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Longitudinal tracking of triple labeled umbilical cord derived mesenchymal stromal cells in a mouse model of Amyotrophic Lateral Sclerosis

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ABSTRACT

The translational potential of cell therapy to humans requires a deep knowledge of the interaction between transplanted cells and host tissues. In this study, we evaluate the behavior of umbilical cord mesenchymal stromal cells (UC-MSCs), labeled with fluorescent nanoparticles, transplanted in healthy or early symptomatic transgenic SOD1G93A mice (a murine model of Amyotrophic Lateral Sclerosis). The double labeling of cells with nanoparticles and Hoechst-33258 enabled their tracking for a long time in both cells and tissues. Whole-body distribution of UC-MSCs was performed by in-vivo and ex-vivo analyses 1, 7, 21 days after single intravenous or intracerebroventricular administration. By intravenous administration cells were sequestered by the lungs and rapidly cleared by the liver. No difference in biodistribution was found among the two groups. On the other hand, UC-MSCs transplanted in lateral ventricles remained on the choroid plexus for the whole duration of the study even if decreasing in number. Few cells were found in the spinal cord of SOD1G93A mice exclusively. No migration in brain parenchyma was observed. These results suggest that the direct implantation in brain ventricles allows a prolonged permanence of cells close to the damaged areas and makes this method of tracking reliable for future studies of efficacy.

1. Introduction

Over the last two decades, cell-based therapy has been evaluated in cardiovascular, oncologic, autoimmune and neurodegenerative diseases. In the neurological field preclinical studies have demonstrated the potential of stem cell injection (Lindvall and Kokaia, 2006). On the other hand, their clinical application is still limited and controversial (Viswanathan and Keating, 2011). Thanks to their self-renewal and differentiation abilities, stem cells had been originally considered as a possible tool to replace damaged cells by an initial selective migration to the injured area and a subsequent differentiation into the affected neurons (Bjorklund and Lindvall, 2000). Unfortunately, this fascinating mechanism of repair rapidly faded away and the hope of an effective care for neurodegenerative disorders by topic transplantation of highly committed neural stem cells and/or embryonic totipotential cells was more and more neglected. On the contrary, there are growing evidences of an alternative endocrine-like mechanism of action of stem cells in different murine models of acute and chronic neurological disorders that does not involve any specific cell differentiation toward the neural lineage (Silani et al., 2010; Uccelli et al., 2011, 2012). This mechanism, commonly referred as bystander effect, is mainly based on the secretion of trophic factors, anti-inflammatory cytokines, immunomodulatory agents and soluble molecules even far from the diseased area by transplanted cells. This experimental evidence greatly enlarged the spectrum of potential cell candidates, in particular stromal cells derived from extra-embryonic tissues, and paved the way to alternative systemic routes of administrations, in addition to the intraparenchyma implantation (Corti et al., 2004; Zhao et al., 2007; Knippenberg et al., 2012a; Mitrecic et al., 2010; Willing et al., 2008). The possibility to perform preclinical studies by systemically injecting cells in the bloodstream (IV) or, more locally intracerebroventricularly (ICV), has been adopted in many models of neurodegenerative disorders. All these premises prompted the scientific community to focus the attention on the effect of

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systemically injected fetal stem cells in different models of neurodegenerative disorders. In particular a large number of studies have been recently carried out in mouse models of Amyotrophic Lateral Sclerosis (ALS).

ALS is a fatal neurodegenerative disorder characterized by a selective and widespread degeneration of lower and upper motor neurons (Gordon, 2013; Rowland and Shneider, 2001). The main symptomatic features of ALS are muscular atrophy, motor paralysis and difficulties in speaking, swallowing, chewing and breathing (Harms and Baloh, 2013). Respiratory failure is the most common cause of death for ALS patients, which occurs within 3–5 years from the diagnosis (Al-Chalabi and Hardiman, 2013). The effect of Riluzole, the only drug approved by the Food and Drug Administration more than twenty years ago is very modest in prolonging the life of patients without ameliorating their quality of life, even lower than palliative cares (e.g. tracheostomy and invasive ventilation) (Cheah et al., 2010; Musaro, 2013). For these reasons, alternative and innovative therapeutic strategies are strongly and urgently required. Interestingly, it has been recently reported that both the ICV and the IV infusions of ematopoietic and mesenchymal stem cells induced a protective effect in two different models of ALS. Since no clear localization of stem cells within the areas of degenerating motor neurons was found, it was suggested that this was likely the response to the production of anti-inflammatory and immunomodulatory factors produced by the stem cells even far from the damaged areas (Garbuzova-Davis et al., 2008; Canzi et al., 2012; Bigini et al., 2011).

However what is the fate of the cells in terms of biodistribution, organ accumulation, potential target migration and clearance after a systemic or ICV administration(s) is not clearly defined due to the lack of proper markers to track these cells in both healthy and diseased experimental subjects.

Different strategies to follow the fate of transplanted stem cells in preclinical models of human disorders have been reported (Wang and Moore, 2012). In particular, the FDA approved Superparamagnetic Iron Oxide nanoparticles (SPIOn) can provide an easily transferable and non-invasive system to follow stem cells using magnetic resonance imaging. An innovative approach to further increase the reliability of stem cells tracking in different murine models of ALS by specifically labeling the cytoplasm with SPIOn and the nucleus with Hoechst-33258 has been developed by our group (Canzi et al., 2012; Bigini et al., 2012). Although this strategy enabled us to determine the interaction of various types of fetal stem cells in several models of motor neuron degeneration at different times points, perplexities were raised about the biocompatibility and the lack of cell perturbation after SPIOn internalization (Calero et al., 2014; Li et al., 2013; Reddy et al., 2012). To overcome this problem, fluorescent, biocompatible and long lasting traceable poly (methyl methacrylate) nanoparticles (PMMA-NPs) have been recently developed for the tracking of human amniotic fluid cells by ex-vivo analyses. The reliability of our approach was furthermore evaluated by in-vivo studies where the co-incubation of SPIOn and PMMA-NPs confirmed the presence of the two tracers in transplanted cells for at least three weeks after administration (Cova et al., 2013). On the basis of these results in the present study we proposed to label human umbilical cord mesenchymal stromal cells (UC-MSCs) with PMMA-NPs, that segregate into the cytoplasm, and with the nuclear dye Hoechst-33258 before to inject them ICV or intravenously in both healthy and early symptomatic SOD1G93A mice in order to track them at different time points during the disease progression. UC-MSCs were selected because they represent an innovative pool of MSCs, with a simpler, safer and cheaper collection from donors compared to the bone marrow stem cells and the cord blood mononuclear cells. In addition, mesenchymal stromal cells have shown a strong immunomodulatory and cyto-protective activity in different preclinical models of acute inflammation (Griffin et al., 2013; Stagg and Galipeau, 2013; Uccelli et al., 2008). To optimize the tracking procedures cells were labeled with PMMA-NPs, that segregate into the cytoplasm, and with the nuclear dye Hoechst-33258. Two different fluorophores, Rhodamine B (RhB) and a deep infrared dye (DIR) respectively, were conjugated to NPs in order to combine in-vivo and ex-vivo analyses. The labeling enabled us to follow the fate of systemically administered UC-MSCs for a prolonged temporal window that was hypothesized sufficient for the cells to exert a therapeutic response in SOD1G93A mice. This is the first example in preclinical ALS studies in which the tracking of the same type of stem cells is reported by using two different ways of administration and where this longitudinal tracking has been carried out in living mice by whole body scanning with optical imaging system. The results obtained by this work can be therefore considered as a robust pre-requisite to plan future experiments in which the cell-host interaction could be easily correlated with the therapeutic efficacy, in a sort of theranostic approach, independently of the pathological model or the cell-type utilized.

2. Materials and methods

2.1. Nanoparticles synthesis and characterization

Poly (methyl methacrylate) nanoparticles (PMMA-NPs) were used to label stem cells thanks to their biocompatibility and to their low level of biodegradability (Cova et al., 2013). They were obtained from a co-polymerization between methyl methacrylate (MMA) and a macromonomer of 2-hydroxyethyl methacrylate (HEMA) covalently bound to RhB, through an emulsion free-radical polymerization process. DIR was not loaded during NP formation due to high temperature and to the presence radicals; therefore a post-synthesis process has been adopted (Sitia et al., 2014).

For our investigation the following nanoparticles have been synthesized:

- Positive 50 nm PMMA-NPs (number of NPs/ml $H_2O = 2.01E * 10^{14}$; polymer concentration = 50 mg/ml)
- Positive 200 nm PMMA-NPs (number of NPs/ml $H_2O = 4.62E * 10^{12}$; polymer concentration = 50 mg/ml)

Details on synthesis and NP characterization are reported in our previous study (Sitia et al., 2014).

2.2. UC collection and cell culture

Fresh human umbilical cords (UC) were collected from the Operating Room of the Obstetrics and Gynecology Unit at A.O. Papa Giovanni XXIII in Bergamo (Italy). Informed written consent was obtained from each donor mother according to the guidelines of ethical committee of the A.O. Papa Giovanni XXIII, as required by the clinical trial "Umbilical Cord Derived Mesenchymal Stromal Cells For The Treatment of Severe Steroid-resistant Graft Versus Host Disease" (clinicaltrials.gov ID NCT02032446) approved by "Istituto Superiore di Sanità" and "Agenzia Italiana del Farmaco". After cesarean sections, UC-MSCs have been isolated from whole UC by tissue mechanical disaggregation and cultivated as previously described (Capelli et al., 2011). Briefly, the UCs were cut into 5 cm long segments which were longitudinally cut and split open to expose the inner surface. Each UC segment, subsequently minced in very small fragments, was transferred into 150 mm cell culture Petri dishes (Corning) containing MSC expansion medium consisting of alpha-Minimum Essential Medium (MEM) (Life-Technologies) enriched with 5% human platelet lysate obtained from healthy donors, 50 μg/ml gentamicin (Fisiopharma) and 2 UI/ml Heparin (Hospira). They were maintained at 37 °C in a humidified atmosphere with 5% CO_2 for 6–7 days after which the UC tissue was removed and the adherent cells were allowed to expand for an additional week. After approximately 14 days, the adherent cells were harvested by TrypLe Select 1X (Life-Technologies) treatment and re-plated in T175 flasks (BD Falcon) in MSC expansion medium for further expansion.