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# Administrations of human adult ischemia-tolerant mesenchymal stem cells and factors reduce amyloid beta pathology in a mouse model of Alzheimer's disease

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ABSTRACT

The impact of human adult ischemia-tolerant mesenchymal stem cells (hMSCs) and factors (stem cell factors) on cerebral amyloid beta (Aβ) pathology was investigated in a mouse model of Alzheimer's disease (AD). To this end, hMSCs were administered intravenously to APPPS1 transgenic mice that normally develop cerebral Aβ. Quantitative reverse transcriptase polymerase chain reaction biodistribution revealed that intravenously delivered hMSCs were readily detected in APPPS1 brains 1 hour following administration, and dropped to negligible levels after 1 week. Notably, intravenously injected hMSCs that migrated to the brain region were localized in the cerebrovasculature, but they also could be observed in the brain parenchyma particularly in the hippocampus, as revealed by immunohistochemistry. A single hMSC injection markedly reduced soluble cerebral Aβ levels in APPPS1 mice after 1 week, although increasing several Aβ-degrading enzymes and modulating a panel of cerebral cytokines, suggesting an amyloid-degrading and anti-inflammatory impact of hMSCs. Furthermore, 10 weeks of hMSC treatment significantly reduced cerebral Aβ plaques and neuroinflammation in APPPS1 mice, without increasing cerebral amyloid angiopathy or microhemorrhages. Notably, a repeated intranasal delivery of soluble factors secreted by hMSCs in culture, in the absence of intravenous hMSC injection, was also sufficient to diminish cerebral amyloidosis in the mice. In conclusion, this preclinical study strongly underlines that cerebral amyloidosis is amenable to therapeutic intervention based on peripheral applications of hMSC or hMSC factors, paving the way for a novel therapy for Aβ amyloidosis and associated pathologies observed in AD.



## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia in the Western world. AD is neuropathologically characterized by the formation of extracellular senile plaques, primarily composed of polymerized amyloid beta (Aβ) peptides and the intracellular deposition of hyperphosphorylated tau proteins into neurofibrillary tangles (Selkoe, 1991). According to the amyloid cascade hypothesis, the extracellular deposition of Aβ is a critical and central event in the disease process leading to the formation of neurofibrillary tangles, neuroinflammation, cell death, and dementia (Hardy and Allsop, 1991). There is no cure on the horizon for this devastating disease that represents one of today's major healthcare challenges due to its severe socio-economic burden (Brookmeyer et al., 2007; Waite, 2015).

Human adult ischemia-tolerant mesenchymal stem cells (hMSCs) constitute a promising therapeutic approach for the treatment of various neurodegenerative disorders including AD (Fan et al., 2014). However, food and drug administration-approved clinical trials currently evaluating the impact of hMSC on AD are marginal. Therefore, an hMSC-based AD therapy represents a large untapped potential waiting to be exploited. Several preclinical research laboratories have reported a beneficial effect of mesenchymal stem cells on cerebral amyloidosis, adult neurogenesis, or memory impairments in models of AD (e.g., Salem et al., 2014; Yan et al., 2014; Yun et al., 2013). However, the majority of these therapeutic initiatives rely on the direct stem cell delivery into the brain

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through intracerebral or intracerebroventicular injection. These routes of administration entail a major hurdle for clinical applications due to their invasiveness and possible complications (Reyes et al., 2015). This difficulty has certainly hampered to a large extent the clinical translation of these preclinical findings. In contrast, intravenous delivery is fast, easy and complications are rarely observed. To date, only a limited number of preclinical studies have evaluated the impact of intravenous hMSC injections on cerebral amyloidosis (e.g., Kim et al., 2013).

In this present study, we investigated the impact of hMSC and mesenchymal stem cell factors (SCF) on cerebral Aβ pathology in a mouse model of AD. The hMSCs used in this study are bone marrow derived, ischemia-tolerant, cultured under a controlled, low physiological level of oxygen (5%), and manufactured under clinical good manufacturing process conditions. Importantly, these hMSC express negligible levels of human leukocyte antigen-D related (HLA-DR) cell surface receptor and have higher migratory ability as compared to cells cultured at the normal oxygen level (Vertelov et al., 2013). Quantitative reverse transcriptase polymerase chain reaction (RT-PCR) biodistribution revealed that intravenously delivered hMSC are detected in the brain at 1 hour after delivery, decreasing after 1 day, and subsequently dropping below detection level at 1 week after injection. Brain immunohistochemistry demonstrated that intravenously injected hMSCs could be detected in both the cerebrovasculature and in the brain parenchyma. A single intravenous hMSC injection was effective in reducing soluble cerebral Aβ levels at 1 week after delivery in both early and late stage of plaque development. The levels of several major Aβ-degrading enzymes were significantly increased in hMSC-treated mice. In addition, the levels of several proinflammatory cytokines such as Tumor Necrosis Factor alpha (TNFα) were decreased at 1 week after hMSC injection. As a complement to single hMSC delivery, the impact of a repeated intravenous hMSC delivery was investigated on cerebral amyloidosis in APPPS1 mice. Repeated delivery of hMSC (1 injection/wk for 10 weeks) safely reduced cerebral Aβ plaques in both young and aged APPPS1 animals analyzed 1 week after the last injection. Concomitantly, microglial coverage was diminished in hMSC-treated APPPS1 mice. No increase of vascular amyloid or manifestation of microhemorrhages was observed following the repeated intravenous hMSC delivery. Last but not least, in the absence of hMSC injection, proteins secreted by hMSC in culture could also significantly reduce cerebral amyloidosis in APPPS1 mice following repeated intranasal applications for a total of 3 weeks.

In summary, our preclinical results demonstrate that single and repeated intravenous administration of hMSC safely reduces Aβ pathology in the APPPS1 model of AD. The successful reduction of cerebral amyloidosis following intranasal application of hMSC factors invites the next step, that is, clinical studies using these factors as complementary treatment to stem cell injections.

## 2. Materials and methods

### 2.1. Animals and experimental study design for in vivo experiments

For the in vivo experiments, APPPS1 transgenic mice were obtained from M. Jucker (HIH, Tübingen, Germany) and maintained at the EPFL animal core facility. The mice coexpress under the control of the Thy-1 promoter the KM670/671NL Swedish mutation of human amyloid precursor protein (APP) and the L166P mutation of human presenilin 1 (PS1) and usually show the first amyloid plaques in the cortex at an age of 6–8 weeks. No gender effects in Abeta levels and amyloid deposition are observed in this mouse model. Minimal vascular Aβ is observed and is predominantly restricted to the pial vessels (Radde et al., 2006). APPPS1 mice were generated on a C57BL/6 background and both male and female APPPS1 mice as well as aged-matched control nontransgenic littermates were used. Mice were housed in groups of 5 in pathogen-free conditions until the beginning of the injection experiments, after which they all were singly housed (treatment and control groups). All animal procedures were performed according to the guidelines of the local authorities and Swiss animal protection law. APPPS1 mice received either a single intravenous hMSC injection or a weekly intravenous hMSC injection (500,000 cells/injection) for a total of 10 weeks. One injection consisted of a delivery through the tail vein of 500,000 cells in 100 μL of Lactated Ringer solution (LRS). The animals were sacrificed 1 week after the (last) injection. To facilitate the administration procedure, the animals were accommodated into a restraining box and the tail vein revealed by transillumination with an optical fiber. For intranasal application, the following procedure was used to deliver the soluble hMSC factors. The animal was restrained by hand without anesthesia, making sure to apply just enough firmness to the skin around the neck to prevent the mouse from turning or twisting out of the restraint, although avoiding to pull the skin so tightly that the animal cannot breathe. For intranasal delivery, using a 10-μL pipette, the required amount of soluble hMSC factors to be inhaled was placed at the nares of the animal. The animal was kept restrained on its back until the material disappeared into the nares. Regular observation of the animals during the experiments revealed no overt behavioral change due to the treatments.

### 2.2. Preparation of hMSC for injection

Stem cells were obtained from Stemedica Cell Technologies (San Diego, USA). The cells are equivalent to commercially available stem cells from ThermoFisher Scientific "StemPro BM MSC" (part number A15653). The required number of vials of frozen cells was thawed in a 37 °C water bath (1 vial contains $15 \times 10^6$ cells in 1 mL of freeze media with 10% DMSO). Vials were kept in the water bath until a small (~2–3 mm) ice crystal remained. The content of the vials (1 mL) was transferred into the 225 mL centrifuge tube. About 20 mL of prewarmed LRS was gently added (drop wise) and gently mixed. Then, prewarmed LRS was slowly added to a volume of 180 mL and mixed until homogenous. The tube was centrifuged at $600 \times g$ for 5 minutes at room temperature. The pellet was then decanted in the 225 mL tube down as close to the pellet as possible and the supernatant discarded. Subsequently, 20 mL of prewarmed LRS was gently added and mixed. Prewarmed LRS was slowly added to a volume of 180 mL and mixed until homogenous. The tube was centrifuged at $600 \times g$ for 5 minutes at room temperature. The pellet was decanted in the 225 mL tube down as close to the pellet as possible, and the supernatant again discarded. Then, 20 mL of chilled LRS was slowly added to the 225 mL tube and mixed until homogenous. Additional chilled LRS was slowly added to the 225 mL tube to a volume less than the required cell dose. For cell count, 100 μL was set aside. The cells were counted to determine yield and viability. The required volume of chilled (2 °C–8 °C) LRS was slowly added to a concentration of $5 \times 10^6$ cells/mL based on the cell count. The cells were injected directly after their final formulation preparation.

### 2.3. Histology and immunohistochemistry

One week after the last hMSC delivery, mice were deeply anesthetized and perfused transcardially with ice-cold phosphate buffer saline (PBS) (pH 7.4 for 2 minutes) followed by 4% paraformaldehyde in ice-cold phosphate buffer saline PBS (8 minutes). Brains were removed and postfixed overnight in the same fixative followed by 48-hour incubation in 30% sucrose at the temperature