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Bone marrow-derived mononuclear cells therapy for ischemic stroke

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Abstract

Background: Nowadays, the cerebrovascular event is the second cause of death and the third cause of disability worldwide. In the last few decades, stem cell-based approaches are widely analyzed as a potential treatment for this disease. One of these types of cells are bone marrow-derived mononuclear cells (BMMNCs).

In this review, we analyzed 9 completed clinical trials with the use of BMMNCs in patients with ischemic stroke, which we found in the clinicaltrials.gov and PubMed databases, using the keywords "stroke" and "bone marrow mononuclear cells". Our goal was to analyze the safety and efficiency of this therapeutic approach, as well as the optimal therapeutic time window, transplantation route and cell dose used.

The best stroke phase to apply this therapy is the subacute stage. Higher numbers of CD34+ cells, derived from BMMNCs were correlated with a trend toward a better outcome. All the clinical trials support the idea that BMMNCs transplantation is a safe therapy.

Conclusions: In conclusion the author points out that the autologous transplantation of BMMNCs is harmless and not associated with severe complications. Although some clinical studies stated a better outcome in patients treated with BMMNCs, further clinical trials are needed to establish their therapeutic efficiency.

Key words: ischemic stroke, bone marrow mononuclear cells, transplantation, treatment.

Introduction

The cerebrovascular event (stroke) is a medical condition in which the blood flow to the brain is diminished due to arterial ischemia or arterial rupture. Usually this results in severe brain damage, which includes neuronal death, microvasculature disturbances, local inflammation and acid-base imbalance. Stroke is the second cause of death and the third cause of disability worldwide. About 87% of strokes are ischemic, the rest being hemorrhagic. Disability affects 75% of stroke survivors enough to decrease their employability [1]. There were many efforts to elaborate a pharmaceutical medication that would reduce the severity of stroke and support intensive therapy. These led to some achievements, for example the production and use of tissue Plasminogen Activator (tPA), which can be administered in ischemic stroke patients and contribute to degradation of blood clots. Unfortunately, the time window for application of this therapy is a serious limitation, so than it cannot be administered to patients who have suffered an ischemic stroke for more than 4.5 hours after onset. As a result, very few patients benefit of tPA therapy; a study that reviewed records from the National Inpatient Sample from the U.S.A. has shown that from 2005 to 2011, overall 3.8% of patients received tPA, although with the number growing each year [2].

Another important therapy that has evolved in recent years is the mechanical thrombectomy. It implies the use of cerebral clot extracting devices in acute large-vessel occlusion, which results in vascular recanalization. However, this treatment also has some limitations: it is indicated for patients with acute ischemic stroke due to a large artery occlusion in the anterior circulation, who can be treated within 24 hours of the time last known to be well. According to some clinical studies, only 9-10 % of ischemic stroke patients can qualify for mechanical thrombectomy [3-8].

In the last few decades, stem cell therapy is being regarded as a promising therapeutic approach for stroke patients. There are several cell types that could be transplanted in the post stroke patient and have the potential to improve the outcome: bone marrow-derived mononuclear cells (BMMNCs), bone marrow mesenchymal stem cells (BMSCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs) and multilineage-differentiating stress-enduring (Muse) cells, to name just a few.

BMMNCs are a group of cells which contain lymphoid cells, myeloid cells, hematopoietic and mesenchymal stem cells. Preclinical studies have shown an efficiency of treatment with such cell types, by means of different mechanisms of actions, such as neurogenesis, angiogenesis, arteriogenesis and modulation of inflammation [9, 10, 11]. BMMNC autologous transplantation has some remarkable advantages over transplantation of other cell types. These cells can be rapidly prepared for transplantation within hours after harvest; there is no need for *in vitro* expansion in a culture medium, there is no risk of immune reaction associated with their transplant and there are no ethical issues regar-ding such a therapeutic approach.

In this review we have analyzed 9 completed clinical trials with BMMNC autologous transplantation as a treatment for ischemic stroke patients. The aim of this review is to analyze the safety and efficiency of this therapeutic approach, as well as the optimal therapeutic time window, transplantation route, cell dose and to discuss the correlation between these variables and patient outcomes. Secondly, we analyze and discuss the correlation between BMMNC transplantation and the levels of some relevant blood markers, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF) and β-nerve growth factor (β-NGF) and the relation between these levels and such biological processes as neurogenesis, arteriogenesis, angiogenesis and inflammation.

Material and methods

We have analyzed the clinical trials regarding BMMNC therapy for ischemic stroke with published results, which we found in the databases Pubmed and Clinicaltrials.gov. As a selection filters, we have used the keywords: "stroke" and "bone marrow mononuclear cells", and selected just articles in the English language. After processing the materials according to the search criteria, we found 12 finished clinical trials with the use of BMMNCs in order to treat ischemic stroke (excluding case-report studies). The final bibliography of this review included 9 clinical studies, which were considered to be representative and sufficient to describe the overall situation of cerebrovascular event therapy with BMMNC autologous transplantation, including the safety and clinical efficiency of this treatment method.

Clinical studies

The 9 clinical trials analyzed relate to the use of BMMNCs in order to treat ischemic stroke. Their importance consists, firstly, in confirming the BMMNC autologous transplant safety for stroke survivors and lack of association with severe complication. Secondly, some of these trials have also showed that this method of treatment could improve the patient's outcome. Nowadays, it became clear that for the proper understanting of the correlation between BMMNC transplantation and the patient's health condition after the treatment, much more clinical studies are needed.

Valeria Battistella et al. study [12] included 6 patients who had suffered ischemic stroke 59-82 before they received BMMNC intra-arterial transplantation, aged between 24 and 65 years, in their study. The mean quantity of infused

Table 1

Completed clinical studies concerning BMMNC transplantation in ischemic stroke

Study reference	Route of administration	Patient's age (years)	Time period of administration after stroke onset	Number of BMMNCs transplanted	Period of follow-up	Patients treated for ischemic stroke/ Total of patients treated with BMMNCs
Valeria Battistella et al. 2010 [13]	IA	24 - 65	Day 59 - 82	3.058×10^8	180 days	6/6
Sean I. Savitz MD et al. 2011 [14]	IV	55.6 ± 15	24 - 72h	8 patients: 10^7/ kg 1 patient: 7×10^6/ kg 1 patient: 8.5×10^6/ kg	6 months	10/10
Francisco Moniche et al. 2012 [15]	IA	66.9 ± 13.9	Day 5 - 9	1.59×10^8	6 months	10/10
Maurício A. G. Friedrich et al. 2012[16]	IA	30 - 78	Day 3 - 7	22.08×10^7	6 months	20/20
Alok Sharma et al. 2014[17]	IC	27 - 79	4 - 144 months	10^6/ kg	6-54 months	14/24
Kameshwar Prasad et al. 2014 [18]	IV	50.7 ± 11.6	7 - 30 days	280.75×10^6	1 year	58/58
Akihiko Taguchi et al. 2015[19]	IV	57 - 75	7 - 10 days	6 patients: 2.5×10^8 6 patients: 3.4×10^8	6 months	12/12
Azza Abass Ghali et al. 2016[20]	IA	46 - 66	12 – 32 Days (mean = 22 days)	10^6	12 months	21/21
Ashu Bhasin et al. 2016[21]	IV	Group I: 48.6 ± 7.1 Group II: 48.1 ± 9.1	3 months – 1.5 years	10^6/ kg	12 months	10/10

IV – intravenous, IA - intra-arterial, IC - intrathecal.

* Information about the group that has received BMMNCs infusion is undisclosed.

cells was 3.058×10^8 (range between 1×10^8 and 5×10^8). Also, the authors have investigated the distribution of BMMCs labeled with ^{99m}Tc 2 and 24 h after transplantation and observed that the infused cells were localized in the brain, although at 24 h, cell homing could only be visualized in the brains of two patients. 2 patients suffered seizures approximately 200 days after the cell infusion and were placed under an extended follow-up. At the 180 day of follow-up all patients had improved NIHSS (National Institute of Health Stroke Scale) scores in comparison with the pre-transplantation values (range – 1 to 8 points). This study confirms that BMMNC autologous transplantation is safe for ischemic stroke patients and can lead to an improvement in patient outcomes, but the absence of a control group should be pointed out as a study limitation.

Sean I. Savitz et al. [13] have included 10 patients with acute ischemic stroke in their open-label prospective study. Within 24-72 hours after the stroke onset, the BMMNCs were infused intravenously. 8 patients received approximately 10^7 cells/kg, one patient received 7×10^6 cells/kg and the other one revived at 8.5×10^6 cells/kg. Two patients had infarct expansion between enrollment and harvest and subsequently underwent hemicraniectomy. One patient died on the 40th day after enrollment in the experiment due to a pulmonary embolism related to the stroke and the patient's request to discontinue medical therapy. The Median NIHSS score was 13 before harvest of the BMMNCs, 8 – on 7 day after BMMCS infusion, and 3 – 6 months after BMMCS infusion. At 6 months, all surviving patients had shifted down by at least 1 point on the mRS (modified Rankin Scale) compared to day 7. 7 out of 10 patients achieved a BI (Barthel Index) ≥ 90. Also when comparing with the historical controls, the majority of the BMMNC treated patients were within the 95% confidence interval (CI) range or showed a better outcome at 90 days on the mRS scale. This study confirms that BMMNC transplantation is a safe treatment for ischemic stroke patients and may lead to a better outcome, but the lack of a control group should be noted as a limitation.

Francisco Moniche et al. [14] have completed a single-blinded (outcomes assessor) controlled Phase I/II study. They included 20 ischemic stroke patients, from which 10 formed a BMMNC treated group, and 10 formed the control group. The mean NIHSS score was 15.6 in the BMMNC treated group and 15.0 in the control group (P=0.82). Autologous transplantation was done 5 to 9 days after stroke onset. BMMNCs were injected in the M1 segment of the infarct-related MCA (medial cerebral artery) at low pressure. A mean of 1.59×10^8 cells were transplanted in the BMMNC treated group, from which a mean of 3.38×10^6 were CD34+ cells. 2 patients from this group had an isolated partial seizure (at 3 months). In both cases an antiepileptic drug was administered and there were no recurrent seizures. There were no statistically significant differences in the neurological function at 180 days of follow-up. At 6 months, a greater insignificant proportion of BM-MNC-treated patients had mRC modified Rankin Scale scores of ≤2 (20% versus 0%, P=0.47). There was a trend towards a better outcome when higher numbers of CD34+ cells were injected, especially in the BI Barthel Index at 1 month after transplantation (P=0.09). Higher significance levels of β-nerve growth factor (β-NGF) appeared in BM-MNC-treated patients than in control subjects: after 8 days β-NGF levels were 12.8±2.7 in BMMNC treated group versus 3.9±2.5 I control group (P=0.029). This study shows that BMMNC autologous transplant is safe for ischemic stroke patients, and confirms that BMMNC infusion is associated with an elevated level of β-NGF in the blood.

Maurício A. G. Friedrich et al. [15] included 20 patients with moderate to severe acute middle cerebral artery infarcts in their study. The mean baseline NIHSS score was 17 ± 5.6 (median 15.5; range 9–28). The mean time from stroke onset to treatment was 6 ± 1.8 days (range 3–10) and the mean BMMNCs in the infused solution was 22.08×10^7 cells (range 5.1×10^7-60×10^7). There were no serious adverse effects related to the experimental procedure. 2 patients died during the follow-up. One of them was discharged in a good condition but suffered an acute myocardial infarct 43 days after treatment. The other patient has undergone a hemicraniectomy 2 days after intra-arterial infusion and responded well to this procedure. However, he died 61 days after the IA ABMMC infusion from infectious complications related to an elective cranioplasty. A significant reduction of NIHSS score between the pretreatment period and 180 days after transplant was observed (p<0.001). 6 patients (30%) achieved satisfactory clinical improvement in functional recovery at 90 days. A total of 8 patients (40%) achieved a mRS ≤2 at 90 days. This study confirms that intra-arterial BMMNC transplantation is safe and can lead to a better clinical outcome for ischemic stroke patients. The main limitation is the absence of a control group.

Alok Sharma et al. [16] have included 24 patients in their study, 14 of which had suffered an ischemic stroke, and 10 who had suffered a hemorrhagic stroke. Between 24h and 48h before cell harvesting, patients were infused with granulocyte colony stimulating factor. Patients were infused with a quantity of $10^6\times$ kg of body weight of BMMNCs, intrathecal, in the L4-L5 lumbar space. The authors have concluded that out of 24 patients 12 have shown improvements in ambulation, 10 in hand functions, 6 in standing balance, 9 in walking balance, and 10 patients in functional status. Also, it was observed that patients aged less than 60 years showed a high improvement percentage compared with older patients. Also, the percentage of improvement was higher in patients whose stroke episode happened less than 2 years prior, as compared to patients whose stroke episode happened more than 2 years prior to the study. Out of 24 patients, 9 had affected higher mental functions. 2 out of these 9 patients showed an improvement in higher mental functions after BMMNC transplantation and neurorehabilitation. Patients were followed-up for a minimum of 6 months to a maximum of 4.5 years. None of the patients had any major adverse events. This study confirms that BMMNC transplantation using the intrathecal

route is safe and has the potential to lead to a better outcome, but it should be pointed out that 14 out of 24 patients have suffered the ischemic stroke, the remainder having suffered a hemorrhagic stroke. The main limitation of the study is the lack of a control group.

Kameshwar Prasad et al. [17] have conducted a phase II, multicenter, parallel group, and randomized trial with a blinded outcome assessment that included 120 patients that had suffered from ischemic stroke. In the marrow mononuclear stem cells (BMSCs) treated group, 58 patients were intravenously infused with BMSCs (initially there were 60 patients, but 2 missed because of withdrawal and logistical difficulties). Other 60 patients formed the control group. The mean number of BMSCs infused was 280.75×10^6 cells. The transplantation took place between 7 and 30 days after the stroke onset (median of 18.5 days). 5 (8.4%) out of 59 patients in the BMSC group and 5 (8.3%) out of 60 in the control group died before day 180. Three more patients died at day 195, day 206, and day 221 in the BMSC group. No significant differences in the NIHSS score and changes in infarct volume at day 90 and day 180 were observed between the BMSCs and the control group. The BI score on day 90 and day 180 of the both groups was also similar. Analysis adjusted for infarct volume, baseline NIHSS, and baseline BI did not change the results. Scores of mRS in the control group versus the BMSC group at day 180 showed no difference. No relationship was observed between cell dose and outcomes. This study confirms that BMSC transplant is safe for ischemic stroke patients but does not present any improvements in outcomes correlated with such a therapeutic approach.

Akihiko Taguchi et al. [18] have conducted a phase1/2a clinical trial and included 12 patients that have suffered an ischemic stroke of embolic etiology in their study. Patients were aged between 57 and 75 years old (mean age=67.4 ± 5.4 years). Mean NIHSS scores were 16.6 ± 4.7 and 16.3 ± 3.3 on admission and day 7 after stroke, respectively. The BMMNC transplantation took place on day 7-10 after stroke. A group of 6 patients were intravenously infused with a mean number of $2.5\pm0.5\times10^8$ cells, and another group of 6 patients were infused with a mean number of $3.4 \pm 1.3\times10^8$ cells. Patients were followed up 6 months after treatment, and serious adverse effects were observed in two patients. One of them experienced aspiration pneumonia and sepsis 3 months after cell therapy. An independent data monitoring committee concluded that cell transplantation had no association with the occurrence of aspiration pneumonia and sepsis. The other patient experienced a recurrent stroke. The independent data monitoring committee concluded that the association between cell transplantation and the recurrent stroke in this patient was unclear. Mean NIHSS scores on day 7 after stroke and day 30 after cell transplantation were 16.3 ± 3.3 and 11.6 ± 4.8, respectively. Mean improvement in NIHSS score was 4.8 ± 4.6 ($P < 0.01$, 95% CI). Although there were no statistically significant differences between the low-dose and high-dose groups, administration of the higher dose of BMMNCs consistently showed a trend towards an improved neurological recovery. Also, comparing patients who received cell therapy with historical controls, a trend favoring improvement was observed in the group treated with bone marrow mononuclear cells. Significant differences were observed between the two groups in NIHSS scores at the time of discharge ($p < 0.05$) and change of the NIHSS score between day 7 after onset of stroke and discharge ($p < 0.05$). This study confirms that BMMNC autologous transplantation is safe for ischemic stroke patients and has the potential to enhance neurological improvements. The main limitation of this study is the absence of a control group.

Azza Abass Ghali et al. [19] included 39 patients with sub-acute cerebral infarct in their study. The patients had suffered stroke from 1 week up to 3 months before they were included in the study. At that time, their National Institutes of Health Stroke Scale (NIHSS) scores were between 4 and 20. 21 patients were in the group treated with BMMNCs transplant, and 18 patients were in the control group. Three days before the procedure, patients received a daily subcutaneous injection of granulocyte colony stimulating factor (Pegfilgrastim). The BMMNCs treated group received a quantity of approximatively 1×10^6 BMMNCs, by infusion in the ipsilateral carotid artery. The time period of BMMNCs administration after stroke onset was between 12 and 32 days, with a mean of 22 days. At the beginning of this study, there were no significance and differences between both groups in NIHSS ($p = 0.364$), modified Rankin Scale (mRS) ($p = 0.452$), Barthel index (BI) ($p = 0.84$) scores were not significant and different in both groups. At the fourth month of the follow-up, a significant improvement in NIHSS within each group was observed, but without statistically significant comparisons ($p = 0.376$). After 12 months of follow-up both groups showed significant improvement in mRS and BI but aslo without statistical significance on comparison, with $p = 0.290$ for mRS and $p = 0.745$ for BI, respectively. The language deficit, which was evaluated via the Arabic version of the Comprehensive Aphasia Test, was also insignificant in both groups initially ($p = 0.513$); at the end of follow-up there was a marked improvement in both groups, but again without any statistical significance on comparison ($p = 0.691$). There were no severe complications during the treatment and follow-up which could be associated with the BMMNC autologous transplantation. This study confirms that such treatment is safe for ischemic stroke patients, but does not prove any improvement in outcomes associated with BMMNC transplantation.

Ashu Bhasin et al. [20] have carried out a randomized placebo-controlled clinical trial. 20 patients that have suffered an ischemic stroke and 20 age-matched healthy controls were included in this study. 20 patients were randomized and formed 2 groups, with 10 patients in each of them. One group was treated with BMMNC autologous transplantation and the other group with infused placebo. The subjects were diagnosed with ischemic stroke from 3 months up to 1.5 years before being included in the study. The BMMNC treated group received 10^6 BMMNC/kg. After 2 months,

there were no statistically significant differences between BMMNC treated group and the control group, according to modified Barthel index (mBI) (p=0.31) and Fugl Meyer (FM) scale for upper limb (p=0.25). Modified Ashworth scale (MAS) and the Medical Research Council (MRC) for muscle strength were statistically insignificant between the 2 groups (p>0.05). Also, the vascular endothelial growth factor (VEGF) and the brain-derived neurotrophic factor (BDNF) levels were found to be more elevated in BMMNC treated group compared to the control group, but without statistically significant differences (VEGF: 442.1 vs. 400.3 pg/ml, p = 0.67; BDNF: 21.3 vs. 19.5 ng/ml). There were no severe complications during the treatment or follow-up. This study confirms that BMMNC treatment is inoffensive for ischemic stroke patients.

Discussion

Therapeutic time window

There are reasons to consider the optimal therapeutic time window for BMMNCs autologous transplantation to be the subacute stage of the ischemic stroke, although there are some studies that suggest that this treatment could be effective even during the chronic stage [12]. One of the reasons to administrate BMMNCs in an optimal therapeutic time window is that these cells could support the endogenous neurogenesis, especially during its peak after stroke. In rodent stroke models, neural stem cells in the poststroke brain, in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus were observed, all of them capable of differentiating into new neurons. Between 7 and 10 days after stroke, there seems to be an increase in mitotic activity whithin the SVZ, then a decrease during weeks 3-5 is observed, and thereafter it continues at lower levels over the course of the following year [21, 22]. Other studies have pointed out that administration of BMMNCs in rodents between 2 and 14 days after stroke lead to significant positive effects [23].

A histopathological study conducted by Nakayama D et al. [24] has shown that the peak in endogenous neurogenesis in stroke patients occurs on the fourth day and 10-24 days after stroke. Temporal profiles of 2 markers in poststroke cortex: nestin- and musashi-1-positive cells were provided. Also, according to these temporal profiles, day 17 after stroke onset is the last day in which the levels of both of these markers were elevated at the same time, although the level of Musashi-1-positive cells were found to be raised up to 24 days after stroke.

In the first 24-72 hours after stroke, patients are usually neurologically unstable. In the sudy conducted by Sean I. Savitz MD et al. [13], the patients were treated with BMMNCs within 24-72 h after stroke. 2 out of 10 patients had infarct expansion between enrollment and harvest, and required hemicraniectomy after transplantation. In the study conducted by Maurício A. G. Friedrich and colleagues [15] it was also reported that a patient developed hemorrhagic transformation of his infarct before the BMMNCs trasplantation (before day 3 poststroke), and a hemicraniectomy was performed 2 days after the IA infusion of BMMNCs.

In the Francisco Moniche study [14] the patients have been treated with BMMNCs between 5 and 9 days after stroke. Although no correlation between the functional status and the amount of transplanted BM-MNCs was detected, there was a trend towards a better outcome when higher numbers of CD34+ cells were injected, especially in the Barthel Index BI at 1 month after transplantation (r=0.57, P=0.09). Also, higher significance levels of β-nerve growth factor appeared in BM-MNC-treated patients than in control subjects; after 8 days these were 12.8±2.7 versus 3.9±2.5, respectively (p=0.029).

In the study conducted by Maurício A. G. Friedrich [15] the patients were treated with BMMNCs within 3 to 7 days from stroke onset, and satisfactory clinical improvement occurred in 6/20 (30%) patients at 90 days. 8 out of 20 patients (40%) showed a good clinical outcome.

In the study conducted by Akihiko Taguchi [18] patients have been treated with BMMNCs within 7-10 days after stroke. Although there were no statistically significant changes on NIHSS, iB (BI) and mRS between the patients that were treated with BMMNCs IV and the control group that was not, when comparing patients who received cell therapy with historical controls, a trend favoring improvement was observed in the group treated with bone marrow mononuclear cells. Also, the author has pointed out that analysis of cerebral blood flow and metabolism in patients after autologous BMMNC transplantation showed a trend favoring an increase in rCBF (regional cerebral blood flow) and $rCMRO_2$ (regional cerebral metabolic rate of oxygen).

In the study conducted by Valeria Battistella and colleagues [12], NIHSS scores were improved (range – 1 to 8 points) during follow-up in all patients, although they received intra-arterial BMMNCs 59–82 days after stroke. Even so, it should be noted that the patients from this study had a lower NIHSS score when they were included in this study (range between 4 and 13), comparing to other clinical studies [14, 15, 18].

In the study conducted by Kameshwar Prasad and colleagues [17], the time window for BMMNCs transplantation after stroke onset was 18.5 days (median), in the study conducted by Azza Abass Ghali [19] – the time period of 12 to 32 days, with a mean of 22 days poststroke onset, and in the clinical study conducted by Ashu Bhasin and colleagues [20] – 3 months up to 1.5 years after stroke onset. This time period could be a reason for which they did not point out any beneficial effects in stroke treatment.

Optimal cell transplantation route

An optimal cell delivery route should bypass the peripheral filtering organs, provide a maximal possible cell grafting and confirm a maximal safety for the patient. There were 3 types of transplantation routes used in these 9 clinical trials (Fig.1). In 4 studies, the route of choice was the intravenous route, in other 4 studies – the intra-arterial route, and only one study used the intrathecal route.

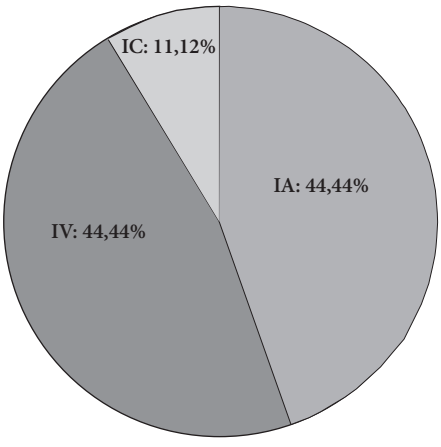

Fig. 1. Diagram showing different BMMNCs delivery routes. IV – intravenous, IA – intra-arterial, IC – intrathecal

There are some concerns about safety regarding the intravenous and intra-arterial delivery routes, namely microemboli formation and development of microstrokes. On the other hand, the intrathecal route may result in most grafted cells, but it is also the most invasive one.

All the clinical trials have confirmed the safety for their chosen delivery route. There were no serious adverse reactions during the treatment or follow-up in all 9 studies linked to any of the chosen delivery routes. BMMNCs have a smaller size, comparing with other stem cells, for example mesenchymal stem cells (MSC), and a preclinical study has shown that infusion of BMMCs resulted in a 30-fold pulmonary passage increase as compared to a single MSC bolus [25]. Also, their smaller size decreases the risk of emboli formation in the blood. In studies that have chosen the intra-arterial delivery route, the infusions were performed using a microcatheter, which is considered to preserve the anterograde blood flow, and therefore to avoid the of microstrokes [26].

Unfortunately, only one study [12] has analyzed the biodistribution of the labeled BMMNCs. It has been concluded that at 2h after transplantation, the ^{99m}Tc-labeled cells were present in the brains of all patients, and the activity of the isotope was 0.6–5.1% of the activity in the whole body. At 24h, the cells were seen to be in the brain in only 2 out of 6 patients. Also, the author has mentioned that the absence of labeled cells in the brain of the remaining patients could be due to the decay of the radioactivity compound below the levels of detection and/or to the decrease in the number of cells at the lesion site. It is not possible to compare these transplantation routes and to conclude which one is more efficient, as the BMMNCs were administered in different time windows after stroke and the number of studies is too small. However, some observations could be made concerning a potential superior efficiency of the intrathecal route over the intravenous route. In the study conducted by Alok Sharma and colleagues [16] the patients were treated in the chronic phase (4-144 after onset) with a mean number of 10^6/kg BMMNCs via intrathecal route, and in the study conducted by Ashu Bhasin and colleagues [20] the patients were treated similarly in the chronic phase (3-18 months after onset) with 10^6/kg BMMNCs. The first study has revealed that patients had a better outcome, as 38% have improved their functional independence measure (FIM) score, 50% improved in their ambulation, 42% in hand functions, 38% in walking balance and 25% in standing balance. By contrast, the second study did not find any significant improvement in patient's outcome, which can lead to the opinion that at least in the chronic phase the intrathecal route is more efficient. The major limitations here are that the study which used the intrathecal route is uncontrolled and the studies did not use the same clinical outcome measures. Another observation is that 3 out of 4 studies in which the intra-arterial delivery route was used have shown some encouraging results. The study conducted by Francisco Moniche et al. showed a trend towards a better outcome when higher numbers of CD34+ cells were injected [14], in the study conducted by Maurício A. G. Friedrich et al. [15] 40% of patients have shown a good clinical outcome, and in the study conducted by Valeria Battistella et al. [12] improved NIHSS scores during follow-up in all patients have been observed. Some of the limitations here are that the last 2 studies are uncontrolled, and in the study conducted by Valeria Battistella et al. the patients had a lower initial NIHSS comparing to other studies [14, 15, 18].

Cell dose

The range of the number of BMMNCs infused varies between 10^6 cells to 10^7 / kg cells (fig. 2, tab. 2). Each quantity has proven to be safe for autologous transplantation in poststroke patients.The number of cells to be infused was selected either by extrapolating the dose from rodents to humans based on their weight or brain size or was based on other clinical trials with cell transplantation.

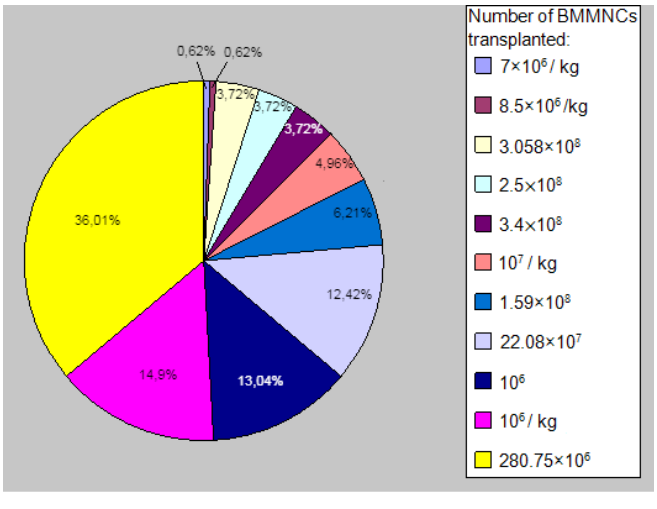

Fig. 2. Chart summarizing the percentage of patients that received certain doses of Bone Marrow-derived Mononuclear Cells (BMMNCs).

It is not possible to make an objective correlation of cell doses with a change in functional outcome as there are other variables that have a marked influence on it, for example

Table 2

A comparison of the different BMMNCs transplantating doses in clinical studies

Authors and year of study	Transplantation dose (cells)	Time period of administration after stroke onset	Route of administration	Improvement in outcome
Valeria Battistella et al. 2010 [13]	$1 \times 10^8 – 5 \times 10^8$ (mean of 3.058×10^8)	Day 59 - 82	IA	+
Sean I. Savitz MD et al. 2011 [14]	7×10^6 / kg - 10×10^6 / kg	24 – 72 h	IV	++
Francisco Moniche et al. 2012 [15]	1.59×10^8	Day 5 - 9	IA	-*
Maurício A. G. Friedrich, et al. 2012 [16]	22.08×10^7	Day 3 - 7	IA	+
Alok Sharma et al. 2014 [17]	10^6 / kg	4 - 144 months	IC	+
Kameshwar Prasad et al. 2014 [18]	280.75×10^6	Day 7 - 30 (median of 18.5 days)	IV	-
Akihiko Taguchi et al. 2015 [19]	6 patients: 2.5×10^8 6 patients: 3.4×10^8	Day 7 - 10	IV	++
Azza Abass Ghali et al. 2016 [20]	10^6	12 - 32 days	IA	-
Ashu Bhasin et al. 2016 [21]	10^6 / kg	3 months - 1.5 year	IV	-

IV – Intravenous; IA – intra-arterial; IC – intrathecal;
"-" – no significant difference in patients outcome;
"+" – an improvement in patients' outcome but no control group in study;
"++" – an improvement in comparison with historical controls;
* – there were no significant differences in neurological function during follow-up, but a positive correlation trend between the number of CD34+ cells injected and Barthel Index was found (r=0.56, P=0.09).

the time window of administration, the route of administration and patient heterogenicity. It should be pointed out that one study [19] has tested 2 different dosages for 2 groups of 6 patients, one of which has received 2.5×10^8 BMMNCs, and the other one – 3.4×10^8 BMMNCs. The author has concluded that administration of the higher dose of BMMNCs consistently showed a trend towards enhanced neurologic recovery, although without statistically significant differences between groups.

Mechanisms of action

The protective mechanisms of action of BMMNCs are thought to be: stimulation of arteriogenesis and angiogenesis, modulation of local and systemic inflammation and secretion of neurotrophic factors.

Arteriogenesis and angiogenesis

After cerebral ischemia, especially after obstruction of the medial cerebral artery (MCA), there is usually a substantial injury of the neural tissue supplied by the artery. Nevertheless, a part of this tissue could be saved, as there are leptomeningeal collateral vessels from the anterior cerebral artery (ACA) and the posterior cerebral artery (PCA), which appears to allow for perfusion of some brain tissues to persist [27]. However, the arteriogenesis is relatively slow and self-limiting and cannot compensate sufficiently for MCA obstruction [28]. Thus, stimulation of arteriogenesis could be an important strategy in the treatment of ischemic stroke. BMMNCs contain endothelial progenitor cells, which have been reported to contribute to revascularization of ischemic tissues [9]. In a preclinical study, Wang et al. reported that transplanted BMMNCs can differentiate into smooth muscle cells (SMCs) and endothelial cells (ECs) after permanent MCA obstruction in rats [29]. The differentiated cells exhibit an increased arteriogenesis (especially for leptomeningeal anastomoses) and angiogenesis by direct incorporation in collateral vessel walls. Other studies, as that conducted by Youshi Fujita et al. [30] did not find any evidence of direct structural incorporation of BMMNCs into ECs. Instead, donor BMMNCs with morphological features of pericytes were observed in the vessel walls. Another study has shown that BMMNC treatment induced an increase in vascular endothelial growth factor (VEGF) and Ser1177 phosphorylated endothelial nitric oxide synthase (eNOs) levels and resulted in an enhanced cerebral blood flow (CBF) in the acute phase [30]. Although the exact mechanism is not known, these preclinical studies show that BMMNCs promote arteriogenesis and angiogenesis through upregulation of eNOs, increasing of VEGF level in the blood, stimulation of endogenous EC proliferation and stimulating the direct differentiation into ECs and pericytes. The VEGF is a key mediator of arteriogenesis and angiogenesis. VEGF has been shown to increase vascular permeability and the proliferation of vascular endothelial cells and to inhibit endothelial cell apoptosis [31]. Unfortunately, there are few clinical trials that have evaluated the level of

VEGF after BMMNCs transplantation. In the clinical study conducted by Akihiko Taguchi et al. [18] a nonquantitative SPECT imaging was performed in a 48h window before cell transplantation, and at 1 and 6 months after cell transplantation the rCBF, $rCMRO_2$ and OEF were measured with a PET imaging. The author has pointed out that the analysis of cerebral blood flow and metabolism in patients after autologous BMMNC transplantation showed a trend favoring an increase rCBF in contralateral hemisphere and an increase in $rCMRO_2$ in both hemispheres. In parallel with the increase of rCBF, a decrease in OEF was observed in contralateral hemisphere. Although, it is important to point out that in 6 out of 12 patients these measures could not be obtained at either 1 or 6 months after treatment because of restlessness of the patient or maintenance/replacement of the PET machine. This study did not show any significant change in vascular endothelial growth factor (VEGF) after BMMNCs infusion. The clinical study conducted by Ashu Bhasin et al. [20] has shown the serum VEGF at baseline was higher in severely affected patients than in moderately affected patients (316.1 vs. 257.4 pg/ml), which remained high at 2 months predicting a good functional recovery. The study has also shown that at 2 months after BMMNCs transplantation, the patients treated with autotransplant had a higher level of VEGF than the control group (mean 453.5 ± 89.1 vs. 408.4 ± 93.3 pg/ml, 95% CI 13.3-6.7, p = 0.96), although without a statistical difference. The author made the conclusion that in chronic strokes (without classification into stroke subtype and volume), VEGF might have been increased already at acute onset in severely affected patients it stimulates angiogenesis and provides neuroprotection.

Modulation of inflammation

The brain responds to ischemic injury with an acute and prolonged inflammatory process, which tends to give rise to cytotoxic damage to the surviving neurons, neural glia and endothelial cells in the peri-infarct area [32]. Some studies have shown the BMMNC infusion can suppress inflammation. The study conducted by Francisco Moniche et al. [33] has shown that there is a negative correlation between the levels of matrix metalloproteinase-2 (MMP-2) at day 4 after transplantation and the number of CD34+ cells injected ($r = -0.667$, $p = 0.071$). Also, lower levels of MMP-2 at day 4 were correlated with lower neurological deficit (NIHSS at day 30) ($r = 0.775$, $p = 0.041$). MMP2 induce shedding of cytokines and growth factors and may contribute to the creation of a chemotactic gradient and subsequent immune cell recruitment to sites of vascular injury [34]. Another study conducted by Francisco Moniche et al. [14] revealed a positive correlation trend between the number of CD34+ cells injected and the BI (r=0.56, P= 0.09). On the other hand, a strong correlation was detected between serum levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) at day 90 after transplantation and the total number of BM-MNCs injected ($r = 0.929$, $p = 0.001$) and BM-MNC per kilogram injected ($r = 0.929$, $p = 0.003$). GM-CSF functions as a cytokine which stimulates stem cells to produce granulocytes and monocytes, thus promoting inflammation.

Secretion of neurotrophic factors and enhancing the neurogenesis

As stated before, the NSCs residing in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus are capable of producing new neurons in adult brains. Moreover, it is known that NSCs develop in the poststroke brain [35].

A histopathological study conducted by Nakayama D et al. has analyzed poststroke cerebral cortices in autoptic human brains and has confirmed that the NCSs are found in the human poststroke cortex [24]. Also, this study has shown that there is a peak in endogenous neurogenesis in stroke patients at the fourth day and 10-24 days after stroke. During this time period, it is absolutely essential to sustain the neurogenesis with neurotrophic factors. The study conducted by Francisco Moniche et al. [14] has shown that higher significance levels of β-nerve growth factor (β-NGF) appeared during the first week in BMMNC-treated patients than in control subjects: β-NGF levels after 4 days were 10.3±3.1 versus 8.5±2.9 (P=0.68) and after 8 days were 12.8±2.7 versus 3.9±2.5 (P=0.029). β-NGF is involved primarily in the growth, as well as the maintenance, proliferation, and survival of neurons. The study conducted by Akihiko Taguchi et al. [18] has shown an increase in brain-derived neurotrophic factor (BDNF) after infusion of 3.4×10^8 BMMNCs (2.721.7 ± 2.052.4 pg/mL at the baseline vs 4.319.0 ± 5.002.8 pg/mL 1 day after transplant) but without any statistically significant changes. Another study has also analyzed the level of BDNF but did not find any statistically significant improvement within 8 weeks between the group treated with BMMNCs and the control group (mean 32.8 ± 9.2 vs. 27.3 ± 9.1 ng/ml).

Conclusions

BMMNC autologous transplant is a safe therapy for patients that have suffered ischemic stroke without any severe complications associated. There are reasons to consider the subacute stage of the stroke to be the optimal therapeutic time window for this method of treatment. Although some clinical studies stated a better outcome in patients treated with BMMNC, further clinical trials are needed to establish their therapeutic efficiency.

Competing interests

The author declares no conflict of interests regarding publication of this paper.

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