# Chapter 4
# Treatment of Autologous Bone Marrow Mononuclear Cells for Acute and Subacute Stroke

## Cell Therapy for Acute/Subacute Stroke

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## Introduction

Stroke is the third leading cause of death in developed countries after heart disease and cancer [1], and the leading cause of disability worldwide. More than 50 % of stroke survivors are unable to completely recover, and 20 % of stroke patients require assistance with their daily activities [2]. Acute ischemic stroke is the most common type and has only thrombolysis as a therapeutic option [3]. Although thrombolytic therapy is effective for acute cerebral ischemia, it must be given within 4.5 h after stroke onset [4], and no definitive treatment exists after that period other than rehabilitation. Thus, development of novel therapies to regenerate neuronal function after stroke is eagerly awaited (Fig. 4.1). Recently, many studies have focused on cell-based therapies to repair the ischemic brain [5–9].

There are two main types of cells to enhance endogenous neurogenesis after stroke, i.e., mononuclear cells and mesenchymal stem cells. Although some clinical trials of mesenchymal stem cells have demonstrated safety, feasibility, and preliminary efficacy in stroke patients [10, 11], autologous mesenchymal stem cells require cell culture to obtain the required dose and cannot be administered in patients with acute stroke. In contrast, mononuclear cells can be prepared rapidly within a few hours and permit autologous administration, which avoids the problem of immunological rejection.

To develop novel therapies for patients after stroke, the therapeutic potential of bone marrow mononuclear cells has been investigated in experimental stroke

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D. C. Hess (ed.), *Cell Therapy for Brain Injury,* DOI 10.1007/978-3-319-15063-5_4

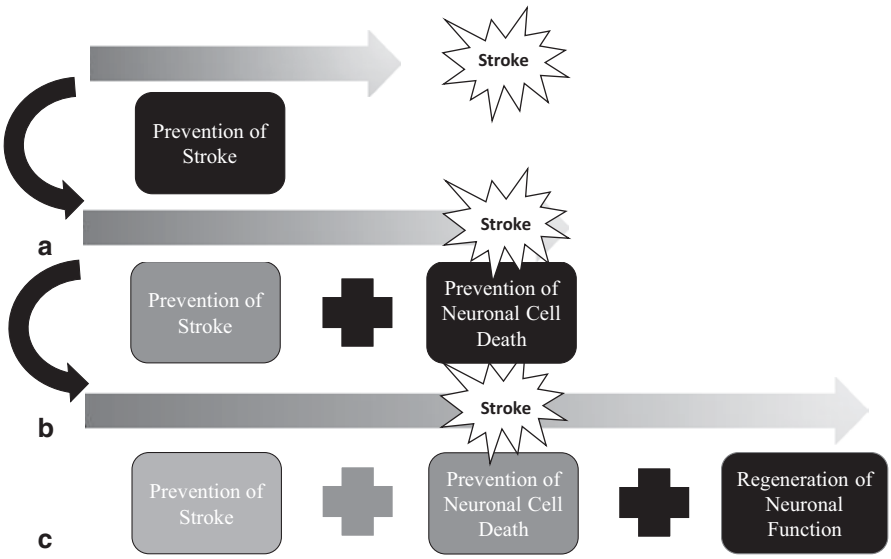


**Fig. 4.1** Change in the strategy for stroke. **a** Treatment of cardiovascular factors and administration of antiplatelet/coagulant drugs significantly contribute to the prevention of stroke onset. **b** In addition to the prevention of stroke onset, development of thrombolysis and neurothrombectomy in the acute period enables prevention of neuronal cell death after cerebral vascular occlusion. **c** Furthermore, establishment of novel therapies that extend the therapeutic time window and broaden treatment options to regenerate neuronal function after stroke is eagerly awaited

models, followed by various clinical trials. This chapter summarizes the findings of recent basic science and clinical studies that have focused on regeneration of the injured brain using autologous bone marrow mononuclear cells in the acute/subacute stage of stroke.

## Neuronal Regeneration After Cerebral Ischemia

Although it had been generally believed that the neuroregeneration in the adult mammalian brain does not occur until the mid-twentieth century, it became recognized that new neurons are continuously generated throughout life in the adult mammalian brain. Under normal, nondisease physiological conditions, neurogenesis is principally restricted to two regions, the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus [12, 13], where unique niche architectures permit continuous neurogenesis [14, 15]. However, accumulating evidence has indicated the presence of neuronal stem cells in a variety of adult brain regions [16, 17]. Moreover, although it remains a matter of controversy as to whether neuronal stem cells in these regions are essentially

similar to SVZ-derived neuronal stem cells, the presence of stroke-induced neuronal stem cells at the cerebral cortex has also been suggested in the adult murine brain [18]. Following an ischemic insult, the proliferation and/or dedifferentiation of endogenous neuronal stem cells is activated in various brain regions, including the SVZ, SGZ, striatum, and cerebral cortex [19] and these neuronal stem cells have been shown to migrate into the injured area, where neurogenesis does not occur under normal conditions [20–22]. Similar to the findings from the murine stroke model, we demonstrated the presence of stroke-induced neural stem/progenitor cells in patients, and that the peak of endogenous neurogenesis is approximately 1–2 weeks after a stroke [23]. These findings indicate the potential for a novel therapeutic strategy using injury-induced neurogenesis for functional recovery in patients with cerebral infarction.

However, the neurogenic response eventually yields only a very small number of mature neurons, as most of these stroke-induced neural stem/progenitor cells do not survive, nor do they contribute to functional recovery after stroke [20]. Thus, appropriate support for the survival of these stroke-induced neural stem/progenitor cells is essential for functional recovery after cerebral ischemia.

## Angiogenesis for the Survival of Injury-Induced Neuronal Stem Cells

In the peri-infarction area, microvascular density decreases [24] and most injury-induced neural stem/progenitor cells are unable to survive there [20]. Angiogenesis after stroke had been investigated as the key element for the survival of injury-induced neural stem/progenitor cells and functional recovery after cortical infarction. Recent studies have indicated that there is a tight correlation between angiogenesis and neurogenesis under both physiological and pathological conditions in the adult brain. In the adult songbird, testosterone-induced angiogenesis leads to neuronal recruitment into the higher vocal center [25]. In the adult rat, endogenous neurogenesis and neovascularization occur in proximity to one another in the cortex following focal ischemia [26]. Moreover, angiogenesis and neurogenesis are regulated by an overlapping set of molecules—for example, sphongosine-1-phosphate plays a critical role in neurogenesis and angiogenesis during embryonic development [27]. The accumulating evidence indicates a close relationship between the vascular system and neurogenesis in the central nervous system, and recent studies that have explored therapeutic strategy have focused on promotion of neurogenesis in association with angiogenesis [6, 9]. Although the coupling and cross talk between endogenous neurogenesis and neovascularization in the cortex of the ischemic brain are still not fully understood, these findings clearly indicate that therapeutic angiogenesis could have a significant role in the functional recovery of stroke patients by enhancing neurogenesis in the poststroke brain.

## Cell-Based Therapy to Enhance Neurogenesis in the Ischemic Brain

To achieve angiogenesis in ischemic tissue, an approach using bone-marrow-derived mononuclear cells, a rich cell source of both hematopoietic stem cells and endothelial stem/progenitor cells, has been proposed. Increasing evidence shows that endothelial stem/progenitor cells play an important role in maintaining vascular homeostasis and repair. Endothelial stem/progenitor cells have been shown to contribute to vascular homeostasis through differentiation to endothelial cells [28] and as a source of numerous growth and angiogenesis factors (e.g., vascular endothelial growth factor (VEGF), hepatocyte growth factor, and insulin-like growth factor I) [29]. Endothelial stem/progenitor cells, mainly obtained from bone marrow cells, have been shown to reduce ischemic damage and enhance functional recovery in experimental models, including limb [30–33], myocardium [34–37], and cerebral ischemia [38, 39] models. Based on these observations, various clinical trials using bone-marrow-derived endothelial stem/progenitor cells are ongoing, with promising results that show improvement of regional perfusion and function in ischemic tissues [40–42].

In addition, we observed that decreased levels of circulating immature bone-marrow-derived cells, such as endothelial stem/progenitor cells, are associated with impaired cerebrovascular function [39] and cognitive impairment [43, 44]. In contrast, high levels of bone-marrow-derived immature cells are associated with neovascularization of the ischemic brain [45].

Based on these observations, we investigated the effect of administrating bone-marrow-derived stem/progenitor cells on stroke using a highly reproducible murine model [46]. We found that transplantation of bone marrow mononuclear cells or hematopoietic stem cells after stroke induces neovascularization at the border of the ischemic zone followed by reconstruction of blood flow, that neovascularization is essential for the survival of neural stem cells in the cortex of ischemic brain, and that the support survival of neural stem cells contributes to functional outcomes improvement [7, 9, 47]. To link these basic findings to clinical trials, we investigated the appropriate cell numbers and optimal therapeutic time window for bone-marrow-derived bone marrow cell transplantation for stroke. We found that the required minimum number of bone-marrow-derived mononuclear cells was $1 \times 10^6$/kg of body weight and the therapeutic time window of administration of bone-marrow-derived mononuclear cells was revealed to be between day 2 and day 14 after stroke [47]. It is notable that this therapeutic time window overlaps with the peak in endogenous neurogenesis after stroke [23]. This positive effect of bone-marrow-derived mononuclear cells was negated by administration of an anti-angiogenesis reagent [7]. These findings suggest that therapeutic angiogenesis, achieved by administering bone marrow mononuclear cells, could be a novel therapeutic strategy for patients after stroke.

Although the mechanisms that link endothelial stem/progenitor cells, including bone marrow mononuclear cell transplantation, and angiogenesis is not fully

understood, a recent study suggested that treatment with bone marrow mononuclear cells at the acute stroke stage increases cerebral blood flow (CBF) through endothelial nitric oxide synthase (eNOS) activation and NO production which leads to vasodilation, and subsequently promotes angiogenesis [48].

Based on these findings, clinical trials of the administration of autologous bone marrow mononuclear cells for patients in the acute/subacute stroke stage have been initiated in many institutes, including our hospital.

## Clinical Trials Using Bone Marrow Mononuclear Cells in Patients After Stroke

We conducted a clinical trial to enhance neurogenesis and functional recovery through activating angiogenesis in patients with cerebral infarction. Our trial was an unblinded, uncontrolled phase 1/2a clinical trial aimed at investigating the feasibility and safety of autologous bone marrow mononuclear cell transplantation in subacute stroke patients. Major inclusion criteria were patients with cerebral embolism, a National Institute of Health Stroke Scale (NIHSS) score higher than nine on day 7 after stroke and the improvement in the NIHSS in the first 7 days after onset of less than six points. On day 7–10 after stroke, patients had 25 ml (low-dose group, $N = 6$) or 50 ml (high-dose group, $N = 6$) of bone marrow cell aspiration from the posterior iliac bone under local anesthesia. Autologous bone-marrow-derived mononuclear cells were purified by the density gradient method and administered intravenously on the same day as the aspiration. Primary outcome measures were: worsening NIHSS score (primary safety outcome measure) and change in the NIHSS score evaluated on day 7 after onset of stroke and day 30 after cell transplantation (primary efficacy outcome measure). We also evaluated the changes in regional cerebral blood and regional cerebral metabolic rate of oxygen consumption using steady-state $^{15}O$ positron emission tomography at 1 and 6 months after cell transplantation. The results of the study showed that administration of autologous bone marrow mononuclear cells in patients with severe stroke was both feasible and safe. Furthermore, the positive trends favoring neurologic recovery and improvement in CBF and metabolism in poststroke patients receiving cell therapy underscored the potential of this approach. Details of the results are now under submission. The clinical findings further support our hypothesis that bone marrow mononuclear cells transplantation after stroke improves CBF and neuronal activity that results in acceleration of functional recovery (Fig. 4.2). Similar clinical trials to ours, such as transplantation of autologous bone marrow mononuclear cells in stroke patients, are being carried out in other countries, including the USA, India, Brazil, and Spain with promising results [49–52]. Though the route of administration (intravenous or intra-arterial) and the stage of stroke (acute or subacute) vary, no side effects or safety problems with cell therapy have been reported. The current status of most of these ongoing clinical trials can be searched through http://clinicaltrials.gov/.

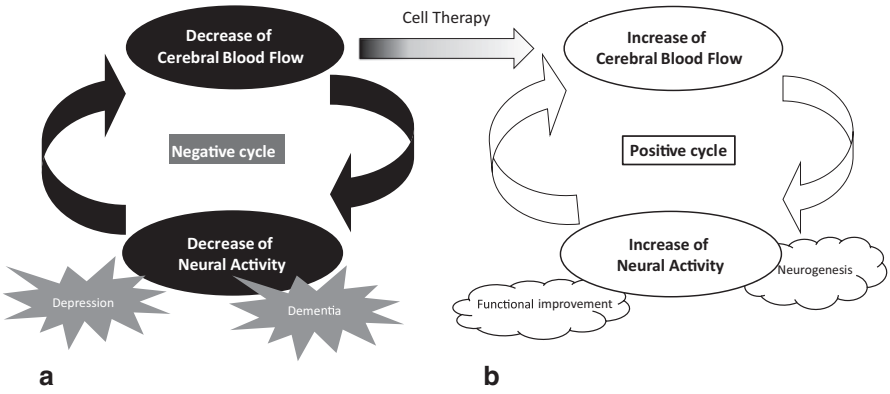


**Fig. 4.2** Activation of microvasculature by cell therapy. **a** Cerebrovascular damage is closely related with cognitive impairment and depression with impaired neural activity and cerebral blood flow. **b** Cell therapy is expected to activate the cerebral microvasculature that enhances neurogenesis and functional recovery after stroke

## Further Development of Cell Therapy for Patients After Stroke

Although several studies have indicated that bone-marrow-derived cells could be a source of endothelial cells [53, 54], a growing body of clinical and experimental evidence indicates that the number of injected cells reaching the brain parenchyma seem to be small, i.e., preclinical studies indicate that approximately 0.02–1 % of injected cells home into the brain [55–57]. Despite significant activation of angiogenesis by cell transplantation, the survival of transplanted cells has rarely been observed in experimental models; thus, the differentiation of the stem cells into endothelial cells in the ischemic brain may not play a critical role in angiogenesis after stroke. These findings suggest that cells do not need to remain in the brain to generate functional improvement. Therefore, many investigators have been focusing on where the cells go and what they do. Schwarting et al. suggested that homing of injected cells to spleen suppressed the infiltration of immune cells, such as T cells and monocytes, into the ischemic cerebral tissue, and consequently the infarct size was reduced [55]. Recent studies have reported that higher radioactive counts were observed in the lungs and spleen at 2 h post injection after technetium-99m labeled bone marrow mononuclear cell transplantation in animals and patients after stroke [51, 56]. The therapeutic effect of bone marrow mononuclear cells is achieved, we believe, by the activation of the systemic microvasculature as well as a local response. It is likely that multiple cytokines, growth factors, and cell adhesion molecules are involved, and the balance between these molecules may determine the fate of injured brain tissue.

In conclusion, the positive results of experimental stroke model and clinical trials indicate the potential of cell therapy for stroke patients, and larger scale,

randomized controlled clinical trials are desirable in order to prove the efficacy and long-term safety of such treatment. Furthermore, elucidation of the therapeutic mechanism is one of the key elements in developing novel strategies to improve functional recovery in patients after stroke.

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