Introduction: We examined endothelial progenitor cells (EPCs) as transplant cells, which can perform both neurogenesis and angiogenesis in the damaged spinal cord. EPCs were reported to exist as CD34+ cells in peripheral blood(1). CD133+ cells are a subset of CD34+ cells, are more immature than CD34+ cells(2), and have a very high potential for proliferation and differentiation(3). These characteristics suggest that CD133+ cells may prove beneficial as transplant cells for Spinal cord injury (SCI) treatment. The purpose of this study was to investigate the therapeutic effects of CD133+ cells on spinal cord contusion injury and clarify the mechanism of regeneration.

Materials and Methods: Adult male athymic nude rats were anesthetized with pentobarbital sodium, and a laminectomy was performed at the T7 level of the spinal cord. A 25g rod was placed on the spinal cord for 90 seconds to induce a contusion lesion followed previous report. We injected 100μl of PBS alone in the control group and 1x105 CD133+ cells/100μl of PBS in the experimental group by a single intravenous injection immediately after the injury. Hind-limb motor function was scored with the BBB scale. Rats in both groups were harvested at 3weeks after transplantation and the cavity was measured using Scion Image computer analysis software (Scion Corporation, Frederick, Maryland, USA). For morphometric evaluation of capillary density at injured spinal cord, histochemical staining with isoelectric B4 antibody was performed. Capillary density was evaluated using fresh axial section. For immunofluorescent staining, ideal frozen sections were used for immunohistochemical staining with following antibodies: HNA and hMit for detection of intravenously administrated CD133+ cells, vWF for detection of endothelial cells, NF for detection of survived axons, Gap43 for detection of regenerated axons, and CXCR4 for detection of neural progenitor cells. The mRNA expression analysis of SDF-1, CXCR4, and VEGF in both groups was performed by real-time PCR. Our research methods were reviewed and approved by the ethical committee of Hiroshima University.

Results: After 1 week, the BBB score of the experimental group demonstrated significant improvement compared to that of the control group at every week up to the sixth week (*p<0.05, **p<0.01) (IMAGE.1.A). Macroscopic findings demonstrated that injured area of experimental group (IMAGE.1.B) was thicker in diameter than that of control group (IMAGE.1.C). The area of the cavity in the injured spinal cord (IMAGE.1.D) was significantly smaller in the experimental group than that in the control group (*p<0.05).

At 1 day after injury, double positive cells that were stained with HNA (red) and isoelectin B4 (green) were observed on the surface of the injured spinal cord in the experimental group (IMAGE.2.A). At 3 days after injury, double staining for NF (green) and Gap43 (red) showed that the expression of Gap43 around the injured axons was much greater in the experimental group (IMAGE.2.B) than that in the control group (IMAGE.2.C). The number of CXCR4+ cells (red) in the experimental group (IMAGE.2.D) was significantly greater than that in the control group at 3 days after injury (*p<0.05) (IMAGE.2.E). At 1 week after injury, the number of vessels stained with isoelectin B4 was significantly greater in the experimental group. (IMAGE.3.A) than that in the control group (IMAGE.3.B) (*p<0.01). At 3 days after injury, mRNA expression of SDF-1 and CXCR4 in injured spinal cord was significantly greater in the experimental group than that in the control group (*p<0.05) (IMAGE.3.D). At 1 week after injury, the expression of VEGF in the experimental group was significantly greater than that in the control group (*p<0.05) (IMAGE.3.E).

Discussion: We demonstrated that the administration of human peripheral blood-derived CD133+ cells accelerated functional recovery and reduced the cavity area of an injured spinal cord in a rat SCI model. SCI is aggravated not only by the primary damage through trauma, but also by local ischemia, which may contribute to secondary degeneration(4,5). In this study, after cell transplantation, the expression of VEGF increased and significant intrinsic angiogenesis was observed. CD133+ cells transplantation might improve the microenvironment in the injured spinal cord through angiogenesis mediated by up-regulation of VEGF. The expression of SDF-1-CXCR4 significantly increased at the injury site in the experimental group, suggesting that the administration of CD133+ cells might activate SDF-1-CXCR4 axis. These recruited CXCR4+ cells could play an important role for tissue regeneration. CD133+ cells are easily isolated from peripheral blood, do not require incubation, and can be isolated for autologous transplantation. Together, these characteristics make CD133+ cells attractive for future clinical applications in the treatment of SCI.


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