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In vitro Study to Identify Effects of Foetal Haemoglobin Inducing Agents on Primary Human Erythroid Cells of Beta-thalassaemia Major Patients

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Induction of foetal haemoglobin (HbF) ameliorates the severity of beta-thalassaemia by addressing the fundamental cause of the disease. Patients with high levels of HbF beyond infancy have protection from disease complications. The objective of the current study is to identify the effect of HbF inducing agents in vitro in human erythroid cells of beta-thalassaemia major patients. Haemopoietic stem cells (HSCs) were isolated from the peripheral blood of three beta-thalassaemia major patients. Mononuclear cells were separated after fractionation on Histopaque®-1077 Hybri-Max, followed by the isolation of CD34+ HSCs using positive selection by magnetic activated cell sorting. Using a three-phase liquid culture protocol, HSCs were then expanded and differentiated into mature erythroid cells. Primary human erythroid cells at day 7 of the culture were incubated with hydroxyurea(20μM), busulfan(30μM), vorinostat(0.5μM) and valproic acid(1000μM) for 72 hours. Effects of these compounds on cell expansion, viability and morphology were measured using standard laboratory methods. Negative controls were tested in parallel. Ethical approval was obtained from the ethics committee of the faculty of medicine, university of Kelaniya. Busulfan, vorinostat and valproic acid significantly decreased the erythroid cell proliferation compared to controls. Fold expansion and viability of hydroxyurea treated erythroid cells were similar to control cells. Morphologically, vorinostat treated cells were unhealthy. Cells treated with hydroxyurea, busulfan and valproic acid were in at basophilic erythroblast stage compared to the controls indicating that these compounds do not affect cellular differentiation. In conclusion, hydroxyurea did not alter the cell expansion, viability or differentiation of erythroid cells of beta-thalassaemia major patients in vitro, favouring its role as a pharmacological agent to induce HbF.

Keywords: *beta-thalassemia, primary human erythroid cells, foetal haemoglobin induction*