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In vitro study to identify effects of fetal haemoglobin inducing agents on primary human erythroid cells

N. Yasara[1], A. Manamperi[1] and S. Mettananda[1,2] *

[1] *Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka*
[2] *University Paediatrics Unit, Colombo North Teaching Hospital, Ragama, Sri Lanka*

Beta-thalassemia is an autosomal, recessively inherited monogenic disorder characterized by defective beta-globin synthesis. Deposition of excess alpha-globin in erythrocytes and their precursors due to unbalanced globin synthesis is the main underlying pathophysiology. Clinical data show that induction of fetal haemoglobin (HbF) in erythroid cells ameliorates the disease severity and associated complications. Hence, improving HbF via up-regulating gamma-globin gene expression has been identified as a novel treatment. The aim of our *in vitro* study was to identify the effects of fetal haemoglobin inducing agents in human erythroid cells. Haematopoietic stem cells (HSC) from umbilical cord blood samples collected from three healthy human placentas were isolated. Firstly, mononuclear cells were separated from the interface after fractionation on Histopaque®-1077 Hybri-Max and CD34+ HSCs were isolated using positive selection by magnetic activated cell sorting. HSCs were then expanded and differentiated into mature erythroid cells using a three-phase liquid culture protocol. Primary human erythroid cells at day 7 of the culture were incubated with hydroxyurea (20 μM), butyric acid (10 μM), 5-azacystidine (10 μM), decitabine (5 μM), busulfan (30 μM), vorinostat (2.5 μM) and valproic acid (1000 μM) for 72 hours. Effects of these compounds on cell expansion, viability, morphology, as well as α-, β- and γ-globin mRNA levels were measured using standard laboratory methods. Negative controls were tested in parallel. Compared to other compounds, hydroxyurea and butyrate treated erythroid cells displayed a significantly high mean fold expansion and viability. Significantly higher gamma-globin mRNA levels were observed in hydroxyurea treated cells (Mean relative expression: 186 ± SEM16) compared to negative control cells (Mean relative expression: 137 ± SEM14). Highest γ/β globin mRNA ratios were observed in busulfan (12.6 ± SEM2.9) and decitabine (12.1 ± SEM3.3) treated erythroid cells. In conclusion, hydroxyurea induces gamma-globin expression and decitabine and busulfan favourably alter the γ/β-globin mRNA ratios *in vitro* in human erythroid cells.

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E-mail: sachith.mettananda@kln.ac.lk