Histol Histopathol (2006) 21: 1209-1216

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The application of stem cells in the treatment of ischemic diseases

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Summary. Ischemia causes oxygen deprivation, cell injury and related organ dysfunction. Although ischemic injury may be local, it involves many biochemical changes in different cell types. The ability of stem cells to differentiate into different cell lineages provides the possibility of their use in treating a variety of diseases requiring tissue repair or reconstitution, such as stroke, ischemic retinopathy, myocardial infarction, ischemic disorders of the liver, ischemic renal failure, and ischemic limb dysfunction. Several cell types including embryonic stem cells, various progenitor and stem cells of hematopoietic or mesenchymal origin have been used in attempts to reconstitute injured tissue. Xenologous or autologous stem cells may be administered either through the peripheral vascular system or directly by regional injection. The stem cells are then guided to the infarct site by homing signals. Either by cell differentiation or paracrine effects, stem cells or progenitor cells participate in the reconstruction of a favorable microenvironment resulting in neovascularization and tissue regeneration that eventually improve the physiological function of organs with ischemic damage.

Key words: Stem cells, Ischemic diseases

Introduction

Infarction or ischemia occurring in different organs is a common cause of clinical illness. Occlusion of blood flow from the supplying artery leads to ischemia and oxygen deprivation with associated cell injury to organs. The consequences of ischemia depend on the tissue area involved in the development of the occlusion, the presence of collateral circulation, and the vulnerability of a given tissue to hypoxia. Neurons are subject to irreversible damage when deprived of their blood supply for 3 to 4 minutes and myocardial cells die after 20 to 30 minutes of ischemia (Cotran et al., 1994). Thus, persistent ischemia results in irreversible tissue injury and necrosis.

Ischemia destroys parenchymal cells, vascular cells and nerve cells in affected organs. Cell necrosis triggers a vigorous inflammatory response and a degradation of the extracellular matrix, which is followed by necrotic tissue removal, tissue replacement and tissue remodeling. These structural changes markedly increase mechanical and functional stress on organs resulting in progressive dysfunction (Cotran et al., 1994). The number of cells with proliferating capacity for regeneration in the highly differentiated organs, such as the brain and the heart, is less than in other less differentiated tissues, which therefore have a higher ability for cellular regeneration (Cotran et al., 1994).

Stem cells posses the capacity to self-renew and develop into functionally specialized cells. Implanted stem cells can be integrated into various host organs, survive, and reverse different ischemic deficits (Cogle et al., 2003). Adult stem cells, fetal transplants, immortalized cell lines, and bone marrow stromal cells have now shown promise in experimental models of ischemic diseases including stroke, cardiac infarction, ischemic retinopathy, and limb ischemic injury. Accumulating evidence from animal and human studies shows the possibility that cell therapy exerts a therapeutic effect on human diseases and provides a promising strategy for regenerative medicine.

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Sources of stem cells

Stem cells are capable of self-renewal and have the ability to differentiate into one or more specialized cell types for tissue formation (Cogle et al., 2003). Embryonic stem cells derived from the inner cell mass of blastocysts are totipotent cells that can differentiate *in vitro* into a variety of cell types (Thomson et al., 1998). Adult stem cells which exist in various organs are derived either from hematopoietic cells or mesenchymal cells (Pittenger et al., 1999; Jang et al., 2004; Sakaguchi et al., 2005). They are often multipotent or unipotent, display considerable plasticity and can transdifferentiate into various lineages of cells depending on their functions and locations. Bone marrow is the main reservoir of adult stem cells. Bone marrow-derived stem cells can differentiate into several nonhematopoietic cell types including skeletal muscle, adipocytic, chondrocytic, osteocytic lineages, and hepatocytes (Petersen et al., 1999; Pittenger et al., 1999; Orlic et al., 2001a). However, cells from the umbilical cord blood provide an alternative rich source of hematopoietic and mesenchymal stem/progenitor cells. Recently, mesenchymal stem cells which show multilineage potential and which can be used in the treatment of stroke or cardiac repair, have been isolated from the Wharton's jelly of the human umbilical cord (Wang et al., 2004).

Mechanisms of cell repair

During the inflammatory and granulation formation phase that occurs after ischemic insult, there is a significant increase in various cytokine and growth factor expressions. Interactive regulation of signaling among stem cells and progenitor cells, peripheral circulation, and the infarcted tissue is important in conducting the process of mobilization, homing, incorporation, survival, proliferation and differentiation of stem cells, which leads to organ regeneration. Signaling factors such as G-CSF (Granulocyte colony-stimulating factor), GM-CSF (Granulocyte-monocyte colony-stimulating factor), SCF (stem cell factor), SDF-1 (stromal cell-derival factor-1), TNF-α (tumor necrosis factor-α), IL-8 (interleukin-8), IL-10, EPO (erythropoietin), and VEGF (vascular endothelial cell growth factor) are important for the inflammation and are involved in trafficking, proliferation and differentiation processes of stem cells (Vandervelde et al., 2005). Cytokines and growth factors interact with their receptors expressed on endothelial progenitor cells, hematopoietic progenitor cells or stem cells, and promote migration of these cells to the site of injury. VEGF (Asahara et al., 1999) and GM-CSF (Takahashi et al., 1999) were found to augment mobilization of endothelial progenitor cells from bone marrow, increase circulated endothelial progenitor cell level and improve neovascularization of injured tissue. In addition, endothelial progenitor cells can be mobilized by other proangiogenic growth factors such as SDF-1, placental growth factor, and erythropoietin (Hattori et al., 2001, 2002; Heeschen et al., 2003). G-CSF and SCF were used to mobilize hematopoietic stem cells and improve cardiac regeneration experimentally (Orlic et al., 2001b). SDF-1 and its receptor CXCR4 form an important chemotaxic axis in stem cell mobilization which has been reported to be involved in the chemoattraction of bone marrow-derived cells for cardiac repair after myocardial infarction (Kucia et al., 2004). Local administration of SDF-1 can also enhance endothelial progenitor cell recruitment and neovascularization (Askari et al., 2003).

The fate of the implanted stem cells seems to be determined by the niches in which they engraft rather than by an intrinsic cellular program (Orkin and Zon, 2002). Human mesenchymal stem cells, when engrafted into murine hearts, seem to transdifferentiate into cardiomyocytes that are indistinguishable from those of the host (Toma et al., 2002). The transdifferentiation of stem cells to cardiomyocytes is prominent in injured tissue and they may express cardiac-specific contractile proteins like cardiac troponin T and phospholamban. In contrast, the stem cells transduced by *MyoD* gene cultured in vitro did not show these proteins (Toma et al., 2002). Bone- marrow-derived stromal cell injection improved the function of the murine ischemic hindlimb, with elevated bFGF (basic fibroblast growth factor) and VEGF protein levels were found in adductor muscle. Moreover, colocalization of VEGF and murine-marrow derived stromal cells was observed within adductor tissue (Kinnaird et al., 2004).

Stroke

Stroke is a leading cause of death and disability worldwide, and there is currently no effective treatment enhancing stroke recovery (van Gijn and Dennis, 1998). However, one potential strategy for the treatment of stroke is transplantation of bone marrow stem cells. These cells appear to cross the blood brain barrier after intravenous administration and selectively migrate to the ischemic hemisphere of the damaged brain to improve neurological recovery (Li et al., 2002).

In stroke treatment through administration of stem cells, two main determinants are critical for the colonization and transdifferentiation of stem cells into a variety of tissues: (i) ischemic tissue damage, and (ii) the availability of a large number of circulating stem cells (Orlic et al., 2001b). Under ischemic conditions, circulating stem cells appear to selectively migrate to ischemic regions to support plasticity and functional recovery of damaged tissue (Orlic et al., 2001b). In our rat brain ischemic model, the brain expressed SDF-1 and its receptor CXCR4 after focal cerebral ischemia. Subcutaneous administration of G-CSF induced increases in bone marrow cell mobilization and targeting to the brain that improved neural plasticity, enhanced vascularization, and reduced the volume of cerebral

infarction. We have suggested that cerebral ischemia enhances hematopoietic stem cell plasticity and provides an environment that enhances the differentiation of hematopoietic stem cells into the original lineage cell types of the damaged organ, such as endothelial cells and neurons (Shyu et al., 2004). Subcutaneous administration of G-CSF may provide a basis for the development of a non-invasive autologous therapy for cerebral ischemia.

Peripheral blood hematopoietic stem cells have already been used in transplantation for the regeneration of non-hematopoietic tissues, such as skeletal muscle, the heart (Orlic et al., 2001b) and neurons (Sigurjonsson et al., 2005). Although the concentration of peripheral blood hematopoietic stem cells under steady-state conditions without any cytokine induction is very low (Elfenbein and Sackstein, 2004), G-CSF has been shown to mobilize hematopoietic stem cells into the peripheral blood from bone marrow (Demetri and Griffin, 1991), thus, amplifying the concentration of peripheral blood hematopoietic stem cells. We have also demonstrated that the subcutaneous injection of G-CSF combined with mobilized peripheral blood hematopoietic stem-cell transplantation could be of practical use in the treatment of chronic stroke in rats (Shyu et al., manuscript submitted).

Under ischemic conditions, circulating stem cells appear to selectively migrate to ischemic regions to support the plasticity and functional recovery of damaged tissue (Orlic et al., 2001b). Recent reports have indicated that SDF-1 is a strong chemo-attractant for $CD34^+$ cells which express CXCR4, the receptor for SDF-1, and play an important role in the trafficking of hematopietic stem cells between peripheral circulation and bone marrow (Petit et al., 2002). We have shown that SDF-1 exhibits neuroprotective effects, which plays a role not only in stem cell differentiation but also in ischemia-induced trafficking of stem cells from peripheral blood to the damaged brain (Shyu et al., manuscript submitted). Locally over-expressed SDF-1 induced by intracerebral administration after cerebral ischemia may chemo-attract hematopoietic stem cells, and also provide an environment that enhances differentiation of hematopoietic stem cells into the various lineages of cells in the damaged brain.

Finally, methods used to serially monitor cell migration after injection and the fate of cells after transplantation are important if stem cell therapies are to be used successfully to treat cerebral ischemia in a clinical setting. Using the transfection agent Effectene® to increase the labeling efficiency of paramagnetic particles [gadolinium-diethylene triamine penta-acetic acid (Gd-DTPA)] in stem cells (Frank et al., 2002; Crich et al., 2004; Modo et al., 2004) and sequentially tracing the migration of labeled stem cells in an ischemic rat brain with 3.0-Tesla magnetic resonance imaging, we reported recently a non-invasive technique base for tracing stem cell fate in stroke treatment (Shyu et al., manuscript submitted).

Ischemic retinopathy

Retinal ischemic diseases resulting from central and branch retinal arterial occlusion, carotid artery disease, or other ocular disorders (e.g., diabetes mellitus, hypertension, or glaucoma) are among the most common cause of visual impairment and blindness (Ffytche, 1974). Retinal ischemia may be caused by systemic abnormalities such as severe left ventricular failure, hypovolemic shock, atherosclerosis, and hyperlipidemia (Hayreh and Podhajsky, 1982; Hayreh, 1995), or more commonly by local circulatory failure, all of which can result in irreversible morphological and functional changes. Its major pathogenic characteristics are reduced perfusion of retinal vessels, increased vascular permeability, degradation of the surrounding matrix and leakage of serum substances into the eye, and finally intraocular proliferation of new but defective blood vessels (Burgos et al., 1997; Hofman et al., 2001; Vincent et al., 2002). Such ischemia-induced retinal neovascularization can cause retinal edema, hemorrahge and retinal detachment. It was generally believed that it is not possible to repopulate the lost retinal cells and repair the retinal injury in these diseases. Although an increasing number of treatments have been shown to interrupt the “ischemic cascade” and attenuate the detrimental effects of retinal ischemia, success in the laboratory has not been translated to the clinic (Osborne et al., 2004).

Recently, however, a novel therapeutic strategy based on the use of stem cells for the treatment of ischemic retinopathies has been developed. Stem cells with their regenerative potential should be able to promote angiogenesis in the degenerative retina or block its progress in proliferative eye diseases. Kurimoto et al. (2001) found that intravitreally injected adult rat hippocampus-derived neural stem cells (AHSCs) have the capacity to migrate and integrate into the host retinas of adult rats in a retinal ischemia-reperfusion model which induced transient ischemia with high intraocular pressure, while none of the cells migrated into the retina without ischemic injury. Guo et al. (2003) showed that the green fluorescent protein-labeled AHSCs transplanted to the subretinal space can readily incorporate into host retinas that have undergone ischemic injury. Many cells migrate to specific retinal cellular layers, where they undergo morphologic differentiation to resemble known retinal neurons, this process extends to the optic nerve. It has also been demonstrated that self-renewing adult haematopoietic stem cells can serve as blood vessel precursors during retinal neovascularization (Grant et al., 2002). In a model for retinal degeneration, intravitreal injection of adult haematopoietic stem cells were able to rescue and maintain a nearly normal retinal vasculature in neonatal mice as well as stabilize degeneration in adult mice. Intraocular administration of adult haematopoietic stem cells transfected with T2-tryptophanyl-tRNA synthetase, an inhibitor of retinal angiogenesis, was shown to block

normal retinal angiogenesis in neonatal mouse eyes (Otani et al., 2002). Moreover, inhibition of neovascularization under ischemic conditions may exacerbate the progressing ischemia (Smith, 2002). Stem cells are therefore ideal candidates to perform reconstruction of the neural circuitry of ischemic-injured retina. Enhanced integration and differentiation of transplanted cells, leading to functional restoration may benefit currently untreatable ocular diseases. Additionally, the use of stem cells as drug delivery vehicles has the potential to selectively and continuously deliver therapeutic molecules to the back of the eye for prolonged periods of time. As this is where most vision-threatening pathologies occur, intraocular administration of stem cells offers an elegant approach to deliver anti-angiogenic drugs to areas of neovascularization and gives hope for the development of an effective therapy for retinal ischemic diseases.

Myocardial infarction

Myocardial infarction leads to myocardial remodeling and subsequent reduced cardiac performance. Cardiac stem cell therapy has been conducted on patients with acute myocardial infarction and chronic ischemic heart failure through the use of different cell types including skeletal myoblasts (Taylor et al., 1998), bone marrow cells (Orlic et al., 2001a), endothelial cells (Kamihata et al., 2001), mesenchymal stem cells (Tomita et al., 1999), and resident cardiac stem cells (Dawn et al., 2005). A number of clinical trials have shown significant improvement in left ventricular function and a significant reduction in infarct size in patients with acute myocardial infarction (Assmus et al., 2002; Strauer et al., 2002; Wollert et al., 2004). There is no significant difference in the effect of ventricular function improvement using either blood-derived endothelial progenitor cells or bone marrow-derived cells (Assmus et al., 2002). An improvement in quality of life and cardiac function has been reported after injection of autologous unfractionated progenitor cells from both circulating and bone marrow-derived cells into patients with acute myocardial infarction (Assmus et al., 2002; Strauer et al., 2002) and chronic ischemic heart disease (Perin et al., 2003). Bone marrow-derived stem cells delivered to the heart were shown to transdifferentiate into cardiac myocytes and to rescue cardiac function after infarction (Orlic et al., 2001a,b). In a rat model, injecting autologous mesenchymal stem cells into the peri-infarct zone of myocardium increased capillary density and left ventricular contractility. It also increased bFGF, VEGF, and SDF-1 in the heart and down-regulated proapoptotic protein Bax in the ischemic myocardium (Tang et al., 2005). In addition to the mesenchymal stem cells or bone marrow-derived cells illustrated in these studies, there is also a possibility that autologous cardiac stem cells could be isolated from small myocardial biopsies, expanded in vitro, and subsequently administered to the same patient for clinical application. These cardiac stem cells may direct themselves to the interstitial compartment of the heart by migrating through the vascular endothelium within a few hours after intra-arterial delivery, resulting in limited infarct size, attenuated left ventricular remodeling, and ameliorated left ventricular dysfunction (Dawn et al., 2005).

The repair effect of stem cells and their fate in the treatment of myocardial infarction are still controversial. It has been suggested that the ischemic myocardium environment is a niche for endothelial cell differentiation of mesenchymal stem cells. Although mesenchymal progenitor cells injected into the infracted myocardium augment myocaridal vessel density and improve the left ventricular performance (Wang et al., 2000), whether bone marrow-derived cells can regenerate cardiomyocytes through transdifferentiation has been extensively questioned. Kajstura et al. (2005) found that regenerated myocytes and newly formed endothelial and smooth muscle cells in the infarct area were of bone marrow-derived stem cell origin, and they claimed bone marrow-derived stem cells can differentiate into cardiomyocytes. However, bone marrow cells had no apparent effect on the growth behavior of the surviving myocardium (Kajstura et al., 2005). In contrast, another group found that most of the engrafted cells lost their smooth muscle phenotype and acquired an endothelial phenotype (Davani et al., 2003). Furthermore, the engraftment of transplanted bone marrow-derived stem cells or a purified population of hematopoietic stem cells in the infracted myocardium seemed to be transient, and most of the engraftment was lost by day 28 after administration (Nygren et al., 2004; Kajstura et al., 2005). In addition, the engrafted cells expressed hematopoietic phenotypes. A low frequency of bone-marrow derived cardiomyocytes was observed outside the infracted myocardium, and these had been exclusively derived through cell fusion (Nygren et al., 2004; Kajstura et al., 2005).

Improvement in heart function may be the result of the activation of angiogenic activity of the endogenous cells of the myocardium by bone marrow cells but not from myocardial regeneration (Balsam et al., 2004). Myocardial neovascularization seems to be dependent on the number of administered endothelial progenitors trafficking to the infarct zone and protection against apoptosis, induction of endogenous cardiomyocytes proliferation and results in improvement of cardiac function (Schuster et al., 2004).

Ischemic disorders of liver

In contrast to other major organs, the liver has great regenerative capacity. It is known that hepatocytes, the major liver parenchymal cells, infused via a portal route can rapidly replace severely damaged liver parenchyma. Therefore, mature hepatocytes themselves, or a fraction thereof, are thought to be the best cell population for cell therapy (Overturf et al., 1999). Hepatocyte

transplantation can be a bridge to, or even an alternative to liver transplantation in some clinical settings. However, shortage of donor hepatocytes limits clinical application (Horslen and Fox, 2004).

The presence of Y chromosome-positive hepatocytes in livers from female individuals who have undergone sex-mismatched bone marrow transplantation suggests that bone marrow cells can convert to hepatocytes (Theise et al., 2000). Murine studies have clearly demonstrated the colocalization of stem cell markers and the hepatocyte marker in liver cells that was thought to be a result of the transdifferentiation of bone marrow cells into hepatocytes (Lagasse et al., 2000). *In vitro* and *in vivo* evidence from the hematopoietic stem cells induced by the paracrine factor of damaged hepatocytes shows that hematopoietic stem cells can express hepatocyte-specific genes. That hematopoietic stem cells can transdifferentiate to functional hepatocyte-like cells without fusion further supports these findings (Jang et al., 2004). In addition, embryonic stem cells and cord blood cells can also differentiate into hepatic cells *in vitro* (Kakinuma et al., 2003; Kuai et al., 2003). Although another group has argued that fusion of stem cells and pre-existing hepatocytes, rather than transdifferentiation, contributed to the colocalization of hepatocyte-like markers (Vassilopoulos et al., 2003), further studies have identified that macrophages were the principal fusion partners of host hepatocytes (Willenbring et al., 2004).

No matter what the mechanism, only about 1% of parenchymal hepatocytes or less are repopulated by transplanted stem cells in most human and animal studies (Masson et al., 2004). So far, only two studies have reported a high percentage of liver repopulation as well as functional improvement. Wang et al. (2002) clearly demonstrated that donor hematopoietic stem cells and their progeny could home to the injured liver and fuse with host hepatocytes, which then proliferated and eventually replaced more than 30% of overall liver parenchyma by 22 weeks. In another study, transplantation of unfractioned bone marrow cells lead to 25% repopulation of the recipient liver cells by 4 weeks in a chronic liver injury mouse model (Terai et al., 2003).

Mature hepatocytes are fully functional and hepatocyte transplantation is feasible for liver cell replacement, but the lack of donor cells is still a major problem. Oval cells (liver stem cells) are difficult to isolate, which limits their therapeutic value. Therefore, various stem cells derived from other origins could be used as the cell source for treating the injured liver. Hepatocytes derived from bone-marrow cell may contribute to liver repair; although a low percentage of engraftment provides little help in most situations. Mass production of stem-cell derived hepatocytes *in vitro* or stem cells engineered *ex vivo* with high survival advantage might be a an advantageous strategy in the future.

The liver is very resistant to hypoxia compared with the other major organs. In patients suffering from ischemic insult, hypoxic injury of brain, heart and kidney develops rapidly and fatally before ischemic liver injury becomes significant. In humans, a healthy liver can tolerate continuous occlusion of total blood inflow for 40 minutes. The dual blood supply system (portal vein and hepatic artery) further enhances its ischemic resistance. Consequently, hepatic ischemia is a rare event and is seldom thought to be an independent pathologic condition. Although the studies discussed in this section were performed in animals with hepatotoxin-induced hepatic injury, it is believed that a similar principle can be applied in the treatment of varying liver parenchymal diseases, including liver ischemic disease.

Other ischemic disorders

Stem cells can also provide renoprotection. Intravascular administration of mesenchymal stem cells after renal ischemia reveals renoprotection with improved renal function, higher proliferative and lower apoptotic indexes (Togel et al., 2005a). This effect is reflected by the increased SDF-1 expression in the postischemic and reperfusion kidney which recruits different leukocyte populations, including bone marrow-derived stem cells, to participate in repair (Togel et al., 2005b).

Additionally, it has been demonstrated that the introduction of blood-derived angioblasts or *ex vivo* expended endothelial progenitor cells into ischemic hind limb of mice accelerated the rate of blood flow restoration and capillary density, and significantly reduced limb loss rate (Schatteman et al., 2000). Injection of murine marrow-derived stromal cells increased adductor muscle levels (local production) of bFGF and VEGF protein, which was associated with reducing the incidence of autoamputation and attenuated muscle atrophy and fibrosis.

Perspective

There are clearly many developments needed before stem cell therapy becomes a realistic option for the clinical treatment of ischemic diseases, and to date, no double-blind randomized clinical trials have been conducted. Furthermore, the safety of cell treatment and the selection of cell type in selected patients require further confirmation.

Since the scar tissue developed after the ischemic damage may form a barrier limiting the integration of the implanted cells (Reinecke et al., 1999), how to deliver enough cells for the therapy to be effective is a further concern. Additionally, long-term survival for most of the cells delivered to the ischemic tissue is limited and there is also a significant loss of cells from the injection site within 1 day of cell administration (Muller-Ehmsen et al., 2002). Although intravenous administration is the easiest to implement, the hindrance of a greater number of infused cells by other organs will

reduce the number of cells homing to and populating the injured tissue. Targeted and regional administration of cells is therefore preferred (Strauer et al., 2002).

Finally, efforts to promote cell survival and long-term engraftment and to investigate the mechanisms of cell replacement are necessary to improve outcome. Further research is needed to explore the therapeutic merits of cell transplantation as well as any possible adverse side effects or complications.

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Accepted May 9, 2006