Reduced intensity conditioning haematopoietic stem cell transplantation with mesenchymal stromal cells infusion for the treatment of metachromatic leukodystrophy: a case report

We report the case of a 23-year-old woman who presented with an adult form of metachromatic leukodystrophy (MLD) evolving over one year with a progressive neurological deterioration. A non-myeloablative matched related haematopoietic stem cell transplantation (HSCT) with concomitant mesenchymal stromal cells (MSCs) infusion was performed. Engraftment occurred rapidly with no significant toxicity or side effects following the MSC infusion. At a follow up of 40 months, the patient had a stabilisation of all neurological manifestations of her disease. This case report suggests the feasibility and the potential efficacy of reduced intensity conditioning (RIC) allogeneic HSCT combined with MSC infusion for patients with the adult form of MLD.

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Introduction

Metachromatic leukodystrophy is an autosomal recessive genetic disease characterized by the demyelination of the white matter in the central nervous system and the peripheral nerves. Patients typically present with a progressive deterioration of motor and neurocognitive function. The disease is due to a deficiency of the enzyme arylsulfatase-A (ASA) resulting in the accumulation of sulfatide compounds in neural and non-neural tissues.[1] Allogeneic SCT has been used as a treatment for MLD with an improvement in survival for transplanted patients compared to non-transplanted patients.[2-3] However, failure to obtain an improvement or stabilisation of the disease is still common in juvenile and adult forms of MLD.[3] Clinical benefits are usually obtained if the allogeneic SCT is performed in the pre-symptomatic stage.[3-4] Mesenchymal stromal cells are pluripotent cells capable of differentiation into various tissues, including bone, cartilage, muscle, adipose tissue and neuronal elements.[5-6] These cells can also reconstitute the bone marrow stroma, are the source of several haematopoietic growth factors and have immunoregulatory properties.[7-8] MSCs can be isolated from bone marrow and expanded *ex-vivo* for clinical use.[9] In the setting of allogeneic HSCT, MSCs have been shown to improve or accelerate haematopoietic recovery and decrease the risk of GVHD.[8] More importantly MSCs express high amounts of arylsulfatase-A, which could therefore migrate to and repair ASA deficient tissues.[10] A study by Koç *et al.* showed that infusion of MSCs in patients with MLD previously treated with allogeneic stem cell transplantation could improve nerve conduction velocities.[11] For these reasons, we decided to perform a non-myeloablative HSCT with MSC coinfusion in an adult patient with progressive MLD.

Case report

A 23-year-old woman was admitted to our institution for the treatment of a progressive MLD with a non-myeloablative HSCT and MSC infusion. The patient presented with an adult form of MLD diagnosed thirteen months before the transplantation when she complained of a seven month history of slowly progressive instability. She had previously been healthy except for a grade one oesophagitis treated with rabeprazole. Over the following year, her instability worsened with progressive bilateral leg weakness and difficulties in walking associated with increasing difficulty in concentration. At the time of transplantation, her major neurological symptoms were instability, proximal weakness of the lower limbs, intentional and postural tremor of the right arm and memory disturbance. Magnetic resonance imaging (MRI) revealed lesions of the white and grey matter of the brain. Neurophysiologic testing demonstrated a slowing of nerve conduction velocities in the upper limbs. The level of urinary arylsulfatase A was low at 0.19 nmol/min/mL (reference range = 0.42-1.67 nmol/min/mL).

Allogeneic HSCT was discussed with the transplant team and a non-myeloablative conditioning regimen was proposed in order to reduce the morbidity and mortality of the procedure. This procedure was approved by the hospital's Ethical Review Board committee with the patient signing a written informed consent. The donor for the haematopoietic stem cells (HSCs) and MSCs was her HLA-identical sibling brother. Twenty millilitres of bone marrow were collected from the donor for the *ex-vivo* isolation and expansion of MSCs. MSC expansion was performed in a commercial serum-free medium (UltraCulture, Cambrex, Walkersville, MD) supplemented with a serum substitute (Ultroser, Pall Biosepra, Cergy-Saint-Christophe, France) as previously reported by our group.[12] Peripheral blood stem cells were mobilized with granulocyte-colony stimulating factor (G-CSF). The conditioning regimen of HSCT consisted of fludarabine (30 mg/m^2, day -4 to -2) combined with anti-thymocyte globulin (Fresenius, Munich, Germany) (10 mg/kg, day -3 to -1). GVHD prophylaxis post-transplantation consisted of mycophenolate plus cyclosporin. The infusion of peripheral blood stem cells contained 5×10^6 CD34$^+$ cells/kg immediately followed by the infusion of 1×10^6 cells/kg of *ex-vivo* expanded allogeneic MSCs. MSCs were infused fresh, at passage 2. The criteria's for clinical use were: spindle shape morphology, absence of contamination by pathogens (bacteria, fungal and mycoplasma), viability and typical phenotype (positivity for CD73, CD90, CD105 and negativity for CD34, CD45, CD31). CFU-F assay was used to evaluate the number of mesenchymal progenitors in the sample and the multilineage capacity of MSCs was also tested as previous described.[12]

The patient had no significant complications in the

post-transplant period. No immediate or delayed side effects following MSC infusion were observed. At day 8 post transplantation, she had a complete haematologic recovery. The platelet nadir was 72.000/mm^3 with only 4 days of neutropaenia less than 500/μL. No platelet or red cell transfusions were required. She developed neither acute nor chronic GVHD nor any infectious complications. The CD3 chimerism was 83% at the day 30 days and remained stable (between 80-90%) during the follow-up. The CD33 chimerism was 425 at 30 days, increased to 73% at day 90 and remained at the same level on the future analysis. Six months after the transplantation, we performed a chimerism analysis on MSCs obtained from recipient bone marrow culture and we obtained a full recipient MSCs chimerism. The arylsulfatase-A level at 16 months post-transplantation revealed an increase of the enzyme level to the normal range at 0.63 nmol/min/mL(reference range = 0.42-1.67 nmol/min/mL). Cerebral MRI performed at one and three years after the transplantation showed stable cerebral lesions. With regard to her neurological symptoms, the patient has had no further deterioration with a stabilisation of all her previous clinical manifestations.

Discussion

MLD is an inherited progressive neurodegenerative disorder characterized by a deficit in the lysosomal enzyme, arylsulfatase-A, resulting in an inability to degrade sulphated glycolipids. The disease involves a progressive deterioration of motor and neurocognitive functions with a decreased life expectancy. HSCT has been used in some lysosomal and peroxisomal storage disorders and can prevent the progression of the disease by providing a source of normal enzyme from the donor cells.[2] However, failure to stabilize the quality of life and the progression of neurological symptoms after HSCT is frequent, even with 100% full donor chimerism. Furthermore, this treatment has been mainly effective in presymptomatic MLD patients.[3,4] One must consider that allogeneic HSCT is an intensive procedure with significant morbidity and mortality. Complications such as acute graft-versus-host disease or serious infections may worsen the patient's quality of life in addition to the debilitating effects due to MLD.

Recently, Koç *et al.* have demonstrated an improvement in electrophysiologic testing in patients with MLD who were treated by MSC infusion.[11] MSCs are pluripotent cells capable of differentiation into various cells types and for its ability to migrate to and restore deficient tissues.[5,6] In the allogeneic transplant setting, MSCs have been used for their immunosuppressive properties and for their support of haematopoietic function. Accordingly, we decided to perform an HLA-identical sibling HSCT combined with an infusion of expanded MSCs in our patient. At the time of transplantation, the patient's disease had been progressive over the preceding 12 months with a low expected probability of stabilisation or improvement of her symptoms. To reduce the toxicity of the transplant conditioning regime in this patient, we decided to perform a reduced intensity HSCT. It is known that patients who have not been exposed to chemotherapy prior to the transplant conditioning are at higher risk of graft failure, especially with RIC regimens as was utilised in this patient.[13] The addition of MSCs in our patient may have played a role in engraftment. This potential effect could be explained by the ability of MSCs to reconstitute the bone marrow stroma and to act as a source of several haematopoietic growth factors. The role of MSCs in improving engraftment with HSCT has been supported by clinical trials. This was first reported by Koç *et al.* who described a rapid haematopoietic recovery after co-infusion of expanded autologous MSCs and HSCs in patients treated by high doses chemotherapy for breast cancer.[9] Similarly, Lazarus *et al.* reported a prompt haematological recovery when HSCs were infused with MSCs in the allogeneic transplant setting.[14] More recently, Leblanc *et al*. have shown that co-infusion of MSCs and HSCs in patients with graft failure or poor engraftment can enhance haematopoietic engraftment.[15]

Our patient had a complete stabilisation of her neurological symptoms due to her MLD following transplantation with this being maintained at 40 months. Indeed we observed an increase in the level of arylsulfatase-A after the transplantation. Although this could be unrelated, there are several possible mechanisms in which the addition of MSCs may have contributed to the arrest of disease progression in our patient. Firstly, it has been demonstrated that MSCs possess high levels of lysosomal activity and their associated enzymes. When fibroblasts from MLD patients are co-cultured with MSCs, ASA released by the MSCs into the culture media is taken up by the affected fibroblasts.[10] The clinical effect of normalising the ASA level should first stabilise neurological symptoms and in the long term slowly improve peripheral nerve function by passive transfer of the enzyme into affected neural tissue. Secondly, under normal circumstances haematopoietic stem cells do not cross the blood brain barrier. In contrast, MSCs have been demonstrated in *in-vivo* models to be able to cross into the central nervous system following intravenous injection and thus provide active enzyme.[16,17] These cells are also capable of migrating into the brain and differentiating into astrocytes.[18,19] Thirdly, MSCs are also able to differentiate into neuronal cells and therefore have the potential to repair neuronal defects.[20,21] However, repair of nerve demyelination by Schwann cell differentiation would take several months to show any clinical improvement given the slow remyelination of peripheral nerves. In their trial Koç *et al.* observed that there was an improvement of nerve conduction velocity in the MLD patients treated with MSC infusion. This progressive increase was observed over a follow-up longer than one year, however, without obvious clinical implica-

tions. It is difficult to establish if this improvement was due either to an *in vivo* differentiation of the MSCs into neuronal cells or to a restoration of the deficient enzymes. Nonetheless, this is the sole existing study that has reported the benefit of MSC infusion in the treatment of MLD.[11] This may be due to the limited number of patients treated with allogeneic stem cell transplantation but particularly by the fact that MSC infusion is an experimental treatment not accessible in all transplant centres. Many questions still remain regarding the clinical application of MSC therapy including the optimal cell dose required, the appropriate timing of infusion with or without repeated infusions post-transplant, which patient may have the greatest benefit and importantly the long term side effects of this treatment. In view of the recent literature, it will be interesting to follow the clinical and therapeutic impact of this new approach in the coming years.

In conclusion, this case report supports the feasibility and the potential efficacy of RIC allogeneic HSCT with MSC infusion for patients with MLD. The addition of MSC at the time of transplantation may help to prevent graft failure, accelerate the haematopoietic recovery and restore arylsulfatase-A levels in order to arrest and possibly repair MLD-associated deficits. In the future, repeated infusions of MSCs after HSCT might further improve the outcome of patients with MLD by controlling the enzyme deficiency and decreasing the risk of GVHD and graft failure.

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