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Stem cell therapies in preclinical models of stroke. Is the aged brain microenvironment refractory to cell therapy?

Raluca Elena Sandu [b], Adrian Tudor Balseanu [b,1], Catalin Bogdan [b], Mark Slevin [c], Eugen Petcu [d], Aurel Popa-Wagner [a,b,*]

[a] Department of Psychiatry, University Hospital Rostock, Germany
[b] University of Medicine and Pharmacy of Craiova, Chair of Biochemistry, Neurobiology of Aging Group, Romania
[c] Department of Healthcare Science, Manchester Metropolitan University, Manchester, UK
[d] Griffith University School of Medicine, Gold Coast Campus, QLD 4222, Australia

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ABSTRACT

Stroke is a devastating disease demanding vigorous search for new therapies. Initial enthusiasm to stimulate restorative processes in the ischemic brain by means of cell-based therapies has meanwhile converted into a more balanced view recognizing impediments that may be related to unfavorable age-associated environments. Recent results using a variety of drug, cell therapy or combination thereof suggest that, (i) treatment with Granulocyte-Colony Stimulating Factor (G-CSF) in aged rats has primarily a beneficial effect on functional outcome most likely via supportive cellular processes such as neurogenesis; (ii) the combination therapy, G-CSF with mesenchymal cells (G-CSF + BM-MSC or G-CSF + BM-MNC) did not further improve behavioral indices, neurogenesis or infarct volume as compared to G-CSF alone in aged animals; (iii) better results with regard to integration of transplanted cells in the aged rat environment have been obtained using iPS of human origin; (iv) mesenchymal cells may be used as drug carriers for the aged post-stroke brains. Conclusion: While the middle aged brain does not seem to impair drug and cell therapies, in a real clinical practice involving older post-stroke patients, successful regenerative therapies would have to be carried out for a much longer time.

1. Age as a key factor in preclinical stroke studies

Cerebral ischemia is a common disease in the older population and the second most common cause of death in Europe, and the third leading cause of death in Canada and the United States (Lloyd-Jones et al., 2010; Roger et al., 2012).

Age is the principal nonmodifiable risk factor for cerebral ischemia. The incidence of stroke increases significantly with age in both men and women, with half of all strokes occurring in people over the age of 75, and one-third in the population over age 85 (Roger et al., 2012). Further, there are gender differences in the incidence of stroke by age subgroups. The incidence of stroke is higher in men up to age 75, similar in the 75–84 age group, and higher in women in the age group older than 85 (Roger et al., 2012). This may be attributed to the longer life span in women. There are many other gender differences with regard to stroke outcome, risk factors, treatment, and mortality that have more complicated and unexplained underlying etiologies. The age-associated decline in functional reserves is most pronounced in advanced age of 85 or older and implies an impaired response to stressors and illnesses.

Overall, age-associated changes also show great variability among individuals and are often impacted by genetic and long-term lifestyle factors (Wolfson et al., 2009; Tacutu et al., 2010; Tacutu et al., 2011).

2. Stroke models using aged animals are clinically more relevant

Studies of stroke have demonstrated an age and gender effects on incidence, functional recovery and mortality, not only in humans but also in animal models (Bergerat et al., 2011; Gokcay et al., 2011). Indeed, the age-dependent increase in the evolution of ischemic tissue into infarction strongly suggests that age is a biological marker for the variability in tissue outcome in acute human stroke (Ay et al., 2005).

Over the past 20 years, suitable models for stroke in aged rats have been established Bacigaluppi et al., 2009. All are based on the middle cerebral artery occlusion (MCAO). MCAO has been produced with permanent or transient occlusion for 30–120 min using a thrombus through intraluminal filament occlusion or a hook attached to a micromanipulator, or by occlusion of distal branches of the MCA, while long-term hypoxia-ischemia could also be induced by unilateral common carotid

* Corresponding author at: Department of Psychiatry, University of Medicine Rostock, Gehlsheimerstr. 20, 18147 Rostock, Germany.
E-mail address: aurel.popa2011@googlemail.com (A. Popa-Wagner).
[1] Equal contribution.

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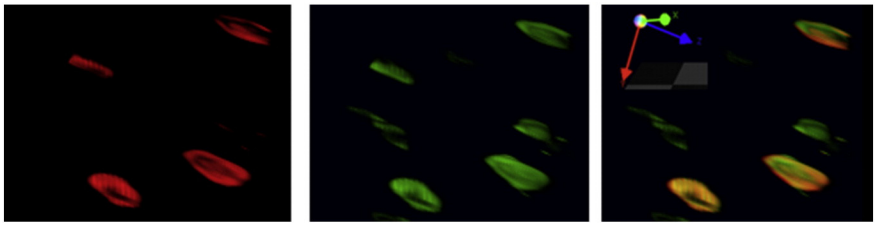

Fig. 1. Three-dimensional imaging of several colocalized BrdU (red)/NeuN (green) cells in the SVZ of the infarcted hemisphere. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

artery occlusion (reviewed in Popa-Wagner et al., Frontiers Cell Neuroscience 2014).

Since epidemiological studies have shown that human stroke occurs more often in late middle age (50–70 years) than in older subjects (over 70 years) (Feigin et al., 2003) justify the use of stroke models in middle aged animals (Popa-Wagner et al., 2007).

3. Limited beneficial effects on mortality, behavioral recovery and neurogenesis of G-CSF in aged rats

The hematopoietic factor Granulocyte-Colony Stimulating Factor (G-CSF), has been shown to reduce infarct volume and improves behavioral outcome after various types of experimental stroke (Shyu et al., 2004; Lee et al., 2005; Xiao et al., 2007; Han et al., 2008). Under ischemic conditions, G-CSF inhibits programmed neuronal cell death (Komine-Kobayashi et al., 2006) and stimulates neural progenitor cell differentiation. These mechanisms and others, including immunomodulation and blood vessel plasticity, are currently thought to be responsible for infarct size reduction and improved functional outcome in young-adult rodent stroke models (Minnerup et al., 2008).

One potential weakness of the preclinical dataset is, however, the lack of proof in aged subjects. It is in fact a general drawback of preclinical evaluations of candidate stroke drugs that due to cost effectiveness and practicability most studies were done in young animals. A lack of data from aged subjects in preclinical studies may at least in part explain the failure of candidate neuroprotective drugs in clinical trials. The aged brain has compared to the young brain an enhanced susceptibility to stroke and displays a limited recovery from an ischemic injury (Popa-Wagner et al., 1998; Badan et al., 2003; Rosen et al., 2005). Therefore we assessed the treatment effects of G-CSF on mortality, behavioral function, infarct volume, and neurogenesis in 19 to 20 month old male Sprague-Dawley rats subjected to 90 min occlusion of the middle cerebral artery (MCA).

One of the remarkable effects of G-CSF treatment was a significant decrease in the mortality rate. However, there was no significant effect of G-CSF on reducing infarct volumes. In contrast to young animals - where recovery of function is complete even after short terms of G-CSF treatment - functional recovery of motor function (rotarod, inclined plane) in aged animals occurred predominantly during the treatment period and was therefore limited to the first 12 days after stroke onset, except the radial maze the beneficial where the beneficial effect lasted for 21 days. G-CSF treatment also had a beneficial effect on functional recovery of motor function (rotarod, inclined plane) and working memory (radial maze). However, the beneficial effect of treatment was generally limited to the first 12 days post-stroke. A stereological analysis of the number of BrdU labeled cells in the SVZ revealed a significant increase in the number of proliferating cells in G-CSF treated animals compared to vehicle treated animals. Further, the G-CSF treatment increased the number of proliferating cells in the SVZ and the dentate gyrus and increased the number of new born neurons in the SVZ, ipsilateral to the lesion (Fig. 1).

4. Cell therapy of stroke using mesenchymal stem cells

Cellular therapy (Fig. 2) can enhance the endogenous restorative mechanisms of the injured brain by supporting processes of neovascularization, neurogenesis, neural reorganization and functional recovery (Chen et al., 2005; Crigler et al., 2006; Bao et al., 2011; Lim et al., 2011; Hayase et al., 2009; Hsieh et al., 2013; Liu et al., 2014).

Mesenchymal stromal cells (MSCs), derived either from bone marrow or from adipose tissue, have been shown to ameliorate the clinical outcome in experimental model of cerebral ischemia (Kocsis and Honmou, 2012). Administration of MSCs in acute stroke animal models markedly decreased brain infarct size, improved neurological function by enhancing neurogenesis, and showed anti-inflammatory and antiapoptotic effects. Additionally, initial clinical studies using

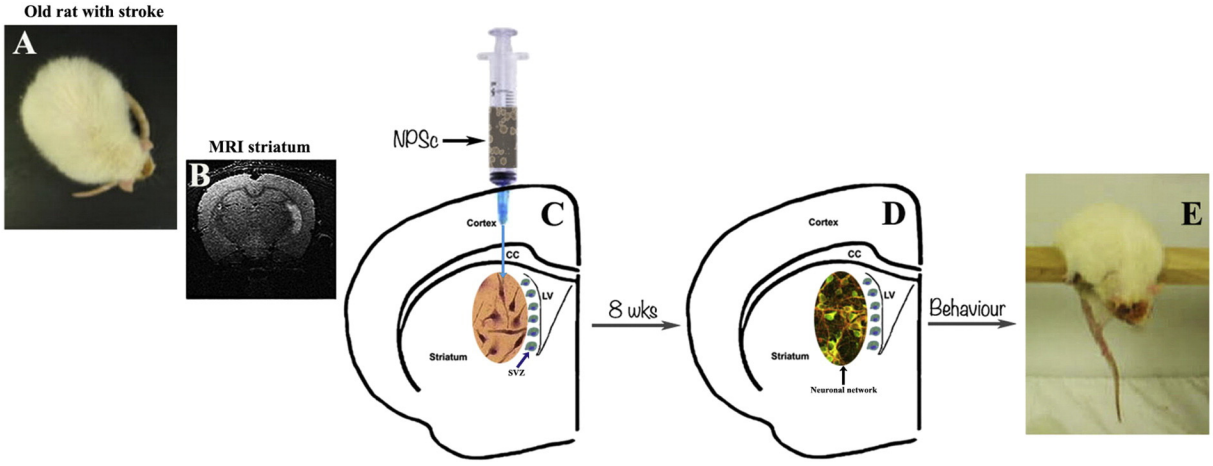

Fig. 2. Stem cell therapy of stroke. (A) Old rat with striatal infarct; (B) Documentation of the striatal infarct by MRI; (C) Injection of NPCs into lesioned striatum; (D) build up of an hypothetical neuronal network weeks later; (E) Behavioral assessment of cell treatment.

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