G-CSF IN ALS: FROM MICE TO HUMANS

Caterina Bendotti, Italy
Grant: € 70 000

There are currently no effective treatments for ALS, and the disease has a dramatic and rapidly fatal outcome**.** Several recent studies focused on the potential use of bone marrow-derived cells (BMC), and in particular on the most immature BM-derived stem cells, to repair nervous system damage such as that occurring in the motor neurons of patients with ALS. The interest in BMC has been bolstered by the observation that specific stimuli and appropriate environmental conditions result in BM stem cell transdifferentiation in vitro into a variety of adult cell types, including neural and glial cells. The use of BM-derived stem cells in vivo induces functional improvements in some experimental models of stroke and Parkinson's disease. In addition, the possibility of neural regeneration from BMC is supported by observations in animal models that BM-derived stem cells can migrate from the peripheral blood (PB) to the central nervous system (CNS) and can stimulate the growth of both microglia and neuroectoderm cells. Similar observations have been reported for human BM-derived stem cells. Since several years, the BMC mobilization procedure is used as a regular part of the transplant procedure in the management of several haematological disorders. Taken together, these observations support the interest to investigate the use of BMC for treating patients with ALS.**.** Due to its potent mobilization capacity, granulocyte-colony stimulating factor (G-CSF) is the cytokine most often employed for transplantation purposes. Some studies suggest that G-CSF has some protective effects in the SOD1 transgenic mice but is not known yet whether this effect may depend on BMC mobilization and migration to CNS. There are also initial data from non-controlled placebo studies that G-SCF could have some effects of slowing disease progression in ALS patients.

Therefore we propose to perform the relevant preclinical studies in order to validate the potential benefit of G CSF and to investigate its mechanism of action to eventually start a clinical trial.
According to this project, we will investigate the effect of G-CSF in the transgenic SOD1G93A mice, the most reliable and well-characterized model of familial ALS. We will compare the effect of two doses of GCSF: 30microg/kg which has been reported to modestly prolong the life span of SOD1G93A mice through a partial direct neuroprotective effect on motor neurons and the dose 300microg/kg which has been demonstrated to exhibit neuroprotection and functional improvements in different experimental models of neurodegeneration by inducing the homing of BM cells in the CNS and enhancing the neurogenesis.
The plan of experiments is the following: as first step three groups of SOD1G93A mice will receive subcutaneously 30microg/kg or 300microg/kg of GCSF or the placebo every day for five days/week for two weeks. These mice will be used: 1) to compare the efficacy of the two GCSF doses to increase the levels of CD34, a marker of myeloid stem cells, in the blood and in the CNS (spinal cord and brain) through a standardized flow cytometry protocol 2) to monitore the levels of GCSF in blood by ELISA and

finally in the CNS tissues. Few mice will receive in addition to GCSF, an intraperitoneal injection of EdU 50/mg/kg (InVitrogen) a marker of proliferating cells. This will allow to compare the effect of the two doses of the GCSF on the number and phenotype of proliferating cells in the CNS (either from BM labelled with CD34antibody or from local microglia proliferation with CD11b antibody). In a second step other three groups of SOD1G93A will receive subcutaneously 30microg/kg or 300microg/kg of GCSF or the placebo every day for five days/week starting at the symptom onset until the animals will reach the end stage of the disease. The disease progression and survival length will be assessed according to protocols standardized and routinely used in Bendotti's laboratory. Blood , spinal cord ,brain and nerves of the mice at the final stage will be collected for subsequent biochemical and histopathological analyses.
The project will start September 15th and we expect to get the results in 18 months.
If GCSF will exhibit positive effect on cell homing in CNS and on the disease progression then a panel of cytokines relevant to the pathogenesis of ALS including those reported to be changed in CSF and Serum of ALS patients after GCSF treatment as reported in a preliminary study by Chio and al. (Muscle and Nerve, in press) will be measured by ELISA and RT-PCR in blood and CNS tissues collected and stored at point 1 and 2. This part of study which will provide further elements to understand the mechanisms of GCSF, will probably require an extension of the grant .

Overall, this study will allow to assess the mechanism of action of a potential beneficial effect of G-CSF in the animal models providing the key informations for planning a future multicenter, double blind, placebo-controlled trial in ALS patients that will be coordinated by Prof. Adriano Chiò, Director of the Turin ALS Center.

TEAM

Dr. Caterina Bendotti is the head of the Laboratory of Molecular Neurobiology, Department of Neuroscience of the Mario Negri Institute of Pharmacological Research in Milan. Dr. Bendotti and her group is internationally recognized for their expertise in animal model of neurodegeneration, specifically for the behavioural and neuropathological characterization of mouse models of ALS. One of her major contribution is the development of guidelines for the use of preclinical animal models of ALS to test therapeutics.

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Granulocyte colony-stimulating factor (G-CSF) is a cytokine largely and safely used in clinic to mobilize large amount of the endogenous BM-derived stem cells (BMSC) into the blood. Since it has been demonstrated that BMSC can migrate from the blood to the damaged regions of the central nervous system, the use of GCSF has been proposed as a potential therapeutic for neurodegenerative diseases including ALS.

Previous studies in a mouse model of familial ALS have reported a beneficial effect of GCSF however, the treatment started long before the appearance of symptoms, a condition, which is far to be translated into the clinical practice. Therefore, the aim of this project was to investigate the therapeutic potential of the G-CSF in SOD1G93A transgenic mice, the most widely used mouse model of familial ALS, starting the treatment at the symptoms onset and to investigate the effect of GCSF on BMSC mobilization and their potential migration in the damaged regions of these mice. Treatment of ALS mice with GCSF clearly increased the number of micorglial cells in the spinal cord. However, in spite of this there is no significant benefit on survival after G-CSF treatment in SOD1G93A mice and that the highest G-CSF dose may be even detrimental in the context of a more severe disease.