KIDNEY FUNCTION

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DEGRADATION OF ENDOTHELIAL SURFACE GLYCOSAMINOGLYCANS AND FIBRONECTIN FOLLOWING TREATMENT WITH ENDOTOXIN AND NEUTROPHILS

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Vascular endothelial damage is a major component of the physiological derangement seen in septicaemia. The sulphated glycosaminoglycans (GAGS) are thought to be involved in regulating endothelial permeability, thromboresistance and leucocyte traffic across the blood vessel wall. Modulation of GAG metabolism may therefore be important in causing the capillary leak and disseminated intravascular thrombosis often seen in bacterial sepsis.

We have previously shown that the cytokines IL1 and TNF modulate glycosaminoglycan metabolism, resulting in a reduction of heparan and dermatan sulphate from the cell surface of cultured human umbilical vein endothelial cell cultures (HUVEC).

In the present study we have investigated the correlation between neutrophil activation and endothelial cell damage. To perform these experiments we have developed an endothelial model of surface injury using a cytochemical technique to visualise both endothelial GAGS and cellular fibronectin.

Results: We have demonstrated that the release of neutrophil proteases and neutrophil heparatinase are determined by the degree of both neutrophil and endothelial activation. When unstimulated neutrophils were added to HUVEC prestimulated with endotoxin, GAGS alone were degraded from the endothelial cell surface. However, when the formyl tripeptide, fMLP, was included in the incubation, we also observed extensive destruction of the protease sensitive fibronectin network. When endotoxin and fMLP were added simultaneously with the neutrophils there was minimal endothelial damage.

Conclusion: These results indicate that both neutrophil and endothelial activation is necessary to cause the breakdown of endothelial GAGS and fibronectin. These results may be important in understanding the mechanisms of neutrophil induced endothelial damage in inflammation.

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ENZYMATIC CHANGES IN THE CSF IN CHILDREN WITH NEUROINFECTIONS. Irja Lutsar, Milvi Topmann, Sulev Haldre, Tiina Talvik
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Damage within the central nervous system(CNS) causes a leakage of intracellular enzymes into the cerebrospinal fluid (CSF).

The CSF consentrations of lactic dehydrogenase (LDH), creatine phosphokinase (CPK), creatine kinase BB (CK-BB) and aspartataminotransferase (AST) were measured in children with viral meningoencephalitis-group(Gr):A, with bacterial meningitis (divided poor prognosis-Gr:B and good prognosis Gr:C) and with meningismus (Gr:D) on admission and after treatment to establish whether any of those enzymes would correlate with the outcome.

The activity of LDH, CPK and AST was determined by photocolourimetric assay (Labsystem) and CK-BB by ELISA.

Results: Determination of enzymes on admission.

Patients	CK-BB(ng/ml)	CPK(U/l)	LDH(U/l)	AST(U/l)
A (n=15)	32.8±15.7**	6.1±5.2	29.3±23.2 *	4.2±2.4 *
B (n=8)	19.5±23.4	6.1±7.3	120.1±109.8	21.2±23.5
C (n=18)	49.0±16.6	4.8±2.7	47.1±63.8	8.1±2.9
D (n=16)	39.6±7.1	5.4±4.1	18.1±10.3	5.1±2.6

*p<0.005 (with respect to patients without meningitis)
**p<0.001 (with respect to good prognosis)

During the treatment the activity of CPK increased from 4.8 to 6.9 in Gr:C and decreased from 6.1 to 2.5 in Gr:B.

Conclusion:A significant rise of LDH and AST activity, low amount of CK-BB and decrease of CPK activity in the CSF during the treatment correlated with poor prognosis in children with CNS infections.

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PHARMACOKINETICS OF VANCOMYCIN (V) IN TERM AND PRETERM NEONATES. A.Pecar, W.Lindner, V.Mönch, G.Münch, R.Roos.
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We examined half time (Pt 1/2) and distribution volume (Vd) of V in 22 (16 preterm and 6 term) critically ill neonates. V was dosed: 20 mg/kg/d OD (<800g), 30 mg/kg/d OD (800-1200g), 40 mg/kg/d TID (>1200g), as 60 min infusion. Target concentrations were 20-40 mcg/ml (pk) and <10 mcg/ml (tr). 88 drug levels were drawn: peak (pk) level 60 min after end of infusion, trough (tr) level prior to the next dose. V concentrations were measured by fluorescence polarisation immunoassay (TDX Abbott). Data are m ± sD:

n	weight (g)	PCA (wks)	Pt1/2 (h)	Vd (l/kg)	(mcg/ml)
<30wks	871±168	28±1.5	9.0±1.7	0.68±0.2	pk 43.6±13
n=20	(640-1230)	(25.5-29)	(6.4-10.5)	(0.5-1.2)	tr 9.5±4.2
30-37wks	1431±255	32±1.8	7.7±4.3	0.71±0.3	pk 36.8±8.7
n=32	(1040-1950)	(30-36)	(3.1-17.2)	(0.5-1.5)	tr 8.3±4.2
>37wks	3196±1126	45±5.7	6.4±3.8	0.83±0.2	pk 29.7±12.8
n=36	(1510-5100)	(38-58)	(2.7-15.5)	(0.6-1.5)	tr 8.3±9.2

Results: Pt 1/2 is decreasing with PCA, but Vd is not related to PCA in our population. 36% (16/44) of pk levels were not within the therapeutic range: 2 below, 14 above the range. 30% (13/44) of tr levels were above the therapeutic range. In 64% (28/44) the dosing schedule was changed according to pharmacokinetic data: increased dose (7), decreased dose (15), lengthened interval (12), shortened interval (3). The adjusted average dosage in our population was: for PCA < 30 wks: 17 mg/kg every 25 h; PCA 30-37 wks: 17 mg/kg every 20 h; PCA > 37 wks: 18 mg/kg every 19 h. Conclusion: Therapeutic drug monitoring of V is essential because of high interpatient variability, to avoid toxicity and to ensure therapeutic blood levels. We suggest a revision of current dosing recommendations in preterm neonates.

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A POSTNATAL INCREASE IN CONTACT INHIBITION REDUCES HYPERPLASTIC GROWTH IN RENAL EPITHELIAL CELLS. Eva Bratt, Anita Aperia and Stefan H Larsson.
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During postnatal maturation, the proximal tubule undergoes rapid growth, initially mainly hyperplastic, later hypertrophic. The mechanism behind the reduction of hyperplastic growth is unknown. Proliferation was studied in proximal tubule cells (PTC) from infant (I) and adolescent (A) rats after 2 days in primary culture and analyzed by 3H-thymidine autoradiography. The rate of proliferation was determined as the percentage labelled nuclei (LI).

The cells grew in colonies with contact inhibited central cells and rapidly growing peripheral cells. The growth rate was determined in each layer of cells from periphery (layer 1) to center (layers 5-6). In layer 1 I and A cells proliferate at the same rate (44±4% vs 41±4% NS). Moving into the center proliferation in A cells fell faster than in I cells. Compared to layer 1, proliferation in layers 5-6 was inhibited by 87±4% in A cells whereas in I cells by 59±5% ($p<0.05$).

To investigate possible mechanisms explaining the low degree of contact inhibition in I cells, we tested their sensitivity to growth factors. Removing serum from the culture medium lead to a more marked inhibition of I than A central cells (84±5% vs 54±5% $p<0.05$). TGFβ1 (0.1 pM), a well characterized inhibitor of epithelial growth, inhibited I central cells by 66±7% whereas no significant effect was seen in A cells.

Conclusion: An increasing contact inhibition in rat PTC may explain the postnatal reduction in hyperplastic growth. A reduced sensitivity to growth stimulatory factors rather than an increased sensitivity to growth inhibitors is suggested.

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CORRELATION BETWEEN BRAIN NATRIURETIC PEPTIDE AND ATRIAL NATRIURETIC PEPTIDE LEVELS IN NEONATAL PLASMA. Patricia Hamilton, Krys Matyka, Phornphimol Littleton and Nick Carter. Department of Child Health, St George's Hospital Medical School, London, UK.

Brain natriuretic peptide (BNP), first discovered in porcine brain, is principally produced by the heart. Unlike atrial natriuretic peptide (ANP), BNP is largely derived from the ventricles. In adults plasma BNP levels, like ANP, respond to changes in blood volume and levels of 3.4-9.0 pg/ml have been reported. Fetal and cord blood BNP levels have been reported but we are not aware of further reports on BNP in neonates.

We used specific radioimmunoassays (Peninsula Laboratories) to measure BNP and ANP levels in plasma from neonates with respiratory distress syndrome before and after treatment with exogenous surfactant. The cross reactivity between the two peptides in their respective assays is <0.001%.

Results:

Individual ANP and BNP levels were compared in 41 paired samples from babies aged 4 to 80 hours. ANP levels ranged from 87-2587 pg/ml and BNP levels ranged from 29-445 pg/ml. There was a strong correlation between the concentrations of the two peptides: R=0.76, $p<0.0001$ (Spearman's Rank Correlation test).

Conclusions:

We conclude that levels of BNP in the neonate are much higher than reported in adults and are highly correlated with ANP, although further work is needed to establish its physiological role.

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Na^+,K^+-ATPase IS REGULATED BY GLUCOCORTICOIDS IN AN AGE AND TISSUE DEPENDENT MANNER. Zheng-Ming Wang, Anita Aperia & Gianni Celsi. Karolinska Institutet, St. Göran's Children's Hospital, Stockholm, Sweden.

The adaptation of newborn mammals to extrauterine life is conditioned by increased expression of enzymes that are of importance for specialized tissue function. In the rat, maturation is accelerated around birth in the lung and around weaning in the kidney. The Na^+,K^+-ATPase enzyme is responsible for the active transport of ions across the membrane of each cell at the expense of 30% of total body O_2 consumption. We have reported that in the infant rat renal cortex, glucocorticoids (GC) rapidly increases the abundance of the Na^+,K^+-ATPase mRNA, suggesting a direct transcriptional regulation. We have now compared the effect of a single injection of betamethasone, a synthetic highly specific GC, on the abundance of mRNA for the catalytic α and the regulatory β subunits in lung and kidney from fetal, infant and young rats. In the kidney the most prominent effects on the α mRNA was found at 10 days of age (5.3±0.9 fold). A significant increase was also found in 20-day-old rats (1.6±0.2 fold), but no effect was found in fetal and 5-day-old rats. In the lung the most prominent effect was found around birth, but no effect on α mRNA was found at 10 and 20 days. In the kidney there was a coordinate increase in α and β mRNA subunits, while in the lung, the β subunit was stimulated alone, in 10- and 20-day-old rats.

Conclusion. GC induction of Na^+,K^+-ATPase genes is age- and tissue dependent. The GC sensitive period coincides with the physiological need for organ maturation.