous and intra-arterial routes were shown to equally improve neurological recovery and provide neuroprotection (Gutierrez-Fernandez et al., 2011). In all above-mentioned studies, cell were administered within 24-48 h of ischemia.

Taken together, the delivery route seems to have an impact on the biodistribution of transplanted cells. Intra-arterial administration provides superior delivery of cells to the ischemic brain, although this depends on the type of cells and the experimental model employed.

6. Effective dose and therapeutic time window for cell transplantation

The effective cell dose needed for therapeutic effects in stroke animals is not well known. Intravenous infusion of 10^4 up to 5 x 10^7 human UCBCs improved behavioral deficits in MCAO rats in a dose-dependent manner, when administered at 24 h of ischemia (Vendrame et al., 2004). A dose of 10^6 cells was the threshold to promote functional recovery. Similarly, human umbilical tissue-derived cells at doses more than 3 x 10^6 have improved the behavioral outcome and enhanced several brain repair mechanisms (Zhang et al., 2011). A meta-analysis of 60 preclinical studies also found a dose-response association between the injected cell number and treatment effects (Janowski et al., 2010). Interestingly, the greatest therapeutic benefit is achieved following a single high cell dose injection of human MSCs (3.0 x 10^6) within 6 h of ischemia rather than multiple low dose injections (Omori et al., 2008). Thus, repeated dosing may not provide additional benefit. While the dose in

preclinical studies is established to be around 10^6 cells per animal, it is more complicated to estimate the optimal dose for clinical studies. Dosing should be based on a dose-response curve and a maximum tolerated dose, as suggested by STEPS recommendations (STEPS Participant, 2009). The doses in early phase clinical trials were scaled to body weight and have varied from 5 x 10^7 (twice) (Bang et al., 2005) to 0.5 - 5 x 10^8 (Honmou et al., 2011) per patient.

In most of the experimental studies (67%), cells were given <24 h after ischemia (Hicks et al., 2009a). This is partly because of the opening of the blood-brain barrier after cerebral ischemia, which allows cells to enter the brain parenchyma (Belayev et al., 1996). Also, the expression of various chemotactic signals peaks at this time point and guides the cells towards ischemic areas (Imitola et al., 2004; Wang et al., 2008). However, while early cell transplantation may provide neuroprotection (Homna et al., 2006; Horita et al., 2006), the hostile environment endangers the long-term survival of transplanted cells. Transplantation at later time points may target against secondary neurodegeneration and promote enhancement of the brain's own repair mechanisms (Zhang & Chopp, 2009). Komatsu et al. (2010) showed that MCAO rats receiving MSCs up to 1 month after ischemia showed enhanced functional recovery and associated angiogenesis in cortical areas adjacent to the infarct. Shen et al. (2007) have also showed that cell transplantation 1 month after MCAO is effective by leading to long-lasting behavioral improvement. Behavioral and morphological evidence suggest that the post-stroke brain displays heightened sensitivity to rehabilitative treatment early after the stroke (1 wk), but declines with time (2-4 wk) (Biernaskie et al., 2004). Based on this, the time of cell transplantation could be extended to up to 7 days after ischemia.

7. Clinical perspectives and future directions of intravascular cell therapy for stroke

Promising experimental data have prompted early phase I/II patient studies. In these studies, either bone marrow mononuclear cells or bone marrow-derived mesenchymal stem cells have been used. Three phase I studies explored the use of bone marrow mononuclear (BM-MNC) cells for stroke (Barbosa da Fonseca et al., 2010; Battistella et al., 2011; Suarez-Monteagudo et al., 2009). The most important finding of these studies is that intra-arterial delivery of mononuclear cells directly to the infarcted hemisphere is safe. Interestingly, Barbosa da Fonseca et al. (2010) labeled the mononuclear cells with technetium-99m, and followed the distribution of the cells in six stroke patients. They were able to show that the cells remained at the site of the lesion for two hours, but then the signal disappeared on all but two patients after 24 hours. It is unclear whether this short time of action will be enough for any therapeutic benefit.

Bang and co-workers pioneered in the use of MSCs for ischemic stroke with two studies (Bang et al., 2005; Lee et al., 2010). In the first one, five patients with stroke received autologous MSCs as two intravenous infusions of 5 x 10^7 cells each. The outcome of the patients after one year was compared to 25 randomized controls in an open-label study. The second study followed the same protocol as the first one but included 16 patients and 36 controls, and the patients were followed for five years. Both studies concluded that intravenous infusion of MSCs in stroke patients was safe. There was no apparent increase in mortality, bovine spongiform encephalitis or other zoonoses, arrhythmias, seizures, or

tumors. The true incidence of possible side effects of MSC therapy can, however, only be evaluated after much larger patient groups have been treated and followed. Interestingly, patients showed significant improvement in the Barthel Index (BI) in both studies, and a trend towards improvement in the modified Rankin Scale (mRS) in the first study. There was some concern as to whether the improved functional recovery was upheld with time (Bang et al., 2005), but later this was confirmed as improvement could still be measured at 3.5 years (Lee et al., 2010). Because of the time required to produce the autologous therapeutic cells used, the infusion of the cells occurred rather late, i.e. the first cell infusion was given at weeks 4-5 and the subsequent one at weeks 7-9 after the onset of the stroke. Honmou and colleagues (2011) studied the safety and feasibility of intravenous infusion into stroke patients of autologous MSCs that had been expanded in autologous serum. The cells were infused 33-133 days post-stroke, and the patients were followed and imaged at one year after. No adverse events were recorded.

Several important questions still need to be addressed in both preclinical and clinical testing. It is unclear which cell type is therapeutically the most beneficial. Both BM-MNCs and MSCs have shown promise in preclinical testing. Selecting the best route of therapeutic cell delivery is also a major issue. Presumably, the route yielding the most effective delivery of cells to the injured tissue might offer most therapeutic potential. In a recent study comparing intra-arterial and intravenous MSC delivery, infusion directly into the internal carotid artery resulted in more engrafted cells as well as a more widespread distribution of cells within the infarcted hemispheres of rats (Li et al. 2010). However, this mode of administration was also associated with high mortality as the MSCs were sequestered in the blood vessels of the treated hemisphere and formed micro-occlusions. Careful development of safe but effective modes of administration is required for the advancement of this technique towards the clinic. In contrast to MSCs, BM-MNCs can apparently be administered via the intra-arterial route without harmful effects.

Another important question is the preferred timing of the cell infusion. There is some evidence suggesting that MSCs infused early (within days of the infarct) have more therapeutic efficacy than those administered several weeks after the event (Zhou et al., 2011). Theoretical considerations support these findings: a major mode of action of the MSCs in stroke is attenuation of the post-infarct inflammatory milieu, which is at its strongest during the early days following the insult (Ohtaki et al., 2008). BM-MNCs have the advantage of having no need for cell expansion. Thus, the patient can be treated soon after stroke even if autologous cells are used.

Whether the patient should be given autologous or allogeneic MSCs is a major unanswered question. If the patient is given autologous cells, the therapy will necessarily take place several weeks after the infarct due to the time required to produce the cells. Allogeneic cells offer the critical advantage of being available off-the-shelf. It can be argued that allogeneic cells are quickly rejected and will rapidly lose their therapeutic efficacy, but if only short term action is required e.g., to modulate post-infarct inflammation, allogeneic cells may be an ideal therapeutic vehicle. In experimental animals, allogeneic cells appear to function as equally well as autologous MSCs (Li et al., 2006). Furthermore, risks of tumor formation are reduced, because the allogeneic cells are eventually rejected by the host's immune system (Poncelet et al., 2007).

Finally, the optimal characteristics of the therapeutic cells need to be determined. Special attention needs to be given to the conditions of cell production, because the administration of apoptotic or senescent cells is not only ineffective, but positively harmful (Modo et al., 2003; Prockop et al., 2010). Another important advance will be the adoption of xenofree culture methods for MSCs, reducing the risks of anaphylaxis and transmission of diseases (Horwitz et al., 2002). Modification of the cell surface prior to administration, to improve the delivery or therapeutic efficacy of the cells, is a promising strategy.

Taken together, there is already preliminary evidence for the safety of intravenously delivered BM-MNCs and MSCs for stroke patients. Further work is required to establish this safety profile. However, careful studies that also use an intra-arterial application of cells should proceed. Futhermore, the use of allogeneic MSCs should be explored, allowing treatment during the first week after infarct.

Currently, eight clinical phase I-II studies are underway, assessing the safety and possible efficacy of either fractionated BM cells or culture-expanded MSCs in patients with recent ischemic stroke (Table 2). Three studies will address the feasibility and safety of intravenous

Identifier/Sponsor*	**Cell type**	**Time window**	**Adminis-tration route**	**Comments**
NCT00859014/The University of Texas Health Science Center, Houston, USA	autologous BM-MNCs	24-72 h	i.v.	phase I, non-randomized
NCT01028794 National Cardiovascular Center, Japan	autologous BM-MNCs	day 7-10	i.v.	phase I-II, non-randomized
NCT00473057/Federal University of Rio de Janeiro, Brazil	autologous BM-MNCs	day 3 - 90	i.v./i.a.	phase I, completed
NCT761982/Hospital Universitario Central de Asturias, Spain	autologous BM CD34+ cells	day 5-9	i.a.	phase I-II, non-randomized, completed
NCT01273337/Aldagen, USA	autologous BM ALDH-positive cells	day 13-19	i.a.	phase II, randomized
NCT01297413/Stemedica Cell Technologies Inc, USA	allogeneic MSCs	> 6 mo	i.v.	phase I-II, non-randomized
NCT00875654/ University Hospital, Grenoble, France	autologous MSCs	within 6 wks	i.v.	phase II, randomized trial
NCT01389453/General Hospital of Chinese Armed Police Forces; China	allogeneic cord blood MSCs	day 7-14	i.v.	phase I, non-randomized

*from www.clinicaltrials.com

Table 2. Summary of ongoing clinical trials with intravascular administration of cell therapy in stroke. Only recruiting or completed studies are listed.

BM-MNCs, and one of these will compare intravenous with intra-arterial delivery. Two more trials will utilize BM cells that have been selected using stem cell markers (CD34 or aldehyde dehydrogenase). Three studies evaluate the use of MSCs for stroke, one of them using the patients' own and two using allogeneic cells. In most of these early studies, cells will be administered via the intravenous route. These studies, and others addressing the questions posed above, may help us in ameliorating the devastating consequences of ischemic stroke at the personal and societal level.

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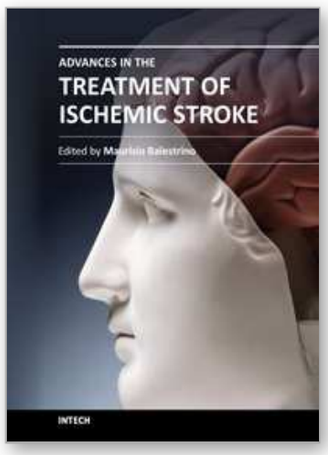

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In recent years research on ischemic stroke has developed powerful therapeutic tools. The novel frontiers of stem cells therapy and of hypothermia have been explored, and novel brain repair mechanisms have been discovered. Limits to intravenous thrombolysis have been advanced and powerful endovascular tools have been put at the clinicians' disposal. Surgical decompression in malignant stroke has significantly improved the prognosis of this often fatal condition. This book includes contributions from scientists active in this innovative research. Stroke physicians, students, nurses and technicians will hopefully use it as a tool of continuing medical education to update their knowledge in this rapidly changing field.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
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InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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