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Research report

Human bone marrow mesenchymal stem/stromal cells produce efficient localization in the brain and enhanced angiogenesis after intra-arterial delivery in rats with cerebral ischemia, but this is not translated to behavioral recovery

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HIGHLIGHTS

- Intra-arterial cell infusion of human mesenchymal cells is efficient technique to target cells into the ischemic hemisphere.
- Intra-arterial cell delivery enhances the perilesional angiogenesis in MCAO rats.
- Cell treatment does not improve functional outcome, when strict behavioral tests were applied.

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ABSTRACT

Intravascular cell therapy is a promising approach for the treatment of stroke. However, high accumulation of cells to lungs and other filtering organs is a major concern after intravenous (i.v.) cell transplantation. This can be circumvented by intra-arterial (i.a.) cell infusion, which improves homing of cells to the injured brain. We studied the effect of i.a. delivery of human bone marrow-derived mesenchymal cells (BMMSCs) on behavioral and histological outcome in rats after middle cerebral artery occlusion (MCAO). Sixty male Wistar rats were subjected to transient MCAO (60 min) or sham-operation. BMMSCs (1×10^6) were infused into the external carotid artery on postoperative day 2 or 7. Histology performed after a 42-day follow-up did not detect any human cells (MAB1281) in the ischemic brain. Endothelial cell staining with RECA-1 revealed a significant increase in the number of blood vessels in the perilesional cortex in MCAO rats treated with cells on postoperative day 7. Behavioral recovery as assessed in three tests, sticky label, cylinder and Montoya's staircase, was not improved by human BMMSCs during the follow-up. In conclusion, human BMMSCs did not improve functional recovery in MCAO rats despite effective initial homing to the ischemic hemisphere and enhanced angiogenesis, when strict behavioral tests not affected by repeated testing and compensation were utilized.

1. Introduction

Stroke is the major cause of adult disability in western countries. More than 50% of surviving stroke patients are left with motor disabilities despite some spontaneous recovery over the ensuing weeks to months following the ischemic event [1]. At present, physical rehabilitation has remained the only effective approach to facilitate functional recovery in stroke patients.

Motor recovery, its underlying mechanisms as well as therapeutic strategies for stroke have recently been topics of intensive investigation. Potential restorative approaches are now emerging including cell therapy, which act through brain plasticity mechanisms. Cell therapies are clinically appealing, since treatment can be commenced even weeks after the ischemic insult [2–5]. The use of bone marrow mononuclear cells (BMMNCs) or bone marrow-derived mesenchymal stem/stromal cells (BMMSCs) is particularly promising, because they are known to home to the injured tissue and once there to secrete factors that promote brain repair [6]. In

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addition, BMMSCs have low immunogenicity, lack ethical issues and offer the potential for allogenic transplantation. Indeed, there is experimental evidence demonstrating behavioral improvement achieved by administration of BMMNCs/BMMSCs in rats subjected to experimental stroke [7–11].

Intravascular cell delivery has become more common and largely replaced intraparenchymal transplantation in experimental settings [12]. The major advantage is the noninvasive delivery and effective and extensive cell homing in case of large infarcts [13]. Several studies have shown that both intravenous (i.v.) and intra-arterial (i.a.) delivery routes are efficient in reducing infarct size and enhancing motor and cognitive recovery in stroke animals [10,14,15]. However, it is still not clear which of the intravascular delivery routes is the most effective [10,16–18].

We have previously shown that i.v. administered human umbilical cord blood cells or human embryonic stem (ES) cell-derived neural progenitors in rats subjected to middle cerebral artery occlusion (MCAO) [19,20]. Obtained results showed the accumulation of cells primarily into lungs and it was followed by relocation to the liver and spleen but with no signs of engraftment in the brain. It is possible to circumvent the trapping organs by targeting the cells directly to the ischemic brain by using i.a. delivery. Unfortunately this can be associated with complications such as mortality possibly related to micro-occlusions [21,22]. We have developed a novel i.a. infusion technique that takes advantage of the external carotid artery (ECA) stump, which is prepared for filament insertion in the MCAO model [23]. I.a. delivery through the ECA resulted in efficient targeting of cells to the injured hemisphere and it was not associated with any mortality due to microemboli.

The delivery time may also have an impact on the results. In most animal studies, cells have been administered 24 h or less after cerebral ischemia [24]. In stroke patients, most studies have relied on autologous cells, necessitating late cell delivery between 4 and 9 weeks after the ischemic event [25–29]. Acute cell transplantation may provide neuroprotection, whereas transplantation during the chronic phase is thought to promote the brain's own repair mechanisms. In line with this, Komatsu et al. [7] showed that rats receiving i.v. infusion of BMMSCs 7 days after ischemia had a reduced lesion volume whereas rats receiving cells at 1 month showed enhanced angiogenesis near to the border of the ischemic lesion. Interestingly, the therapeutic time window of BMMSCs was postulated to be at least 1 month after the stroke in one study [30].

The limited behavioral recovery observed in previous studies suggests that current intravascular strategies are not optimal [19]. In the present study, we sought to determine whether the transient localization of human BMMSCs in the rat brain using i.a. delivery of cells through the ECA would be able to achieve a behavioral recovery following cerebral ischemia. Cells were infused either 2 or 7 days after MCAO to assess whether the delivery time had any effect on the outcome. Angiogenesis was studied as a possible repair mechanism underlying the therapeutic effect of BMMSCs by applying histology after the follow-up [7].

2. Methods

2.1. Animals

Sixty male Han:Wistar rats (Harlan, Israel), weighing 313–426 g were used in the study. The animals were housed individually in a controlled environment (temperature 21 ± 1 °C, humidity 50–60%, light period 07:00–19:00 h) with access to food (2016S, Teklad) and fresh water available ad libitum. Animal care procedures were carried out according to European Community Council Directives 86/609/EEC guidelines and all procedures were approved by the Animal Ethics Committee (Hämeenlinna, Finland).

2.2. Transient middle cerebral artery occlusion

Anesthesia was induced in a gas chamber (30% O_2/70% N_2O) with 5% halothane for 2-3 min and then maintained at 0.5–1.5% using a nose mask. Body temperature was maintained at 37 °C throughout the surgery by means of a heating pad connected to a rectal probe (Harvard Homeothermic Blanket Control Unit). Cerebral ischemia was induced by the filament technique [31]. The right common carotid artery (CCA) was exposed and a heparinized nylon filament of ∅ 0.28 mm diameter was inserted via the ECA stump into the internal carotid artery (1.8–2.1 cm) to occlude the (MCA). After MCAO (60 min occlusion), the filament was gently pulled out and ECA was closed by electro-coagulation leaving a long ECA stump to permit the subsequent cell infusion. Sham-operated control animals underwent the same procedure but without filament insertion. Buprenorfine (0.03 mg/kg) was administered for postoperative pain. Blood gases were monitored before, during and after MCAO in a separate cohort of animals and the values were within normal ranges.

2.3. Preparation and characterization of human BMMSCs

Cells were obtained from bone marrow aspirates from healthy volunteer donors after signed informed consent according to the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Helsinki University Central Hospital (Finland). BMMSC establishment and characterization has been described [23]. Briefly, BMMSCs were cultured in heparinized low glucose Dulbecco's modified Eagle's medium (DMEM; Gibco, Life Technologies Ltd, Paisley, UK), supplemented with 10% platelet lysate and plasma (Finnish Red Cross Blood Service, Helsinki, Finland), 100 U/ml penicillin and 100 μg/ml streptomycin. The medium was changed twice weekly and the cultures were passaged when subconfluent (70–80% confluency). The subconfluent p3 cells were detached with trypsin (TryPLe Express, Life Technologies Ltd). Cell viability was determined for all the samples by trypan blue exclusion or Nucleocounter NC-100 (Chemometec). Cells were cryopreserved in 180 mg/ml human serum albumin (HSA, Albunorm 200 g/l, Octapharma AG) and 10% dimethylsulfoxide (DMSO, Sigma-Aldrich).

BMMSCs were analyzed for cell surface epitope expression with fluorescent-conjugated antibodies against the following proteins: CD73, CD90 (Stem Cell Technologies), CD105, CD44, CD49d, CD49e, CD59, CD166, CD14, CD19, CD34, CD45 (all from BD unless stated otherwise). The cells were labeled with 2 μl of the antibodies/1×10^5 cells for 30 min at 4 °C and run with a FACSAria (BD) flow cytometer. The results were analyzed with the FlowJo software (version 7.6.1 TreeStar Inc.). Relevant isotype control antibodies were used.

2.4. Experimental groups

Twenty-four hours after MCAO, the forelimb placing test was used to assess the success of the MCAO operation [32]. For the test, the rat was held facing towards the table, resting its forelimbs on the table edge. The forelimb was gently pulled down and subsequent retrieval and limb placement was checked. The test was repeated by holding the head upward at a 45° angle so that the rat was not able to see the table or make vibrissal contact. The lateral placing of the forelimb was obtained by pulling down the forelimb while the rat was placed along the table edge. In addition, forelimb flexion was evaluated when the rat was lifted in the air by the base of its tail. The tests were scored in following manner: 2 points for normal performance, 1 point for delayed/incomplete response and 0 points for no response.