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CONTRACTILE ENDOTHELIN B RECEPTORS ON THE SMOOTH MUSCLE CELLS OF THE HUMAN UMBILICAL VEIN

E MILDENBERGER[1], B BIESEL[2], G SIEGEL[2], HV VERSMOLD[2] [1]KLINIK FÜR KINDER- UND JUGENDMEDIZIN, KLINIKUM NEUKÖLLN , [2]UNIVERSITÄTSKLINIKUM BENJAMIN FRANKLIN (GERMANY)

Background: Endothelin triggers vasoconstriction via activation of endothelin A (ETA) and endothelin B2 (ETB2) receptors on vascular smooth muscle cells and vasodilation by stimulating the release of nitric oxide via activation of endothelin B1 (ETB1) receptors on vascular endothelial cells. It is an open question if the human umbilical vein, besides ETA and ETB1 receptors, also has ETB2 receptors.

Aim: To test if the human umbilical vein has ETB2 receptors. Methods: Strip preparations of human umbilical veins were superfused by Krebs solution. A pretension of 1 g was applied. Preparations were then exposed to the selective ETB receptor agonist sarafotoxin c (Sfc) (10–11, 10–10, 10–9, or 10–8 M). Only one concentration of Sfc was tested in each preparation. The isometric tension developed after the switch to Sfc was expressed as the percentage of that before Sfc exsposure. For each of the four Sfc concentrations used, we calculated the mean relative isometric tension developed by 7 intact and 5 endothelium-denuded preparations.

Results: In intact preparations isometric tension did not change after exposure to Sfc (10–11 to 10–9 M). Only at the highest Sfc concentration (10–8 M) isometric tension was higher than that developed in Krebs solution (149.5 ± 12.5 vs. 100%, $p < 0.05$). In contrast, in endothelium-denuded preparations isometric tension increased after exposure to Sfc (10–11 to 10–8 M). Even at the lowest Sfc concentration (10–11 M) isometric tension was significantly higher than that developed in Krebs solution (111.7 ± 4.3 vs. 100%, $p < 0.05$).

Conclusions: Vascular smooth muscle cells of the human umbilical vein have ETB2 receptors mediating contraction. In endothelium-denuded preparations the vasoconstricting effect of Sfc can be attributed to the activation of ETB2 receptors. In intact preparations this vasoconstricting effect appears to be counteracted in part by the vasodilating effect of the activation of ETB1 receptors on vascular endothelial cells.

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PROPHYLACTIC ANTITHROMBIN TO PREVENT INTRAVENTRICULAR HAEMORRHAGE IN PRETERM INFANTS: A SYSTEMATIC REVIEW.

D MILLAR[1, 2], D BASSLER[1], B SCHMIDT[1, 3] [1]DEPARTMENT OF NEONATAL MEDICINE, MCMASTER UNIVERSITY (CANADA), [2]DEPARTMENT OF NEONATAL MEDICINE, ROYAL INFIRMARY OF EDINBURGH (UK), [3]DEPARTMENT OF CLINICAL EPIDEMIOLOGY AND BIOSTATISTICS, MCMASTER UNIVERSITY (CANADA)

Background: Sick preterm infants have low levels of antithrombin (AT). It has been hypothesised that prophylactic administration of AT would prevent intraventricular haemorrhage (IVH).

Method: We conducted a systematic review and meta-analysis of randomised, controlled trials that compared any dose of AT with placebo or no treatment, in preterm infants. This systematic review was conducted using the methods of The Cochrane Collaboration. Data were extracted from the published papers/abstracts. When appropriate we combined data from different trials into a meta-analysis using a fixed-effects model.

Results: Three randomised trials, two published as full-text articles and one abstract, comprising 280 preterm infants, met our inclusion criteria. The mean gestational age, in the 2 full-text studies, was 28 weeks. The total dose of AT was comparable in these two studies, 500 U/Kg and 400 U/Kg, in divided doses over the first 48 hours of life. IVH was reported in all three trials; none of the individual trials found a significant statistical difference. The pooled analysis (from the 2 full-text trials) for any grade of IVH within the first week of life showed a relative risk that favoured the control group: RR 1.30 (95% CI 0.81 to 2.08). The trials did not report follow-up of IVH beyond 1 week. None of the individual trials demonstrated any significant difference in neonatal mortality. A pooled analysis of mortality in infants with no IVH at randomisation was not possible from the published data.

Conclusions: There is little evidence that prophylactic AT reduces IVH. We cannot recommend the use of prophylactic AT to prevent IVH in preterm infants.

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CONSTITUTIVE INTERLEUKIN-10 EXPRESSION PREDICTS THE RISK FOR BPD IN PREMATURELY BORN INFANTS

P MINOO, A GARINGO [1]DEPARTMENT OF PEDIATRICS, UNIVERSITY OF SOUTHERN CALIFORNIA SCHOOL OF MEDICINE (USA)

Compared to full term, both the cellular ability to synthesize, and the content of IL-10 is decreased in the lungs of preterm infants. In this study, we determined the constitutive and LPS-induced synthesis of IL-10 by lung-derived cells from preterm and term infants and determined whether either of the two measurements is related to gestational age and incidence of BPD. METHODS: Lung cells from preterm and term neonates on postnatal days 1–4 or 4–7 were cultured in vitro in presence or absence of 100 ug/ml of LPS. Secreted IL-10 was measured by ELISA. SUBJECTS: A total of 37 infants; Preterm neonates at gestational ages of 23–25 weeks (Group 1, 19 infants), and 28–34 weeks(Group 2, 14 infants), and 4 full-term infants with meconium aspiration (Group 3). RESULTS: A distinct relationship was found between gestational age and LPS-induced IL-10 response; Group 1 on day 1–4 had significant number of IL-10 non-responders compared to group 2. All neonates in Group 3 had positive LPS-induced IL-10 response. These data suggest developmental regulation of LPS-induced IL-10 response in lung-derived cells. Postnatal development in both groups 1 and 2 was also associated with increased LPS-induced IL-10 responsiveness, indicating postnatal maturation of the IL-10 response mechanism. Importantly, presence of constitutive IL-10 production by lung inflammatory cells from preemies born between 23 and 25 weeks of gestation correlated significantly with, and predicted the incidence of BPD in this group of highly vulnerable infants. Supported by NIH 56590 and the Hastings Foundation.

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NOVEL COMPOUND HETEROZYGOUS MUTATIONS IN SGLT2 GENE ARE RESPONSIBLE FOR AUTOSOMAL RECESSIVE RENAL GLUCOSURIA.

E MIORIN[1], G CIANA[2], S CROVELLA[2], F COLONNA[1] [1]U.O.PEDIATRIA,OSPEDALE DI SAN VITO AL TAGLIAMENTO, ASS6 FRIULI OCCIDENTALE , [2]IRCCS BURLO GAROFOLO (ITALY)

Background: Individuals with primary renal glucosuria have persistent urinary glucose excretion in the presence of normal blood glucose levels, normal oral glucose tolerance test results and in the absence of any signs of a general renal tubular dysfunction. Primary renal glucosuria is postulated to be attributable to mutations in SGLT2 gene, which codes for an active transporter specific for d-glucose, sodium/glucose co-transporter type 2, expressed in the luminal membrane of S1 segment renal proximal tubule cells. Described inheritance patterns have included both autosomal dominant and autosomal recessive mechanisms. Some cases have been associated with selective aminoaciduria, distinctly unlike the generalized aminoaciduria seen in Fanconi's syndrome. We report the case of a 5-months-old girl with primary renal glucosuria associated with overexcretion of glutamic acid and aspartic acid , daughter of non-consanguineous parents, both without renal glucosuria. The magnitude of glucosuria was 6,5 g per day and quantitative urine amino acid screen revealed greatly increased levels of glutamic acid, 2955 nmol/mg Creat. (normal range 252-1280) and mildly increased levels of aspartic acid, 911 nmol/mg Creat. (normal range 230–685).

Aim: To define the molecular basis of renal glucosuria, we performed the SGLT2 mutation analysis in this patient with primary renal glucosuria and urinary overexcretion of glutamic and aspartic acid.

Methods: Genomic DNA was extracted from patient's perypheral blood; PCR amplification was performed using primers specific for the coding region of the SGLT2 gene. PCR aplicons were sequenced with SeqScape 1.o software.

Results: Sequence analysis of SGLT2 gene (exons 1,2,3,5,7,8,12) revealed a compound heterozygosity for two SGLT2 mutations, one in exon 5, 525delC (A169fs186X) and the other in exon 7, G/A R267Q.

Conclusion: These findings suggests that two novel mutations in the SGTL2 gene are responsible for a form of recessive renal glucosuria associated with selective aminoaciduria.

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EFFECTS OF IBUPROFEN AND INDOMETHACIN ON VEGF, IGF-I, AND GH IN THE RAT RETINA, VITREOUS, AND SERUM

HD MODANLOU[1], J HASAN[1,2], JV ARANDA[3], KD BEHARRY[1] [1]UNIVERSITY OF CALIFORNIA, IRVINE , [2]MILLER CHILDREN'S HOSPITAL, [3]CHILDREN'S HOSPITAL OF MICHIGAN (USA)

Background/Aims: IB and IN have been shown to improve oxygen-induced retinopathy in mice. The effects of IB and IN on VEGF, IGF-I, and GH (regulators of growth and retinal development) have not been studied. We hypothesized that the effects of IB and IN are partly mediated via their action on these growth factors.

Methods: Newborn rats (n=3 litters/group; 15 pups/litter) received IP injections of saline (Sal), IB (10 mg/Kg), IB (50 mg/Kg), IN (0.2 mg/Kg), or IN (1.0 mg/Kg) on 1, 2 and 3 days postnatal age. At P14, VEGF, IGF-I and GH levels were determined in the retina, vitreous, and serum.

Results: Low doses of IB and IN had no effect on body weight or linear growth although higher serum IGF-I levels (ng/mL) were noted with 10 mg/kg IBU (386.7±30.1, $p<0.05$) vs Sal (282.1±19.8).). Animals treated with 50 mg/Kg IB had decreased body and organ weights ($p<0.001$ vs. Sal) and higher serum IGF-I levels (474.5±36.6, $p<0.001$ vs Sal). Animals treated with 1.0 mg/Kg IN had higher body weights ($p<0.01$ vs Sal) and linear growth ($p<0.001$ vs. Sal) and lower serum VEGF levels (101.3±12.1 pg/mL, $p<0.05$) vs Sal (141.7±13). In the vitreous, animals treated with 50 mg/Kg IB had higher IGF-I levels (121.9±12.1 ng/mL, $p<0.05$) vs Sal (80.4±4.0) and lower GH levels (2.0±0.39 ng/mL, $p<0.05$) vs Sal (3.53±0.37), while animals treated with 0.2 mg/kg IN had lower VEGF levels (665.6±24.2 pg/mL, $p<0.01$) vs Sal (906.3±31.1). In the retina, VEGF levels (pg/mg protein) were suppressed with 50 mg/Kg IB (143.6±11.4, $p<0.01$ vs Sal (191.2±14.7), but elevated with 1.0 mg/Kg IN (238.7±22.4, $p<0.05$ vs Sal).

Conclusions: High doses of IB increased total serum IGF-I levels, and decreased body and organ weights indicating decreased IGF-I bioactivity. Suppression of retinal VEGF by IB may suggest a potential therapy if administered during the vasoproliferative phase of retinopathy of prematurity.

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ONTOGENY OF VEGF, IGF-I, AND GH IN THE RAT RETINA, VITREOUS AND SERUM

HD MODANLOU[1], Z GHARRAEE[1,2], J HASAN[1,2], J WALTZMAN[2], S NAGEOTTE[2], KD BEHARRY[1] [1]UNIVERSITY OF CALIFORNIA, IRVINE , [2]MILLER CHILDREN'S HOSPITAL (USA)

Background/Aim: Growth factors such as VEGF, IGF-I, and GH are implicated in retinal vascular development. Low serum IGF-I and high serum GH has been shown to be associated with ROP. The ocular compartments are protected by the blood-retinal barrier. We hypothesized that levels of growth factors in the ocular and systemic circulation are different and compartment-specific. We examine the ontogeny of VEGF, IGF-I and GH in rat retina, vitreous, and serum from birth (P0) to P21. Methods. Newborn rats (n=3 litters/group; 15 pups/litter) were sacrificed at P0, P7, P14 and P21. Retinal, vitreous and serum VEGF, IGF-I and GH levels were determined.

Results: VEGF levels were 10-fold higher in the vitreous than serum at all stages of development. Vitreous and serum VEGF levels declined at P7, P14 and P21 ($p<0.05$ to $p<0.001$) compared to P0. In the retina, VEGF levels increased with highest concentrations at P21 ($p<0.05$ vs P0). IGF-I levels in the vitreous were comparable with serum levels and decreased from P7 through P21 ($p<0.05$) compared to P0. IGF-I levels in serum and retina increased with advancing postnatal age. Despite 4-fold higher IGF-I levels in the vitreous than retina at P0, equilibration was achieved at P21. GH levels in the vitreous were 10-fold lower than serum levels and were decreased at P14 and P21 ($p<0.05$ to $p<0.001$) compared to P0 and P7 in both compartments. GH levels in the retina remained unchanged from P0 through P21.

Conclusions: The ontogenic patterns of vitreous VEGF and IGF-I during rat postnatal retinal development imply that the vitreous is a reservoir for these growth factors. VEGF, IGF-I and GH in the retina, vitreous and systemic circulation exhibit compartment-specific differences. These differences should be considered in conditions associated with retinal neovascularization, such as ROP.