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RESEARCH ARTICLE

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A comparison of cryopreservation methods: Slow-cooling vs. rapid-cooling based on cell viability, oxidative stress, apoptosis, and CD34⁺ enumeration of human umbilical cord blood mononucleated cells

Tono Djuwantono^{1,2*}, Firman F Wirakusumah¹, Tri H Achmad², Ferry Sandra³, Danny Halim² and Ahmad Faried^{2,3}

Abstract

Background: The finding of human umbilical cord blood as one of the most likely sources of hematopoietic stem cells offers a less invasive alternative for the need of hematopoietic stem cell transplantation. Due to the once-in-a-life time chance of collecting it, an optimum cryopreservation method that can preserve the life and function of the cells contained is critically needed.

Methods: Until now, slow-cooling has been the routine method of cryopreservation; however, rapid-cooling offers a simple, efficient, and harmless method for preserving the life and function of the desired cells. Therefore, this study was conducted to compare the effectiveness of slow- and rapid-cooling to preserve umbilical cord blood of mononucleated cells suspected of containing hematopoietic stem cells. The parameters used in this study were differences in cell viability, malondialdehyde content, and apoptosis level. The identification of hematopoietic stem cells themselves was carried out by enumerating CD34⁺ in a flow cytometer.

Results: Our results showed that mononucleated cell viability after rapid-cooling (91.9%) was significantly higher than that after slow-cooling (75.5%), with a p value = 0.003. Interestingly, the malondialdehyde level in the mononucleated cell population after rapid-cooling (56.45 μ M) was also significantly higher than that after slow-cooling (33.25 μ M), with a p value < 0.001. The apoptosis level in rapid-cooling population (5.18%) was not significantly different from that of the mononucleated cell population that underwent slow-cooling (3.81%), with a p value = 0.138. However, CD34⁺ enumeration was much higher in the population that underwent slow-cooling (23.32 cell/ μ l) than in the one that underwent rapid-cooling (2.47 cell/ μ l), with a p value = 0.001.

Conclusions: Rapid-cooling is a potential cryopreservation method to be used to preserve the umbilical cord blood of mononucleated cells, although further optimization of the number of CD34⁺ cells after rapid-cooling is critically needed.

Keywords: Human umbilical cord blood, hematopoietic stem cell, cryopreservation, slow-cooling, rapid-cooling, cell viability, malondialdehyde, apoptosis, CD34⁺

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