Abstract

Objectives: Hematopoietic stem cell transplantation (HSCT) is a widely used method for treatment of hematological disorders and some other diseases. However, sometimes a suitable donor of hematopoietic stem cells (HSC) is not found for a patient. Because HSC have been described as cells with low proliferative and metabolic activity, their tolerance to the lack of oxygen or metabolic substrates may be assumed. In this study, we explored cadaveric bone marrow as an alternative source of HSC for HSCT, using a mouse experimental model. In addition, the effect of *in vitro* metabolic inhibition and short-term *in vitro* storage (1 - 4 days) on functional properties of mouse HSC was investigated.

Methods: C57Bl/6 mice (wild-type or $p53^{-/-}$) were used in the experiments. To explore cadaveric HSC, bone marrow (BM) was left in intact femurs at 37°C, 20°C and 4°C under the conditions of ischemia. The bone marrow cells were harvested after defined time periods ranging 0 – 48 hours. For metabolic inhibition, the electron transport chain inhibitor potassium cyanide (KCN) and inhibitor of glycolysis 2-deoxy-D-glucose (2-DG) were used *in vitro*. To determine the impact of ischemia, metabolic inhibition, or *in vitro* storage on transplantability of HSC, the competitive repopulation assay using Ly5.1/Ly5.2 congenic model was used. Besides, live/apoptotic/dead cells ratio in BM subpopulations was measured, and frequencies of LSK SLAM ($Lin^{low}Sca\text{-}1^{+}c\text{-}Kit^{+}CD150^{+}CD48^{-}$) and LSK SP (side population) cells (highly enriched in HSC) were detected by flow cytometry.

Results: Chimerism arisen from the transplanted cadaveric donor bone marrow cells (followed up in recipient's peripheral blood (PB) for 6 months after transplantation) revealed that the long-term repopulating ability of HSC is fully preserved for at least 2 hours, 6 hours and 12 hours of ischemia at 37°C, 20°C and 4°C, respectively. Number of LSK SLAM and LSK SP cells decreased in compliance with the transplantability. Furthermore, the LSK subpopulation (enriched in HSC) contained less apoptotic and dead cells as compared to more differentiated subpopulations of the BM exposed to ischemia at 37°C. Cadaveric $p53^{-/-}$ HSC did not differ from wild-type HSC in their survival or transplantability. The incubations with inhibitors showed the LSK cells as more resistant to KCN in comparison with other populations tested; however the 2-DG inhibition was lethal for all bone marrow cells. Two-day or four-day *in vitro* storage of bone marrow cells at 37°C or 4°C, respectively, did not influence the transplantability of HSC.

Conclusions: Our findings suggest that HSC survive in cadaveric bone marrow for considerable time, without loss of their repopulating ability. They represent the most resistant population of BM to oxygen and metabolic starvation. The HSC survival is significantly extended during *in vitro* storage even without growth factors, thus bone marrow cells should be harvested as soon as possible.