179

LIPID A BINDING AND DEFORMABILITY OF RED CELL MEMBRANE

J. Pöschl, O. Linderkamp
Dept. Pediatrics, Univ. Heidelberg, Germany

Endotoxin may disrupt the microcirculation and cause hemolysis. Lipid A is considered to be primarily responsible for the toxicity of endotoxin. The present study was designed to evaluate the effect of lipid A on adult red blood cell (RBC) deformability. A rheoscope was used to study whole cellular deformability and a micropipette system was used for analysis of RBC membrane elasticity (shear elastic modulus) and RBC geometry (volume and surface area). RBC were suspended in buffer solution containing 1, 10 or 100 µg of lipid A per ml of RBC. Lipid A markedly diminished cellular and membrane deformability of RBC. After 15 min of incubation, 10 µg lipid A/ml RBC decreased cellular deformability by 25% and membrane elasticity by 45%. 100 µg lipid A/ml RBC caused a reduction in cellular deformability of 39% and in membrane elasticity of 65%. 1 µg lipid A/ml RBC did not affect RBC deformation. Volume and surface area of RBC were not altered by lipid A. The effect of lipid A on RBC deformation was time-dependent. The lowest RBC deformability was observed after 20 min of incubation. After 30 min RBC deformability improved and reached pre-incubation values after 60 min. We conclude that lipid A strongly diminishes RBC deformability. We speculate that RBC are able to detoxify lipid A.

180

HEMODYNAMIC CONSEQUENCES OF NEONATAL POLYCYTHEMIA AND HEMODILUTION

V.HA. Mandelbaum, C. Guarjardo, O. Linderkamp
Childrens' Hospital, University of Heidelberg, FRG

Partial exchange transfusion (hemodilution) for neonatal polycythemia is performed to improve overall systemic oxygen transport and blood flow to organs.

Material and Methods: Cardiac output, blood flow velocities (BFV) of the internal carotid artery (ICA) and the celiac artery (CA) were measured by pulsed Doppler ultrasound before and after hemodilution in nine neonates (2.3 - 4.4 kg birth weigth, 33 - 41 wk gestational age). Blood pressure (oscillometry) and hematocrit (microcentrifuge) were measured.

Results:

Hematocrit [Hct]	66.8 ±2.8	54.8 ±2.4	p <
Cardiac index [Q] (ml/min/kg)	250.3 ±47	308.4 ±75	.005
BFV ICA (m/s)	.34 ±.07	.41 ±.11	.01
BFV CA (m/s)	.58 ±.10	.69 ±.09	.01
Blood pressure [P] (mm Hg)	57.6 ±10	56.8 ±9	n.s.
Resistance [P/Q]	25.8 ±10.4	18.9 ±5.9	.05

There was a significant correlation between Q and Hct ($p < .002$). Red cell flow in the aorta, in the ICA and in the CA did not change. Correlations between Q and BFV in the ICA were closer than those between Q and BFV in the CA, particularly before hemodilution.

181

CIRCULATING HEMATOPOIETIC PROGENITOR CELLS IN PREMATURE INFANTS: THEIR IN-VITRO RESPONSE TO ERYTHROPOIETIN AND INTERLEUKIN 3

H.-G. Hoffmann, V. Bier, K.M. Debatin, O. Linderkamp.
Dept. Pediatrics, Univ. Heidelberg, Germany

Burst forming units (BFU-E) in peripheral blood of five premature infants (gestational age 28-32 wk) were studied over a period of 8 weeks. Mononuclear cells were isolated by Ficoll-Hypaque gradient sedimentation on day 3, 14, 28 and 56 of life. $5x10^4$ cells were cultured in 1 ml semisolid medium of 0.9% methylcellulose containing 30% fetal calf serum, $5x10^{-5}$ M ß-mercaptoethanol, 500 U granulocyte macrophage colony stimulating factor (GMCSF) and erythropoietin (EPO) in doses of 0.25-20 U (day 3) and of 1 U (day 14-56). All infants showed in vitro sensitivity to increasing doses of EPO on day 3. Maximum stimulatory response was achieved with 1 U EPO and resulted in 38±13 BFU-E/$5x10^4$ cells. This response was almost ten times higher than in normal children. Peripheral BFU-E remained high until day 28. On day 56 BFU-E fell to 20% of the day 3 values (7±2 BFU-E/$5x10^4$ cells). Additional in-vitro stimulation with interleukin 3 (IL3) resulted in a 40% increase in peripheral BFU-E on day 3 to 25. No response to IL3 was observed in preterm infants on day 56 and in children. This indicates that early BFU-E circulate until day 25. We conclude that peripheral blood in preterm infants contains high levels of EPO- and IL3-sensitive BFU-E. The sharp decline in circulatory erythropoietic progenitor cells correlates with the onset of anemia of prematurity.

182

INCREASED PLASTIC DEFORMATION AND FRAGILITY OF RED CELL MEMBRANES IN TERM AND PRETERM NEONATES: A POSSIBLE CAUSE OF ACCELERATED RED CELL AGING

T. Böhler, A. Leo, P. Ruef, O. Linderkamp
Dept. Pediatrics, Univ. Heidelberg, Germany.

Elastic and plastic deformation (i.e. flexibility and fragility) of single red blood cell (RBC) membranes were studied microscopically in 5 term and 5 preterm neonates and 10 healthy male adults. Three methods were used: (1) A micropipette system was used to determine the membrane elastic shear modulus (µ) and the tension (T) required for local fragmentation of RBC membrane tongues aspirated into pipettes with internal diameters of 1 µm. (2) The micropipette system was also used to study the rate of plastic growth of RBC membrane tethers at given shear stress and the relaxation behavior of the tethers. (3) A flow channel system was applied to estimate µ and the tether growth rate at given shear stress. Salient results were: (1) The resistance to elastic membrane deformation (i.e. µ) was approximately 10% less in term and preterm neonates than in adults. (2) T was $3.8\pm0.8x10^{-3}$ dyn/cm in preterm infants, $6.2\pm0.7x10^{-3}$ dyn/cm in term neonates and $8.1\pm1.0x10^{-3}$ dyn/cm in adults. (3) At a shear stress of 2.5 dyn/cm^2 membrane tether growth was 0 in adults, 0.27±0.9 µm/s in term neonates and 0.78±0.15 µm/s in preterm infants. (4) Tethers were partially reversible with recovery times of about 0.5 s in all three groups. The decreased resistance to plastic deformation and fragmentation may contribute to accelerated aging and shorter life span of neonatal RBC.

183

Deficiency of glucocorticoids receptors (GR) in Hyaline Membrane Disease (HMD): preliminary results.

Storme L*, Formstecher P**, Dautrevaux M**, Pierrat V*, Lequien P*.
Laboratoire de Biologie Cellulaire** et Service de Médecine néonatale*, Centre Hospitalier Universitaire, Lille, France

The role of glucocorticoids (GC) on the maturation of fetal lung is well known. HMD could be due to a quantitative and/cor qualitative anomaly of GR with a resistance to GC. In order to study this hypothesis GR were measured by the method of Scatchard in the cord blood lymphocytes of 8 premature babies. All were born before 35 weeks of gestation. 4 developed HMD (Group I); 4 didn't develope any respiratory disease (Group II). The number of GR/cell was 2700 ±1000 in group I and 9800 ± 1200 in group II ($p<0.001$). The affinity of GC for GR and the level of cortisol were not significantly different in the 2 groups. We suggest that the maturation of GR could be delayed in HMD.
In the second part of the work we demonstrated in vitro that Phorbol mirystate acetate increased significantly the number of GR. This could be a way to a new therapeutic approach in the prevention of HMD.

184

CONTROLLED TRIAL OF HYPOSENSITIZATION IN FOOD INDUCED HYPERKINETIC BEHAVIOUR DISORDER

J.Egger, A.Stolla, Haunersches Kinderspital der Universität München

Enzyme potentiated hyposensitization (EPH) was tested in a double-blind placebo-controlled trial in 36 children with food induced hyperkinetic behaviour disorder. Children with established hyperkinetic syndrome underwent oligoantigenic diet (few foods) treatment for 4 weeks. Those whose behaviour became normal subsequently identified provoking foods by sequential reintroduction of foods. Foods which reproducibly provoked overactivity were avoided. 36 patients in whom provoking foods were established by this method were invited to take part in the hyposensitization trial. They were randomly assigned to treated and control groups. Treated patients received 3 doses of EPH (beta-glucuronidase and low levels of food antigens) intradermally at bimonthly intervalls. Control patients followed the same protocol but received buffer only. After the treatment was completed patients were allowed to eat provoking foods. Of the 18 patients who had received active treatment 15 had become tolerant towards provoking foods compared to 2 of 18 in the control group ($p<0.001$, X2 Test). The results show, that EPH gives significant protection from food induced overactivity, and indicate that food allergy is a possible mechanism of the hyperkinetic syndrome.

References

Egger J, Carter, CM, Gumley, D, Graham, PJ, Soothill, JF; Controlled trial of oligoantigenic diet treatment of the hyperkinetic syndrome.Lancet 1985; I. 540-545

Mc Ewen LM; Enzyme potentiated hyposensitization. Five case reports of patients with acute food allergy. Ann Allergy 1975; 35:98-103