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Analysis of N-Ras gene mutations in medulloblastomas by polymerase chain reaction and oligonucleotide probes in formalin-fixed, paraffin-embedded tissues.

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Precise data on the incidence of transforming ras oncogenes in pediatric tumors and the correlations with the histo-pathological properties of the tumor are very limited. Additionally the presence of ras activation in medulloblastomas has not been investigated so far.
Using a combination of tecniques including specific in vitro gene amplification by polymerase chain reaction (PCR) (Saiki R.K.,1985) and detection of single base mutations by sequence-specific oligonucleotides (SSO) (Farr C.J.,1988) we studied N-ras activation (mutations at codon 12,13 and 61) in 32 medulloblastomas. DNA was isolated from 5-10 µm sections of formalin-fixed paraffin-embedded tissue (according to Impraim C.,1987).
Mutations were found in 3 of 32 examined medulloblastomas. In all cases only mutations of the codon 61 were found : the most frequent was a C-A at position 1 (substitution of a glutamine residue for a lysine).A mutation A-T at position 3 was present in the remaining case. The main advantage of the procedure described are its greatly improved sensitivity, the increased speed by wich tumor samples can be analysed, no longer necessary to use high-M.W DNA and the possibility to use paraffin-embedded sections to analyse various and rare tumors in retrospect.

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EVALUATION OF ERYTHROCYTE ANKYRIN CONTENT IN HEREDITARY SPHEROCYTOSIS

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All individuals with Hereditary Spherocytosis (H.S) have spectrin deficiency. Recently Coetzer and coll. have described two patients with an apparently dominantly inherited HS: red cell membranes were deficient in both spectrin and ankyrin. Ankyrin is an extrinsic protein of the red-cell membrane, which links the cytoskeletal network to the membrane by forming a bridge between spectrin and the transmembrane anion channel, band 3. We have studied ankyrin content in 23 normal subjects and in 30 spectrin deficient HS patients. Erythrocyte ghosts were subjected to SDS-PAGE and the amount of ankyrin in the membrane, expressed as a ratio to the amount in band 3, was evaluated by laser-densitometry scansion of the stained gels. Normal subjects showed ankyrin/band3 ratio ranging between 0.183 and 0.265, the mean value being 0.217 ± 0.028. In 28 out of 30 HS patients ankyrin/band3 ratio was found ranging between 0.178 and 0.267, the mean value being 0.213 ± 0.026 : the difference between the two groups isn't statistically significative ($p > 0,1$). We found instead a marked decrease (to approximately half the control value: ankyrin/band3 0.116 and 0.131 ) in the amounts of ankyrin present in two patients' membrane. Furthermore we observed normal ankyrin and spectrin levels in the parents of both children. Since the other spectrin deficient HS patients are not associated with a concomitant decrease of ankyrin and since ankyrin represents the major binding site of spectrin to the membrane, it is possible that the primary defect in these two patients involves ankyrin and that the deficiency of this protein leads to a defective binding of spectrin to the membrane.

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**Prevention of nucleotide depletion induced by oxygen free radicals (OFR) in endothelial cells (ECs)**

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Upon reperfusion of hypoxic tissue xanthine oxidase (XO) metabolizes hypoxanthine (Hx) to xanthine (X) and uric acid (Ua), OFR are produced and may cause organ damage. XO together with Hx causes adenine nucleotide depletion and death of cultured human umbilical vein ECs. We studied the ability of glutathione (GSH)(5,10,15 mM), superoxide dismutase (SOD)(600 IU), catalase (CAT) (600 IU), alfa-tocoferol (TF) (50 and 100uM), ascorbic acid (C-vit) (10mM), dimethylthiourea (DMTU) (5mM), and dimethylsulfoxide (DMSO) (5 mM) to prevent nucleotide depletion. ECs were labeled overnight with 14C-adenine (100 uM) in culture wells, washed,and incubated with XO (80 mU/ml) and Hx (100 uM),with or without the study compounds for 4h. Nucleotides from cell extracts and medium, and breakdown products (Hx,X,Ua) from medium were separated and counted. In control cells, 60-65 % of initial cellular radioactivity (cpm) remain in adenine nucleotides after 4 h and 30-35 % appear as Hx, X, and Ua in medium. XO with Hx depletes nucleotides (1-5% of cpm in cell nucleotides, 60-70 % in Hx, X, and Ua). The corresponding distributions in the presence of the study compounds were: GSH 10/12/26 % and 62/52/48 % (5/10/15 mM); SOD 15% and 60 %; CAT 18 % and 64 %. The rest of the cpm were as nucleotides in medium. TF, C-vit, DMSO, and DMTU were not effective. TF was not protective even when present in the culture medium for 3 days. We conclude that GSH, SOD, and CAT,even though only partially able to prevent OFR-induced nucleotide depletion, should be evaluated in the treatment of in vivo ischemia-reperfusion damage.

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HIGH DENSITY LIPOPROTEIN (HDL) SUBCLASS DISTRIBUTION AND APOPROTEIN AI (APO AI) LEVEL IMPROVE IN NEWBORNS ON TOTAL PARENTERAL NUTRITION (TPN)

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The effect of TPN with intravenous fat (IVF) on Low Density Lipoproteins (LDL) and HDL, assessed by gradient gel electrophoresis and electron microscopy (EM), and apo AI levels was examined in 22 acutely ill newborns. Blood samples were taken before IVF, on maximal IVF (2.5 g/kg/d) and on full enteral feedings. Before start of IVF most infants (73%), as seen on gge, had a normal HDL subclass distribution with 2-3 major peaks, which increased with IVF. Apo AI levels also increased with IVF and further on enteral feedings (73±10, 93±16, 126±29 g/dl). In contrast to this group, the remaining 27% of the infants showed an abnormal HDL subclass distribution with very little material within but large HDL particles outside the normal HDL region, a profile very similar of Lecithin:Cholesterol-acyltransferase (LCAT) deficiency. This was associated with discoidal particles on EM and very low apo AI (32±11g/dl). The abnormal HDL profile improved with IVF, the discoidal particles diminished and apo AI rose significantly (59±19, 100±30 g/dl), as did LCAT mass (1.2, 1.6, 2.6 µg/ml). In opposite to these striking changes, LDL distribution did not change with IVF or enteral nutrition in the individual patient, nor was there a profound difference between the infants.
Conclusion: TPE/IVF improves HDL morphology, probably by stimulating increased liver synthesis of apo AI and LCAT, whereas LDL subclass distribution is not influenced.

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RECOMBINANT HUMAN ERYTHROPOIETIN (rhEPO) FOR PREVENTION OF ANAEMIAS OF PREMATURITY: A RANDOMIZED MULTICENTRE TRIAL. Michael Obladen, Rolf Maier, Ludwig Grauel, Zuzana Herrmann,Barbara Holland, Frieda Houghton, Gerhard Jorch, Otwin Linderkamp,Paul Scigalla, Charles Wardrop. Department of Neonatology, Univ.-Children's Hospital,Berlin-West,Germany (coordinating Berlin-DDR,Berlin-West,Cardiff,Glasgow,Heidelberg,Mannheim,Münster).

We investigated whether preventative treatment with rhEPO is safe and reduces the need for transfusion in preterm infants. 84 infants of 28 to 31+6 weeks gestation were stratified for artificial (vent,n=41) and spontanous ventilation (spont,n=43) and were randomized to a rhEPO- (n=39) and a control-group (n=45). rhEPO, 30 U/kg was given s.c. every 3rd day from day 4 until day 25. Infants received 2 mg iron per day from day 14. Indications for transfusion were clearly defined. No increase of mortality, necrotising enterocolitis or patent ductus arteriosus was observed in the rhEPO-group. Serum ferritin remained normal in both groups. Reticulocytes were 3.0% in the rhEPO- vs. 2.0% in the control-group on day 25.

| cumulated day 1-25 | blood sampled (ml/kg;mean ± SD) | | red cells transfused (ml/kg;mean ± SD) | |
|---|---|---|---|---|
| | rhEPO | control | rhEPO | control |
| All | 22.0±14.6 | 22.7±11.7 | 11.8±14.4 | 15.1±17.5 |
| Vent | 28.1±17.1 | 26.3±11.5 | 17.2±16.8 | 19.7±19.8 |
| Spont | 15.7±7.4 | 19.6±11.2 | 6.1±8.6 | 11.3±14.8 |

Conclusion: rhEPO, 30 U/kg administered every third day had no adverse effects, increased reticulocyte formation slightly, but did not effectively reduce the preterm infants' need for transfusion.

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INTERNAL CAROTID ARTERY BLOOD FLOW VELOCITIES BEFORE, DURING AND AFTER EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO).

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In 24 infants (mean birth weight 3.3 +/- .09 kg) requiring ECMO, blood flow velocities in the internal carotid arteries were studied using pulsed doppler ultrasound. Systolic, mean and diastolic flow velocities were calculated in cm/s. Four infants had right common carotid repair. Fifteen healthy term babies (mean birth weight 3.4 +/-.11 kg) were used as controls. During ECMO, mean and diastolic flow velocities in the left internal carotid artery increased significantly ($p<.05$) (mean flow velocity 70%, diastolic flow velocity 123%). After ECMO, systolic and mean flow velocities in the left internal carotid artery remained significantly elevated when compaired to control ($p<.05$). During ECMO, 48% of the infants had forward flow in the right internal carotid artery. No differences were found between the blood flow velocities in the left and the forward blood flow velocities in the right internal carotid artery during ECMO. The blood flow velocities in the left and in the right internal carotid artery appear similar in the 4 patients with right common carotid repair. Our results suggest a high prevalence of large collaterals between right and left internal carotid artery. The increase in left diastolic flow velocity may be due to ECMO pump action or decreased resistance. Carotid repair appears effective in restoring normal blood flow velocities.