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Titel des Beitrags:
Analysis and cryopreservation of hematopoietic stem and progenitor cells from umbilical cord blood.

Abstract:
BACKGROUND: Umbilical cord blood (UCB) is an important source of hematopoietic stem and progenitor cells (HSC/HPC) for the reconstitution of the hematopoietic system after clinical transplantation. Cryopreservation of these cells is critical for UCB banking and transplantation as well as for research applications by providing readily available specimens. The objective of this study was to optimize cryopreservation conditions for CD34+ HSC/HPC from UCB. METHODS: Cryopreservation of CD34+ HSC/HPC from UCB after mononuclear cell (MNC) preparation was tested in a research-scale setup. Experimental variations were concentration of the cryoprotectant, the protein additive and cell concentration. In addition, protocols involving slow, serial addition and removal of DMSO were compared with standard protocols (fast addition and removal of DMSO) in order to avoid osmotic stress for the cryopreserved cells. Viability and recoveries of MNC, CD34+ cells and total colony-forming units (CFU) were calculated as read-outs. In addition, sterility testing of the collected UCB units before further processing was performed. RESULTS: The optimal conditions for cryopreservation of CD34+ HPC in MNC preparations were 10% DMSO and 2% human albumin at high cell concentrations (5 x 10^7 MNC/mL) with fast addition and removal of DMSO. After cryopreservation using a
computer-controlled freezer, high viabilities (89%) and recoveries for CD34+ cells (89%) as well as for CFU (88%) were observed. Microbial contamination of the collected UCB samples was reduced to a rate of 6.4%. DISCUSSION: Optimized cryopreservation conditions were developed for UCB MNC in respect of the composition of the cryosolution. In addition, our results showed that fast addition of DMSO is essential for improved cryopreservation and post-thaw quality assessment results, whereas the speed of DMSO removal after thawing has little influence on the recoveries of CD34+ cells and CFU.

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