INFECTIOUS DISEASES

107 GLUTATHIONE RECOVERY STUDIES DURING OXIDATIVE STRESS IN NEONATAL RED BLOOD CELLS

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Red blood cell (RBC) peroxide catabolism, via the synergistic action of catalase and the glutathione recycling system (glutathione peroxidase and reductase), helps protect the lung against oxygen toxicity (Am Rev Resp Dis 1989;140:531). Using serial changes in reduced (GSH) and oxidized (GSSG) glutathione as a marker, the ability of RBCs to deal with a hydrogen peroxide (H_2O_2) load was compared in vitro in preterm (n=8) and term (n=9) babies and adults (n=10). Incubation of RBCs with H_2O_2 caused a rapid depletion of GSH and increase of GSSG, followed by a recovery of GSH and fall of GSSG to initial values. A greater GSH depletion produced a slower GSH recovery time (r=-0.79, p<0.001). Neonatal RBCs showed significantly less depletion and quicker recovery of GSH than those of adults (p<0.001). Partial inhibition of H_2O_2 catabolism by catalase inactivation produced 50% loss of intracellular glutathione and slower GSH recovery (p<0.005) in all subjects, but recovery remained quicker in the babies (p<0.01). There was a positive correlation between gestational age and recovery time (r=0.68, p<0.02). The effective peroxide catabolism in neonatal RBCs may partly compensate for deficiencies in antioxidant defenses of the immature lung.

108 PLATELET ACTIVATION IN THALASSEMIC CHILDREN.

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Thromboembolic events,which are associated with significant morbidity and mortality,occur in β-thalassemia major patients.Eldor et al.(Am.J.Hemat.32:94,1989)reported findings of increased circulating platelet aggregates and short platelet lifespan,suggesting platelet activation.We studied the expression of the platelet selectin GMP-140 on intact cells from thalassemic patients,as a marker of platelet activation.Blood was collected in glutaraldehyde solution from 10 children and from 10 health adult donors.Platelets were isolated and the expression of GMP-140 was measured by flow-cytometry,using the monoclonal antibody CLB-Thromb./6.The mean of positive cells was 18 ± 6 vs 5 ± 2 (Wilcoxon test:p <0.01) Our study indicates that in fact platelets are activated in vivo in children affected with thalassemia major.

109 TUMOR NECROSIS FACTOR alpha(TNF-alpha) PRODUCTION BY MONOCYTES FROM CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS (JRA)

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In an attempt to elucitate the possible role of TNF-alpha in the pathomechanism of JRA,the protocol was designed to determine the level of the TNF production by Mø of JRA patients at two distinct clinical phases: acute stage (AS) and late remission (LR).A sample of 16 JRA children and 16 infection-free matched controls were enrolled to the study. The TNF level in the sera was determined by ELISA test.Spontaneous (NIL) and induced production of TNF was assesed. To induce TNF production, patients Mø were stimulated with LPS and fibroblasts (from healthy donor and a selected child with JRA).The analyses were performed at AS and LR. For statistical evaluation non parametric test was used. Results: Lower TNF levels in the sera of JRA patients at AS in comparison to LR was observed (z=-1.491 p = .07).Mø of AS patients revealed significantly lower (p=.01), and LR children significantly higher (p=.00002) NIL production in comparison to the controls.A similar pattern was observed for Mø after LPS stimulation.The production of TNF by Mø of patients (AS) stimulated by JRA fibroblasts was significantly higher (233 U/ml vs 50 U/ml) (z=-2.273 p=.001) in comparison to the situation when the fibroblasts from a healthy donor were used as stimulators.No such relationship could be observed for the Mø of patients in LR.The results suggest that serum level of TNF and the production of this monokine by Mø of JRA patients may be dependent on the clinical stage (AS vs LR) of the disease.The pattern of TNF production by Mø after stimulation with fibroblasts from JRA patient indicate the possibility that Mø-fibroblasts interaction may participate in the pathomechanism of JRA.

110 E.COLI ENDOTOXIN (LPS) GIVEN IN INTRAVENOUS INFUSION RESULTS IN BLOOD-BRAIN BARRIER (BBB) OPENING FOR NA-FLUORESCEIN (NaF) IN NEWBORN PIGLETS

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Cerebral complications like brain edema, bleeding, thrombosis, etc. are very frequent in the course of neonatal bacterial infections. We investigated in vivo the reactions of pial vessels by fluorescence macroscope giving LPS (E.coli O 111 B 4) to newborn piglets in intravenous infusion in doses 0.1 µg/kg bw./h (Group I, n=6) and 1.0 µg/kg bw./h (Group II, n=6) through 4 hours. 6 animals were given 0.9% NaCl, and served as controls (Group III). The physiological parameters (HR, MABP, CVP, body-temperature, blood gases and acid-base state) were monitorized continuously. Using 1% NaF as BBB permeability tracer extravasation was observed in Group I (128.3±27.7 min after the start of infusion), and in Group II (177.3±28.3 min, N.S. vs. Group I). Brain NaF uptake was higher in Group I (2.1±0.4 µgNaFxmg^{-1} protein/µgNaFxµl^{-1} serum), than in Group II (1.4±0.5 µgNaFxmg^{-1} protein/µgNaFxµl^{-1} serum, N.S. vs. Group I) and was non-detectable in Group III. Continuous administration of both doses of LPS produced hyperdynamic state with increased cardiac output (180% of baseline) and significantly decreased lung-thorax compliance (80% of initial value) at the time of BBB-opening. During endotoxin infusion WBC-counts in sera of treated groups elevated gradually; they reached an approximately 2-fold increase when NaF extravasation occurred, and an almost 7-fold increase 2 hours after the end of the infusions. At this time a moderate metabolic acidosis and pleocytosis was found in Group I (1800±431 cells/µl CSF) and in Group II (345±79 cells/µl CSF, p<0.01). All the parameters studied were within normal range in Group III. It is concluded, that LPS given in similar doses as found frequently in plasma from septic newborns opens the BBB for NaF in piglets. These brain microcirculatory disturbances were accompanied with significant leucocytosis and pleocytosis augmented them. (All values are mean±SE.)

111 NEUTROPHIL ELASTASE IN DIAGNOSIS OF NEONATAL INFECTION.

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Elastase (E) released from neutrophils during phagocytosis is rapidly bound and inactivated by α1-proteinase inhibitor. As previously shown, the complex is a sensitive and rapidly responsive indicator of neonatal sepsis using a time-consuming ELISA method (J. Pediatr. 1986, 108:987). In this 3 center prospective study we measured E with a rapid assay (IMAC-Elastase, Merck; 15 min) and compared it with immature/total neutrophils (I/T) and C-reactive protein (CRP) in infants with suspected infection. Normal IMAC-E values (n=319) were obtained from 125 controls (upper limits: day 0-2, 130 µg/l; day 3-5, 95 µg/l; day 6-28, 65 µg/l). An additional 252 neonates of diverse birth weights and gestational ages were evaluated for infection. Sepsis was proved in 10 and pneumonia (positive tracheal aspirate culture and x-ray) in 23.

	E	I/T	CRP	E+ I/T	E+ CRP	I/T+ CRP	E+ I/T +CRP
Sensitivity*	75	52	52	43	44	23	23
Specificity	62	82	92	93	95	98	100
Pos. Pred. Value	19	25	44	41	52	54	88
Neg. Pred. Value	96	94	94	94	94	92	92

*Values derived from infected (sepsis/pneumonia) vs. non-infected

IMAC-E is a useful adjunct in diagnosing neonatal infection, but combining E with I/T and/or CRP markedly increases PPV.

112 ELEVATION OF PROSTAGLANDIN LEVELS IN PREGNANCIES COMPLICATED BY PREMATURE RUPTURE OF THE MEMBRANES (PROM)

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Paired maternal and fetal prostaglandin levels were estimated in patients with pregnancies complicated by premature rupture of the membranes (PROM). Their results were compared to those of controls who had pregnancies with intact membranes. Fetal samples were obtained by cordocentesis, no patient was in labour at the time of cordocentesis. No control pregnancy was complicated by oligohydramnios or infection and none of the fetuses had renal disease. Prostaglandin levels were assessed by estimation of PGEM levels. Nine patients with PROM were recruited, cordocentesis was performed at a median of 4 days following PROM and at a median of 28 weeks gestational age. 12 controls were recruited, median gestational age at cordocentesis 27 weeks. Maternal PGEM levels were higher in the PROM patients (mean 348pg/ml) than the controls (mean 262 pg/ml), (95% confidence intervals 2.0 to 172), p<0.05. Fetal PGEM levels were also higher in the PROM patients (mean 349 pg/ml) than the controls (mean 216 pg/ml), (95% confidence intervals 41 to 224), p<0.01. We conclude prostaglandin levels are elevated in pregnancies complicated by premature rupture of the membranes.