

5PS-33-05

IN VITRO ERYTHROPOIESIS INDUCED FROM CD34+ STEM CELLS IS INFLUENCED BY THE OXYGEN CONTENT

I. Dorn, J. Ribbat, P. Lazar, S. Boie, H. Kirchner, P. Schlenke

UK S-H, Campus Luebeck, Luebeck, Germany

Background: Up to now, the regulation of human erythropoiesis is only partly understood. There is some evidence that erythroid progenitors in the bone marrow mature along an oxygen gradient under hypoxic conditions, with stem cells residing in the most hypoxic areas and proliferating progenitors in more O₂ rich areas.

Aims: To investigate, which growth factors and environmental conditions are involved in red blood cell development, we established an in vitro model of human erythropoiesis. Besides different growth factors, the influence of the oxygen content on human red blood development was investigated.

Methods: After leukapheresis, human CD34+ cells were isolated using an immunomagnetic separation technique. Purified cells were cultured over 16 days in a two-phase liquid assay (day 1-8: SCF, EPO and insulin-like growth factor 1 (IGF-1); day 9-16: EPO and insulin). Cells were incubated under normoxic (20% O₂), mildly hypoxic (5% O₂) and strongly hypoxic (1% O₂) conditions. Cell growth and vitality were determined by trypan blue staining and microscopic examination. Cell differentiation was evaluated by flow cytometry using antibodies against CD34, 36, 71 and glycophorin A (GPA). Cytospin preparations were made and stained by Pappenheim and neutral benzidine.

Results: Under normoxic conditions absolute cell numbers increased up to 50fold (median 39fold). On day 16 cultures consisted of >95% GPA+ cells with 48% normoblasts and 44% enucleated reticulocytes. Only few non erythroid cells could be observed (2%). Compared to normoxia, proliferation was 3.2fold higher under 5% O₂ (p<0.05) and 3.8fold reduced under 1% O₂. Vitality was always over 80%. Regarding cell differentiation, maturation under 5% oxygen was slightly delayed with more immature basophilic erythroblasts on d9 and more polychromatic erythroblasts instead of normoblasts on d13 (p<0.05). On d16 no more differences between 5% and 20% oxygen could be observed.

Conclusions: The established assay was able to show different stages of human erythropoiesis, including hemoglobin synthesis and terminal enucleation. Compared to normoxia, proliferation was significantly higher under 5% O₂ and reduced under 1% O₂. Furthermore, under 5% O₂, differentiation was significantly delayed, although the outcome of fully matured reticulocytes was not reduced. These results are in line with our hypothesis that the microenvironment of the bone marrow influences proliferation and differentiation of erythroid progenitors. Our next experiments will investigate, if differences in cell cyclus or apoptotic mechanisms are the underlying mechanisms.