

Effect of Mesenchymal Stem Cells on the cerebral microvascularisation in a rat model of stroke: MRI study

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Introduction: Stroke is the leading cause of disability and the third cause of death in the world. Despite active researches, thrombolysis is still the only available and effective treatment as a stroke therapy but its narrow time window limits the number of treated patients. Human mesenchymal stem cells (hMSC) are non-haematopoietic stromal cells that can differentiate into several different cell types and have the ability of relative easy expansion in culture. After systemic infusion, hMSC home to injured tissues [1] and improve functional outcome in rat models of stroke [2]. All these characteristics make hMSC a promising source for tissue repair. However, mechanisms by which hMSC benefit to stroke are still controversial. Among them is enhancement of angiogenesis. The increase in cerebral blood flow that occurs within one to two weeks after stroke has been reported to be physiological and essential for the oxygenation and nutriment support into the lesion. Several studies support the idea that hMSC would enhance angiogenesis and vascular maturation by increasing the secretion of pro-angiogenic factors [3]. Magnetic resonance imaging (MRI) is a non invasive imaging method that can be used to study changes in cerebral blood volume (CBV), vessel size (VSI) and vascular density in a rat model of stroke [4]. However, this MRI method has never been used to study changes induced by hMSC on the cerebral microvascularisation after stroke. The aim of this study is to measure CBV and VSI changes during 21 days in a rat model of stroke after an intracerebral (IC) administration of hMSC.

Material and methods: Twenty Sprague Dawley male rats were randomly allocated in 4 groups: **1)** Rats underwent a transient (90 min) focal cerebral ischemia by occlusion of the right middle cerebral artery (MCAo) at day 0 (D0) and received at D8 an IC administration of 10 μ l of PBS-glutamine into the right striatum (5 μ l) and cortex (5 μ l) (MCAo-PBS, n=4); **2)** Rats underwent MCAo at D0 and received at D8 an IC administration of 4 \times 10⁵ hMSC suspended in 10 μ l of PBS-glutamine (MCAo-hMSC, n=5); **3)** Rats without cerebral lesion received, at D8, PBS-glutamine IC administration (control-PBS, n=6); **4)** Rats without cerebral lesion received, at D8, hMSC IC administration (control-hMSC, n=5). All groups were followed by MRI (7T, Bruker Avance III) during 21 days. T₂-weighted images were acquired at D1, D7, D9, D14 and D21 after MCAo to measure the lesion volume. The apparent diffusion coefficient (ADC) was mapped (TR/TE = 3000/28.6 ms, b=900 s/mm²) at D1, D7, D9, D14 and D21 to estimate brain oedema. CBV and VSI changes were measured at D7, D9, D14 and D21 in the four groups. CBV and VSI values were obtained using a steady approach. Briefly, a multi gradient echo (5 echoes) spin-echo (TE=2.4 ms) sequence (TR=4000 ms, 7 slices, voxel size=128 \times 72 \times 1000 μ m³) was acquired before and 2 minutes after the intravenous injection, via the tail vein, of an intravascular iron-based contrast agent (Sinerem(r), Guerbet Research, France; 200 μ mol iron/kg body weight). At the end of the MRI study (D21), a quantitative immunohistological study of blood vessels was performed (RecA labelling). Paired t-test was used for within-group comparison (right vs left hemisphere) and unpaired t-test for between group comparison (MCAo vs control).

Results: The two groups that underwent MCAo (MCAo-PBS, MCAo-hMSC) exhibited similar initial lesion volume (At D1: 187 \pm 195 mm³ vs 189 \pm 199.0 mm³, respectively) and similar evolution of the lesion volume during 3 weeks, demonstrating the absence of effect of hMSC on the lesion volume. ADC was larger in the MCAo-PBS group at day 14 and 21 than in the control PBS-group (D14: 1059 \pm 23 μ m².s⁻¹ vs 724 \pm 63 μ m².s⁻¹; D21: 1182 \pm 221 μ m².s⁻¹ vs 807 \pm 68 μ m².s⁻¹) (Figure 1). No ADC difference was observed between MCAo groups or between control groups, demonstrating the absence of effect of hMSC on the brain oedema (data not shown). After cerebral ischemia, CBV values were higher in the lesion than in the contralateral hemisphere during 21 days with a peak at D9 and a decrease afterwards (Figure 2). At D9, the ipsilateral CBV was higher in the MCAo-PBS group than in the MCAo-hMSC group (6.3 \pm 0.7 % vs 4.7 \pm 0.2 %) (Figure 2). In healthy rats, no difference in CBV was observed between both control-PBS and control-hMSC groups (data not shown). In the MCAo-PBS group, the VSI increase in the lesion was delayed and occurred at D14 instead of D9 (MCAo-PBS vs MCAo-hMSC at D9: 16.3 \pm 1.0 μ m vs 11.5 \pm 1.4 μ m; D14 = 15.8 \pm 2.2 μ m vs 17.3 \pm 4.7 μ m; D21: 16.1 \pm 2.0 μ m vs 18.1 \pm 2.5 μ m) (Figure 3). In healthy rats, no difference was observed for VSI values between both control-PBS and control-hMSC groups (data not shown). Immunohistology at D21 confirmed the increase in vessel size into the lesion with respect to that of the contralateral hemisphere and the absence of difference between both control groups without lesion and between both MCAo groups.

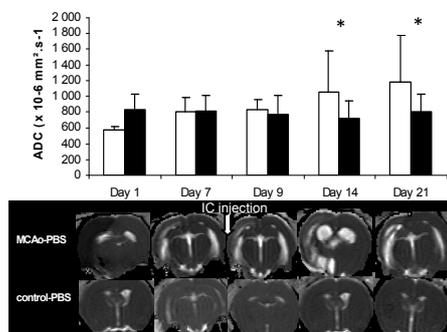


Figure 1. Effect of transient cerebral ischemia on the apparent diffusion coefficient (ADC).

Top: Quantitative analysis of ADC in MCAo-PBS group (white) and healthy control-PBS group (black) in the infarcted hemisphere (mean \pm SD). * $P < 0.05$.

Bottom: ADC maps from representative rats after cerebral ischemia (MCAo-PBS) and in the healthy control group (control-PBS) from day 1 to day 21.

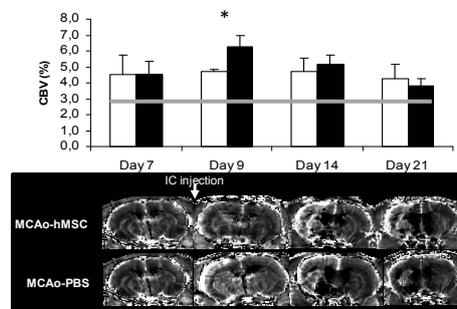


Figure 2. Effect of PBS-glutamine and hMSC IC injection on the cerebral blood volume (CBV) after transient cerebral ischemia.

Top: CBV over time in MCAo-hMSC group (white) and MCAo-PBS group (black) in the infarcted hemisphere (mean \pm SD). The mean CBV in the contralateral hemisphere is represented as a grey line. * $P < 0.05$.

Bottom: CBV maps from representative rats after hMSC or PBS-glutamine IC injection from day 7 to day 21.

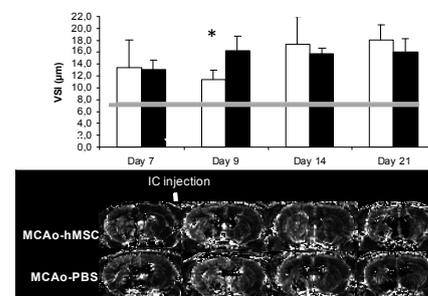


Figure 3. Effect of PBS-glutamine and hMSC IC injection on the vessel size index (VSI) after transient cerebral ischemia.

Top: Quantitative analysis of VSI over time in MCAo-hMSC group (white) and MCAo-PBS group (black) in the infarcted hemisphere (mean \pm SD). The mean VSI in the contralateral hemisphere is represented as a grey line. * $P < 0.05$.

Bottom: Maps of VSI from representative rats after hMSC or PBS-glutamine IC injection from day 7 to day 21.

Conclusions: These results show that our MRI method is suited to study the cerebral microvascularisation *in vivo* and to assess the effect of cell therapy on microvascularisation in stroke models. One day after IC administration, the hMSC have an effect on the cerebral microvascularisation and abolish the CBV increase commonly observed between 9 and 14 days after cerebral ischemia [4]. According to our VSI estimates, hMSC also delay the vasodilation secondary to cerebral ischemia.

References: [1] Detante and al. Cell Transplant 2009; [2] Chen and al. Stroke, 2001; [3] Chen and al. Circ Res 2003; [4] Lin and al. JCBFM 2008