Superparamagnetic Iron Oxide and Protamine Sulfate Do Not Affect the Viability and Multi-Transdifferentiation Capacity of Adult Stem Cells

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Background: Adult stem cells have been studied extensively for their therapeutic efficacy for various diseases. Non-invasive monitor of the implanted stem cells is very important for ensuring the success in stem-cell therapies and to elucidate the potential mechanisms for their therapeutic benefits. Because of its strong T_2* effect, superparamagnetic iron oxide (SPIO) has often been used to label the stem cells in order to visualize them *in vivo* using MR imaging. Protamine sulfate (PS) is often used to facilitate labeling stem cells with SPIO. However, no studies have fully examined the effects of SPIO and PS on the viability and transdifferentiation of adult stem cells. The present study was therefore designed to assess the effects of SPIO and PS on the viability and transdifferentiation of adult stem cells.

Materials and Methods: Adult stem cells used in this study were collected from the subcutaneous adipose tissue of Lewis rats. They were referred to as adipose-derived stem cells (ADSCs). To assess the effect of SPIO and PS on stem cell viability, the ADSCs were divided into four groups. Group-I ADSCs were cultured in a cell-culture medium (CCM); group-II ADSCs were in CCM containing 6g/ml PS; group III was in CCM containing 6μg/ml PS and 50μg/ml SPIO; and group IV in 6μg/ml PS and 100 μg/ml SPIO. During 8-day culture, survival percentages of ADSCs (living ADSCs/total ADSCs x 100%) were measured four times using trypan blue staining. To assess the effect of SPIO and PS on transdifferentiation of ADSCs, another four groups of the ADSCs were first cultured for 2 days in the above four media, respectively. The ADSCs in each group were divided into 3 portions that were then transferred into 3 induction media for adipogenic, osteogenic, and myogenic inductions, respectively. Adipogenic transdifferentiation was assessed by measurement of lipid deposits and mRNAs for lipoprotein lipase (LPL) and peroxisome proliferator-activated receptor (PPAR) using oil-red-O staining and RT-PCR, respectively. Osteogenic transdifferentiation was evaluated by measurement of alkaline phosphase (ALP) and mRNAs for secreted-phosphoprotein (SPP) and γ-carboxyglutamic acid protein (GLAP). Myogenic transdifferentiation was assessed by measurement of mRNAs of α-smooth muscle actin (α-SMA) and myogenin.

Results: Survival percentages of the ADSCs were comparable among the four groups of the ADSCs. By the end of 8-day culture, survival percentages of the ADSCs in all four groups were very high (>98%, Figure 1), indicating that SPIO and PS did not affect viability and proliferation of the adult stem cells. As to transdifferentiation, we found that levels of lipid deposits were not significantly different among the four groups of the adipogenic-induced ADSCs. Moreover, the levels of the mRNAs for LPL and PPAR were very comparable among the four groups. Furthermore, the four groups of the osteogenic-induced ADSCs showed very comparable levels of ALP and mRNAs for SPP and GLAP. Finally, expression of the mRNAs of α-SMA and myogenin were not statistically different among the four groups of the myogenic-induced ADSCs.

Discussion and Conclusion: Our study demonstrated that SPIO and PS did not affect the viability and proliferation of the adult stem cells. More importantly, our study demonstrated that SPIO and PS did not affect multipotent transdifferentiation property of the adult stem cells. We therefore conclude that the SPIO is a safe MR contrast agent for tracking the implanted adult stem cells and PS is a safe SPIO-loading agent.

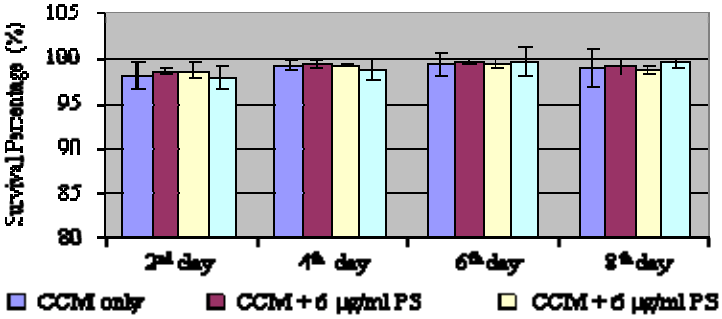

Figure 1. Comparison of percentages of living ADSCs over total ADSCs cultured in different media. The survival percentages were measured at 2nd, 4th, 6th, and 8th day of cell culture. The figure showed that the ADSCs cultured in a different medium had a very high and similar survival percentage, indicating that iron oxide does not affect the viability of the stem cells. PS, protamine sulfate (used to enhance penetration of iron oxide into the ADSCs); CCM, cell-culture medium; SPIO, superparamagnetic iron oxide.