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Author(s)	譚, 成博
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学位論文内容の要旨 (Summary of dissertation)

博士の専攻分野の名称 博士 (医 学)
(Degree conferred: Doctor of Philosophy)

氏 名 譚 成博
(Name of recipient: Chengbo Tan)

Studies on regeneration therapy for central nervous system using bone marrow stromal cells
(骨髓間質細胞移植による中枢神経再生医療の研究)

【Background and Objectives】

Human bone marrow stromal cells (hBMSCs) have been regarded as a potential cell source for ischemic stroke therapy, owing to their potential to differentiate into multiple cell lineages, their neuroprotective effects, and their ability to promote functional neural recovery of patients. Although these results are encouraging, several problems still remain unsolved to impede its clinical application. To solve the problems, we designed new clinical trials called the RAINBOW study, which is a phase 1 study about autologous BMSC transplantation for acute ischemic stroke. In the present study, we aimed to evaluate the feasibility and the efficacy of hBMSC culture with allogeneic PL (Chapter I), the safety of SPIO-labeled BMSCs and the curative effect of cell therapy after stereotactically transplantation (Chapter II) as the preparatory work for RAINBOW study. We aimed to translate these results to the optimal design of the clinical trials.

【Materials and Methods】

Chapter I: Feasibility and efficiency of human bone marrow stromal cell culture with allogeneic platelet lysate-supplementation for cell therapy against stroke

Platelet-concentrates (PC) was harvested from nine healthy volunteers and made into single donor-derived PL (sPL). Three types of PL mixtures (mPL) were made from three different sPLs. Platelet-derived growth factor (PDGF)-BB, transforming growth factor (TGF)- β 1, brain-derived neurotrophic factor (BDNF), and platelet cell surface antigens (CD41 and CD61) were detected by enzyme-linked immunosorbent assay (ELISA). Cell proliferative potential of 10% PL was compared with fetal calf serum (FCS). Cell surface markers of hBMSC cultured with PL (hBMSC-PL) were analyzed. The hBMSC-PLs were incubated with superparamagnetic iron oxide (SPIO) agents and injected into a pig brain. MRI and histological analysis were performed.

Chapter II: Short-, middle- and long-term safety of superparamagnetic iron oxide-labeled allogeneic bone marrow stromal cell transplantation in rat lacunar infarction model

BMSCs were isolated from transgenic rats expressing green fluorescent protein (GFP), and they were labeled with SPIO. A Na/K ATPase pump inhibitor ouabain or vehicle was stereotactically injected into the right striatum of wild type rats to induce lacunar lesion (n=22). 7 days after the insult, BMSCs or SPIO solution were stereotactically transplanted into the left striatum. A 7.0-Tesla MRI was performed to serially monitor the behavior of BMSCs in the host brain. The animals were sacrificed 7 days (n=7), 6 weeks (n=6) or 10 months (n=9) after transplantation, respectively.

【Results】

Chapter I:

MRI and histological analysis were performed. The result showed that twelve types of PLs (sPL: n = 9, mPL: n = 3) were prepared. ELISA analysis showed that PL contained adequate growth factors and a very small number of platelet surface antigens. Two weeks after culture, the proliferation capacity of hBMSC-PLs was equivalent to or higher than that of FCS. No contradiction in cell surface markers were found in hBMSC-PL. When injected into the pig brain, MRI detected the distribution of SPIO-labeled hBMSCs similar to histological analysis.

Chapter II:

MRI demonstrated that BMSCs migrated to the damage area through the corpus callosum. Histological analysis showed that activated microglia were found around the bolus of donor cells 7 days after allogeneic cell transplantation, though immunosuppressive drug was used. The SPIO-labeled BMSCs resided and started to proliferate around the route of cell transplantation. Within 6 weeks, a large number of SPIO-labeled BMSCs had reached the lacunar infarction area from transplantation region through the corpus callosum. Some SPIO nanoparticles were phagocytized by microglia. In 10 months later, the SPIO positive cells were less than the numbers in 7-day and 6-week groups. There was no tumorigenicity and severe injury in all animals.

【Discussion】

In the present study, we found that the content of these growth factors in each PL was relevant to the number of platelets in original PC. Furthermore, the ability of cell expansion correlated with the contents of the growth factors in each PL. When the contents of platelets in original PC reached more than 15 units, the cell proliferating potential of PL was equivalent or much higher compared with the FCS. This indicated that the PL-supplement contains adequate essential growth factors and nutrition as well as FCS for the expansion of hBMSC. Moreover, because there was a smaller difference among mPL compared with sPL regarding the contents of growth factors, pooled PL should be useful in mass production. The present results were used for a quality control of PL produced under good manufacturing practice (GMP).

In addition, cell labeling with SPIO was employed to track donor cells in the host brain by means of MRI. Cultured hBMSCs can uptake SPIO nanoparticles into their cytoplasm when the particles are added to the culture medium. Because they have clearly detectable signal extinctions, the labelled cells were easily tracked anatomically with QSM and T2 MR images. We believe that MRI cell tracking with SPIO based labeling agents is a good resource to monitor cell distribution after hBMSC transplantation. In order to evaluate the safety of SPIO-labeled BMSCs histologically, we used immunohistochemistry with Turnbull blue staining technique. In 10-month group, SPIO-solution or SPIO-labeled BMSCs caused no severe inflammation in host brain, regardless of lacunar infarction or not. We hope this technology can be used for cell therapy in clinical applications.

【Conclusion】

hBMSCs cultured with allogeneic PL and labeled with SPIO may be a valuable, feasible, safe, and effectible for cell therapy against cerebral ischemic stroke. We will translate these results to the protocol of RAINBOW study, and we hope that it will be helpful for the patients who suffer from stroke.