Review Article

Regenerative Potential of Intravenous Infusion with Mononuclear Cells in Cord Blood and G-CSF-Mobilized Peripheral Blood

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Abstract

Up to now most of the cells used in cell therapeutics have been mesenchymal stem cells (MSCs). However, mononuclear cells (MNCs) from cord blood (CB) or G-CSF (granulocyte-colony stimulating factor)-mobilized peripheral blood (mPB) MNCs could be an alternative option because MNCs contain both HSCs (hematopoietic stem cells) and MSCs. In addition, cellular components other than purified HSCs or MSCs could play important roles in tissue regeneration. The optimal route of administration in cell therapy is another important issue. The therapeutic benefits and clinical availability of intravenous infusion of CB- or mPB-MNCs in the field of cell therapy will be briefly reviewed.

Keywords: Cell therapy; Mononuclear cell; Cord blood; Peripheral blood

Introduction

Cell therapy has emerged as an advanced medical technology for restoring damaged tissues and organs. Of the various possible cell types, MSCs have been used most frequently in experiments and in clinical trials in the field of regenerative medicine [1]. Since the technical difficulties of manufacturing MSCs and the regulatory restrictions imposed tend to be barriers to clinical application [2], only a few MSC-based new drugs have been developed so far. Compared with MSCs, MNCs derived from bone marrow (BM), CB, or mPB, has not been frequently used for studying tissue regeneration. Increased efforts to examine the therapeutic benefits of MNCs could provide greater choice for cell therapy, because MNCs are easily obtained and are subject to few regulatory restrictions.

MSCs vs. MNCs for Cell Therapy

Ongoing efforts to restore damaged tissue have employed the currently available stem cells. Recently, the ethical issues surrounding the use of embryonic materials have prompted research using adult stem cells [3]. Hematopoietic stem cells (HSCs) were the first adult stem cells to be discovered followed by MSCs. MSCs are the adult stem cells most often used in clinical studies for the treatment of degenerative diseases. They are multipotent, self-renewing cells that can be found in almost all postnatal organs and tissues [4]. MSCs, which are directly injected into damaged tissues or migrate to them by various routes, can differentiate into multiple cell types, including nerve, bone, cartilage, tendon, and muscle. In addition to the effects of the cells into which the MSCs differentiate, MSCs have been shown to exert paracrine actions that promote the repair of damaged tissues. In the neurologic field, many paracrine effects related to neurogenesis, myelination, angiogenesis, synaptogenesis, apoptosis, etc. have been demonstrated [5]. Although MSCs are generally isolated from BM or CB, these cells have also been reported to exist in PB. Indeed Ukai et al. have suggested that PB-derived multipotent precursor cells could be an important source for cell therapy of stroke [6]. While only small numbers of stem cells exist in PB compared to the number in BM, more numbers can be obtained from the PB-MNC fraction after mobilizing them from BM with G-CSF and/or chemotherapeutic agents [7].

There have been many suggestions that MNCs might also be used for tissue regeneration because it was found that MSCs could be cultured and isolated from the MNC fractions of BM, CB, and mPB

[7-9]. The effects of MSCs and MNCs have been compared in various experimental settings (Table 1) [10-13]. Iwase et al. observed that MSC transplantation caused significantly greater improvement in hindlimb ischemia than MNC transplantation [10]. Mazo et al. also demonstrated that MSCs provided superior long-term benefits to whole BM-MNCs in a rat model of chronic myocardial infarction [11]. On the other hand, Mathieu et al. revealed that cell therapy with autologous BM-MNCs resulted in better cardiac recovery than unmodified MSCs in a canine model of chronic myocardial infarction [12]. In a double-blind, randomized, controlled trial, Lu et al. concluded that BM-MSCs appeared more effective than BM-MNCs in increasing lower limb perfusion and promoting foot ulcer healing in diabetic patients. They observed that human BM-MSCs used for transplantation secreted significantly higher levels of angiogenic factors than BM-MNCs under conditions of normoxia as well as hypoxia [13].

There have recently appeared clinical reports demonstrating the therapeutic potential of autologous BM- or mPB-MNC transplantation in patients with limb ischemia or myocardial infarction. The data suggest that the main benefit of the MNCs results from paracrine effects via secretion of cytokines, fibroblast growth factors, and vascular endothelial growth factors involved in angiogenesis [14-17], inhibition of cardiomyocyte apoptosis [18] and cell-cell interactions [19-21]. The end results of these effects are the replacement of lost myocardial tissue and rescue of ischemic and hibernating tissue, and improvement of left ventricular function. Horie et al. recently showed that patients with limb ischemia given the large number of CD34-positive cells present among G-CSF-mobilized autologous PB-MNCs had a notably better prognosis than other patients [22].

To investigate the possible use of autologous MNCs in the field of neurological disorders using mPB-MNCs as well as CB-MNCs,

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Authors	Experimental model	Administration route	Effect of cell types on the regenerative potential	Reference number
Iwase et al.	Hindlimb ischemia, Rat	Intramuscular	Autologous, BM-MSC>BM-MNC	[10]
Lu et al.	Limb ischemia, Clinical	Intramuscular	Autologous, BM-MSC>BM-MNC	[13]
Mazo et al.	Chronic MI, Rat	Intramyocardial	Allogeneic, BM-MSC>BM-MNC	[11]
Mathieu et al.	Chronic MI, Canine	Intramyocardial	Autologous, BM-MSC < BM-MNC	[12]

MSCs: Mesenchymal Stem Cells; MNCs: Mononuclear Cells; MI: Myocardial Infarction; BM: Bone Marrow

Table 1: Comparative functional studies between MSCs and MNCs for regenerative therapy in various experimental settings.

my colleagues compared levels of inflammatory cytokines and neurotrophic factors in PB-MNCs and mPB-MNCs from children with cerebral palsy (CP) with those from healthy adult donors and from CB-MNCs donated from healthy newborns (unpublished data). Higher levels of interleukin (IL)-6 and lower levels of IL-3 were noted in the mPB-MNCs as compared to the PB-MNCs of the children with CP. The level of brain-derived neurotrophic factor (BDNF) in the mPB-MNCs from the CP children was significantly higher than in the CB-MNCs or mPB-MNCs from healthy adults. The level of G-CSF in the mPB-MNCs from CP children was comparable to that in CB but significantly higher than in the mPB-MNCs from healthy adults. Lower levels of IL-1β, IL-3, and IL-6 and higher levels of IL-8 and IL-9 were obtained from the CB-MNCs and mPB-MNCs from CP children than from healthy adults. A lower level of IL-1β, and a higher level of IL-8 were observed from mPB-MNCs from CP children than from healthy adults. Based on these findings, we proposed that the different levels of neurotrophic factors and anti-inflammatory cytokines in the mPB-MNCs of CP children and the CB-MNCs of healthy children suggested that these cells had potential as new sources for cellular therapy for individuals with neurologic diseases.

These studies also indicated that CB-, BM- and mPB-MNC preparations, without any *ex vivo* manipulation, could be useful for tissue regeneration, because they contain immunomodulatory lymphocytes expressing various cytokines as well as progenitor cells such as MSCs [23].

Routes of Administration for Cell Therapy

There is no consensus with a few comparative functional studies regarding the optimal route of administration for the treatment of acute tissue damage. Forest et al. analyzed the distribution of injected cells in damaged and undamaged myocardium after intracoronary or intravenous BM-MNC delivery. They observed a significant number of cells retained within the heart after intracoronary injection, whereas following peripheral intravenous cell injection no cardiac homing was observed at 24 hours and the injected cells were mainly detected within the lungs [24]. Nevertheless, other functional study demonstrated that intravenous BM-MSCs injection can significantly improve myocardial function, as do intraventricular and intramyocardial MSCs injections [25].

For refractory neurological diseases, intracerebral, intrathecal, intra-arterial, intravenous, and also peri-lesional administration of various stem cells has been tried. A recent meta-analysis of pre-clinical studies of the use of MSCs for ischemic stroke revealed that the effect of MSC therapy varied significantly depending on the administration route (intracerebral > intra-arterial > intravenous, although the effect of the intravenous route was nonetheless very large [26]. Comparative functional studies by administration routes in various experimental settings are summarized in Table 2. Although intravenous administration is less effective than other techniques, it has been reported that intravenously administered CB-MNCs could enter the brain, survive, migrate, and improve functional recovery in

an experimental stroke model [27] as well as in a child with Krabbe disease [28]. Liu et al. also recently demonstrated that intravenously infused mPB-MNCs could survive in the brain of hosts, migrate to the damaged area and express neural markers. In addition, a reduction in motor function impairment, lesion volume and neural cell apoptosis was observed in hypoxia-ischemia rats that received mPB-MNCs [29].

Korf-Klingebiel et al. suggested that a hypothetical paracrine effect of intravenous MNC infusion was responsible for myocardial functional improvement, because MNCs in a quiescent or non-differentiated state were capable of releasing many types of growth factor into the peri-infarction tissue [30]. Others have shown that intravenous administration of murine MSCs improved the outcomes of neural and lung injuries in experimental animal models primarily through paracrine effects and a reduction in the production of pro-inflammatory cytokines at the sites of injury [31,32]. These observations would appear to justify the use of an intravenous route for cell therapy because, in contrast to HSC transplantation, non-hematopoietic applications such as those for cardiovascular or neurological indications do not require permanent graft survival and many of the therapeutic effects of MSCs and MNCs are believed to be due to immunomodulatory mechanisms, such as the secretion of neuroprotective, angiogenetic, and anti-inflammatory cytokines [33-35].

Intravenous Infusion of MNCs for Cell Therapy

The availability of adequate sources of cells and effective routes of administration are central priorities in regenerative medicine. Unfortunately, these two requirements are not often both fulfilled at the same time. However, MNCs are more easily and safely applied in the clinical setting because they are easily obtained and do not provoke the same kinds of legal debates as MSCs whose clinical applications are strictly regulated. In addition, the use of MNCs is based on the therapeutic effect of their paracrine actions rather than requiring the survival of any graft, as well as by their availability and the safety of their administration [14-21,23].

In cases of acute cellular injuries, at which most of experimental and clinical trials have been directed, the migration and homing followed by differentiation of infused MNCs can be explained by the fact that the endothelial barrier is disrupted. However, it is difficult to explain the therapeutic effect of cell therapy as simply due to homing of intravenously administered MNCs because most endothelial junctions remain intact in chronic injury and do not permit cells to migrate into damaged tissues. Suárez de Lezo et al. also explained the therapeutic efficacy of intravenously administered MNCs in chronic myocardial infarction as due to a reversed gradient of chemokines and their receptors [25]. Thus, intravenously administered MNCs may not enter the brain of CP patients in the absence of some challenge such as chemo-radiotherapy that can cause permeability changes in the blood brain barrier (BBB). However, as we learned in experimental work, cytokines that are consistently expressed by CB-MNCs may be able to alter endothelial cells in such a way as to cause alterations in tight junction structure (BBB) and permit the entry of leukocytes

Authors	Experimental model	Cell type	Effect of route on the regenerative potential	Reference number
Wang et al.	Myocardial ischemia, Rat	BM-MSCs	Intravenous=Intraventricular=Intramyocardial	[25]
Mathieu et al.	Ischemic stroke, Animal (Meta-analysis)	MSCs	Intracerebral>Intra-arterial>Intravenous	[12]

BM: Bone Marrow; MSCs: Mesenchymal Stem Cells

Table 2: Comparative functional studies among administration routes for regenerative therapy in various experimental settings.

after intravenous infusion of CB-MNCs [23,27]. In these inflammatory conditions, there can be both intrinsic activation and proliferation of parenchymal microglia. Activated microglial cells could then release neurotrophic factors such as BDNF and glial cell-derived neurotrophic factor (GDNF), remove synapses from damaged neurons and influence the synaptic connectivity of newly-formed neurons [36]. I have also emphasized the importance of the neurotrophic effects of the CB-MNCs for improving neurologic outcomes, because most of the patients that responded showed clinical changes within 1 month after CB-MNC infusion [37], and many reports have demonstrated that CB-MNCs consistently express neurotrophic factors such as nerotrophin (NT)-5, NT-3, BDNF, and other growth factors [33].

Given this background, my colleagues have obtained positive results for the infusion of intravenous autologous CB-MNCs in children with CP in terms of the safety and feasibility of the procedure, as well as its potential efficacy in countering neurological impairment [37]. Another group also recently reported that intravenous infusion of allogeneic CB-MNCs has therapeutic potential in CP [38]. They suggested that infusion of allogeneic CB-MNCs could be an effective procedure, accompanied by structural and metabolic changes in the brain, for ameliorating motor and cognitive dysfunction in children with CP. However, the use of immunosuppressants such as cyclosporin in CP patients receiving allogeneic CB-MNCs must be justified and its potential neurotoxicity must be carefully monitored. This is the reason why my colleagues instituted a double blind, randomized, cross-over study using G-CSF-mobilized autologous PB-MNCs for CP patients. Although the study is ongoing, we have confirmed that G-CSF mobilization and the collection of stem cells in CP children can be carried out safety and the treatment appears to have some positive clinical and radiologic efficacy [39]. mPB-MNCs would have a lot of advantages over CB-MNC therapy in the treatment of CP children provided the efficacy of mPB-MNCs is not inferior to that of CB-MNCs. Potential advantages of mPB-MNCs over CB-MNCs for cell therapy are as follows: first, G-CSF used for stem cell mobilization is already known to have neuroprotective effects, second, separate stem cell preparations could be pooled and aliquots cryopreserved for repeated infusion, third, they can be given to all candidates even if they do not have autologous CBs.

Conclusions

Trials of cell therapy using various manufactured stem cells including MSCs need to be continued in order to develop new cell therapeutics and new techniques for overcoming refractory diseases. Furthermore, as we learned from the transplantation of HSCs, experimental and clinical investigations using MNCs should not be overlooked prior to manufacturing particular types of stem cells.

Acknowledgements

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