27

PREVENTION OF ARTERIOVENOUS FISTULA FAILURE DUE TO THROMOSIS: IS THERE A ROLE FOR THROMBOPROPHYLAXIS?

A Alatkar, JJ Lin, C Crandell, B Adams, and SW Pipe. University of Michigan, Ann Arbor, MI

Background: Development of thrombosis is the commonest cause of arteriovenous fistula (AVF) failure in children with end stage renal disease (ESRD) requiring hemodialysis. We report our experience of using primary thromboprophylaxis (PTP) for prevention of thrombosis at AVF. **Methods & Results:** A strategy of PTP constituted an infusion of unfractionated heparin (UFH, 10 IU/kg/hr) for the first 24 hours after AVF surgery followed by subcutaneous injection of low molecular weight heparin (LMWH, 0.5 to 1 mg/kg/dose) twice daily until AVF was matured and successfully accessed. LMWH therapy was monitored by peak and trough anti-Xa levels. Target anti-Xa levels were maintained in therapeutic range (0.5 to 1.0 IU/ml) for those with history of thrombosis or associated risk factors for thrombosis while remaining patients were maintained in prophylactic range (0.2 to 0.5 IU/ml). Total of 26 AVF were performed on 18 children from Jan 2001 to July 2006: 19 (73%) historical controls; 7 (27%) received PTP. Mean time for AVF maturation was 60 days (range: 33 to 88). Among 19 children, 14 received no thromboprophylaxis while 5 received aspirin (81 mg once daily). Eleven (79%) of 14 AVF in no treatment group failed: 9/14 (65%) due to thrombosis, 2/14 (14%) due to poor growth of venous segment. Among 5 children who received aspirin prophylaxis, 2/5 (40%) AVFs failed. One (20%) developed hematoma and 1 (20%) had poor growth. In PTP group, 2/7 (29%) AVF failed: 1 due to hematoma, 1 due to poor growth. Additional events in PTP group included: vasospasm-induced thrombosis requiring thrombectomy (n=1) and hematoma (n=2). Two children who developed hematoma had anti-Xa levels at 1.56 IU/ml and 0.6 IU/ml respectively. Presently 4/7 (57%) AVFs in PTP group are functioning well. The 7th patient does not require hemodialysis. Three of the 5 children in the PTP group are still on LMWH (mean duration 6 months, mean anti-Xa level 0.6 IU/ml). Mean AVF survival was higher in children who received PTP (Day 100 survival: 57.14±18.7% versus 42.10±11.32% respectively; p 0.20; Figure). Small sample size thus far limits the meaningful statistical analysis. **Conclusion:** Our experience of LMWH thromboprophylaxis appears encouraging for prevention of AVF failure due to thrombosis. Close clinical and laboratory monitoring is required to prevent bleeding complications related to LMWH. More prospective data to expand our sample size will be required to clarify our observation.

28

DEVELOPMENT OF MILK-TARGETED TRANSGENIC ANIMALS EXPRESSING COAGULATION FACTOR VIII BIOENGINEERED FOR HIGH EFFICIENCY SECRETION

HZ Miao[1], ST Butler[2], WH Velander[3], and SW Pipe[1], Virginia Tech[2], Blacksburg, VA, University of Nebraska[3], Lincoln, NE and University of Michigan[1], Ann Arbor, MI.

The lifelong hemorrhagic diathesis of hemophilia A is treated successfully with recombinantly derived factor VIII (rFVIII). rFVIII is presently produced and purified from mammalian cell lines in liquid culture but has proven to be costly. The milk of transgenic livestock can yield an abundant source of complex therapeutic proteins such as recombinant coagulation proteins. Only the pig mammary gland has been shown to carry out all of the post-translational modifications necessary to generate the biological activity and long circulation half-life needed for complex glycoproteins. Velander and colleagues have demonstrated that human recombinant factor IX can be produced at levels up to 100 U/ml. However, yields of rFVIII have been much lower and subject to chain dissociation due to the harsh chelating environment and/or the absence of vWF. Through bioengineering strategies we have developed rFVIII variants with enhanced secretion (F309S/226aa/N6) and stability (IR8). Our goal is to express these rFVIII variants (F309S/226/N6 and IR8) in the milk of transgenic mice and pigs under the murine Whey Acidic Milk Protein (WAP) promoter and downstream 3' UTR elements. Transgenic mice provide both insight into the encoding fidelity of the transgene constructions as generally expressed by mammary epithelia but also provide useful amounts (ie. >1 ml per lactation at >20 ug/ml) of post-translationally modified, recombinant protein for evaluation. We have created 6 murine transgenic lineages that express the rFVIII variants either alone or in combination with vWF and/or alpha1-antitrypsin. We are co-expressing these constructions with vWF to help stabilize the rFVIII variants. We have expressed vWF previously in the milk of transgenic mice at greater than 100 ug/ml. We are also co-expressing alpha1-antitrypsin as this has been shown to dramatically reduce the amount of proteolytic degradation in milk. Trigenic and bigenic combinations of these constructs were mixed equal molar and microinjected into mouse zygotes to produce transgenic mice at the University of Michigan. Founder animal lineages are presently being outbred with wild-type mice. The resulting F1 lineages will undergo induced lactation and the milk analyzed for rFVIII content. Trigenic, transgenic pig fibroblast lines have also been made and lineages are awaiting nuclear transfer in August 2006.

29

CXXC-FINGER PROTEIN 1 REGULATES DNA METHYLTRANSFERASE 1 PROTEIN EXPRESSION AND ACTIVITY.

JS Butler, DG Skalnik, Herman B Wells Center for Pediatric Research and Section of Pediatric Hematology/Oncology, Departments of Pediatrics and Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN.

Epigenetic regulation refers to changes in gene expression that occur without changes in the nucleotide sequence. Cytosine methylation and histone tail modifications are two epigenetic modifications that influence gene expression. Elucidating epigenetic mechanisms of gene regulation is becoming increasingly important as deregulation of epigenetic processes is observed in many diseases, including cancer. The *CXXC1* gene encodes CXXC finger protein 1 (CFP1), a transcriptional activator that specifically binds unmethylated CpG dinucleotides. The specific binding activity of CFP1 makes it unique in that most CpG binding proteins bind methylated CpG dinucleotides and facilitate heterochromatin formation. CFP1 has recently been identified as a member of the mammalian SET1 histone H3 lysine 4 methyltransferase complex. Disruption of *CXXC1* in mice results in an early embryonic lethal phenotype, therefore *CXXC1-/-* embryonic stem (ES) cells were isolated from *CXXC1-/-* blastocysts to further characterize the function of CFP1. Embryonic stem cells lacking expression of CFP1 exhibit multiple epigenetic modification defects including altered histone modifications and reduced global cytosine methylation. DNA methyltransferase 1 (Dnmt1) is the major source of DNA methyltransferase activity in mammalian cells and is responsible for copying methylation patterns during DNA replication. Dnmt1 protein level and methyltransferase activity are decreased by ~50% in *CXXC1-/-* ES cells and are rescued by stable expression of murine CFP1. Dnmt1 transcript level is not reduced in *CXXC1 -/-* ES cells, however. Immunoprecipitation experiments revealed an interaction between CFP1 and Dnmt1 *in vivo*. Regulation of Dnmt1 protein level and activity by CFP1 is an exciting discovery in that it is the first example of reduced Dnmt1 protein without direct disruption of Dnmt1 gene function. The functional significance of this novel intersection of epigenetic regulatory proteins is the principle focus of ongoing experiments.

30

CARDIAC ERK ACTIVATION DURING SUSTAINED ARTERIAL AND VENOUS HYPERTENSION IN THE SHEEP FETUS.

S Jonker[1,2], S Louey[1], K Thornburg[1,2,3], G Giraud[1,2,3,4] 1) Heart Research Center, 2) Dept of Physiology & Pharmacology, 3) Cardiovascular Medicine, Oregon Health & Science University, 4) Portland VA Medical Center, Portland, Oregon

The relationship between fetal cardiomyocyte growth and *in vivo* phosphorylation and activation of the three arms of the MAPK cascade (ERK, p38 and JNK) is only beginning to be understood. Because we know that phosphorylation of ERK is necessary for fetal sheep cardiomyocyte proliferation and enlargement *in vitro*, and p38 phosphorylation has been described in pressure-loaded fetal sheep hearts *in vivo,* we hypothesized that ERK and p38 phosphorylated would be increased in the hearts of fetal sheep with chronic arterial and venous hypertension. **Methods:** Fetal sheep were instrumented with intravascular catheters to permit arterial and venous access for pressure measurements, sampling and infusions. Following recovery, fetuses were infused with plasma protein (~13g/day) for 8±1 days, which we have previously shown to gradually increase arterial and venous pressures. At the conclusion of the experiment (138±1 days of 145 day gestation), fetal body, heart and ventricular freewall weights were measured, and freewalls were frozen in liquid nitrogen. Age-matched fetuses served as controls. Levels of protein expression and phosphorylation were measured by Western blot analysis in right ventricular (RV) extracts (ERK phosphorylation was also measured in left ventricles [LV]). Data were normalized as phosphorylated-to-total protein. Groups were compared by t-tests (data shown as mean ±SD). **Results:** Chronic intravascular infusion of plasma increased fetal arterial and venous pressures by 50% and 60%, respectively (8±1 days compared to baseline). Heart weight was greater in hypertensive fetuses than control fetuses (37±4 vs. 27±7g), but body weights were not different. ERK phosphorylation was 300-400% greater in the ventricles of hypertensive fetuses than control fetuses (LV $p>0.01$; RV $p>0.04$). Although mean p38 phosphorylation was 385% greater in the RVs of hypertensive fetuses than control fetuses, this result did not reach statistical significance ($p=0.07$); phosphorylation of p38 occurred very strongly in some RVs but less in others. Phosphorylation of JNK was not different between hypertensive and control fetuses. **Discussion:** In this model, onset of arterial and venous hypertension is gradual, thus chronic rather than acute activation of ERK (and perhaps p38) may be the most significant signals regulating cardiomyocyte proliferation, enlargement or terminal differentiation previously shown to be occurring in these fetal hearts.

31

ORAL FEEDING DISORDERS AT HOSPITAL DISCHARGE: A UNIQUE MARKER OF DISABILITY?

SB Allen, University of Kansas Medical Center, Kansas City, KS, and CA Castor, CA Lambrecht, and HW Kilbride, Children's Mercy Hospitals & Clinics, Kansas City, MO.

The purpose of this study was to determine the prevalence of oral feeding problems at hospital discharge for preterm infants and to assess if these could be markers of later disability. This was a retrospective review of patients enrolled in a follow-up clinic database. Infants included were ≤ 32 wk GA, seen for the initial clinic visit between January 1, 2002 to December 31, 2004. 71/328 (22%) infants had oral feeding disorders (OFD) as defined by having one or more of the following at hospital dismissal: use of tubes and/or feeding pump, feeding more frequently than every 3 hours secondary to poor volume tolerance, and/or less than 100% of feeding orally. A comparison group of infants was determined by matching each study infant with the next infant in the database without a feeding problem but with the same GA (± 1 wk) and BW (± 100g). Medical complications and limited outcome data were obtained by database review. Group differences were determined by chi-square and univariate analysis of variance with significance indicated by $P<.05$.

There were no group differences in BW (1023 ± 370 and 1026 ± 345 g, OFD and control, respectively), GA (27.6 ± 2.5 wk, both), or gender. OFD infants were, on average, discharged from the hospital 5 weeks later and 880 g heavier than comparisons ($P=.000$). There were no group differences in rates of severe IVH or ROP. OFD infants were more likely to have CLD (52% and 35%, $P=.04$) and be discharged on a home apnea monitor (47% and 25%, $P=.009$). 39% of OFD infants had neither CLD or IVH. A subset of infants had 24-month developmental screening using the Bayley Infant Neurodevelopmental Screener (BINS). Controlling for CLD and maternal race, OFD infants (n=14) tended to have a lower mean BINS scores than comparisons (n=30), 6.9 ± 3.6 and 4.3 ± 3.2, $P=.08$.

Preterm infants appear to frequently have oral feeding problems at hospital discharge. Additional study of this population is needed to determine possible etiologies and long-term implications of delayed oral feeding, which could be a unique marker of disability.

32

ALTERATION OF THE DISTRIBUTION OF ENDOTHELIAL COLONY FORMING CELLS (ECFCS) IN SWINE MODELS OF CARDIOVASCULAR INJURY.

L Huang, Indiana University School of Medicine, Herman B Wells Center for Pediatric Research, Indianapolis, IN; DM Hou, B Johnstone, KL. March, Indiana Center for Vascular Biology and Medicine, Indianapolis, IN; DA Ingram, MC Yoder, Indiana University School of Medicine, Herman B Wells Center for Pediatric Research, Indianapolis, IN.

Endothelial progenitors play an important role in both postnatal vasculogenesis and vascular homeostasis. Based on proliferative potential, we have recently identified a hierarchy of endothelial colony forming cells (ECFCs) in human blood and blood vessels. We questioned whether the hierarchy of ECFCs also existed in the swine and whether a cardiovascular stress would affect this hierarchy. Therefore, we investigated the distribution of ECFCs by a single cell endothelial colony forming assay in normal pigs and pigs following acute myocardial ischemia (AMI) (n=5) or placement of a coronary stent (n=3). In normal animals, the hierarchy of ECFCs was present in circulating blood and aortic endothelium. In experimental pigs with AMI, the percent of single cells derived from peripheral blood that formed colonies doubled after 45 minutes of AMI. Moreover, the percent of high proliferative potential ECFC (HPP-ECFC) (>2000 cells/colony) was significantly increased by16-fold after 45 minutes of AMI compared to baseline (2.5%) and 2-fold higher than the frequency measured from the samples obtained at sacrifice 7 days later (14%). On the other hand, the percent of endothelial cluster (2-50 cells/colony) was reduced more than 3 fold following 45 minutes of AMI. The percent of HPP-ECFC from aortic endothelium in the stented pigs was 3-fold higher than that observed in the control pigs. Likewise, HPP-ECFCs were present in peripheral blood samples taken from stented pigs but were not detectable in the control pigs. These experimental animal observations (1) indicated that ECFCs circulate in the blood and are present in vascular intima in the normal swine and (2) revealed that the distribution of ECFCs in the vascular endothelium and circulation are altered during the early events of cardiovascular injury. Further studies will be required to determine whether the change of ECFCs distribution is resulting from the mobilization of endothelial progenitors from bone marrow or from vascular endothelial intima.