5

EFFECT OF DIET ON THE ONTONGENY OF DRUG BIOTRANSFORMATION IN HEALTHY INFANTS.

MJ Blake, SM Abdel-Rahman, RE Pearce, JS Leeder, GL Kearns, Children's Mercy Hospitals and Clinics, Kansas City, Missouri, U.S.A.

Drug biotransformation can be altered by diet in adults resulting in drug accumulation and toxicity, or in some cases, enhanced elimination. The cytochrome P450 enzymes (CYPs) are a super family of heme-containing phase I enzymes responsible for biotransformation of a significant number of commonly used pharmacologic agents and a large array of endogenous substrates. Previous studies suggest that drug metabolism by CYPs differs between breast-fed and formula-fed infants. The effect of infant diet on the development of functional CYP activity is assessed in this report. Oral doses of caffeine and dextromethorphan were used as pharmacologic probes to track CYP enzyme activity in neonates seen at 6 visits over their first 6 months of life. The caffeine elimination rate constant (k_e) was determined from serum caffeine levels obtained on alternating visits. At all visits, levels of caffeine, dextromethorphan, and their respective metabolites were determined by HPLC in urine samples collected over a 24 hr post-dose period. The serum caffeine k_e of infants at their first study visit (2 weeks post-natal age) was unrelated to post-conceptional age. Caffeine elimination was low in infants at this first visit and displayed a significant positive linear correlation with increasing post-natal age (k_e = .004 (Age [weeks]) + .001; $p<0.001$). Caffeine k_e increased faster in formula-fed infants (slope = .005 95%CI: 0.004, 0.006) than in breast-fed infants (slope = .002 95%CI: 0.001, 0.002) ($p<0.001$) concomitant with increased conversion of caffeine to 3-demethylated metabolites. In contrast, breast feeding significantly increased the urinary fractional molar recovery of 3-methylxanthine (fr3X) [(fr3X) = .004 Age [weeks] + .016; $p< 0.01$] suggesting that this feeding modality produces a relative shunt in metabolism towards 7-demethylated metabolites. Dextromethorphan metabolism, assessed as the fractional molar recovery of 3-hydroxymorphinan (fr3HM), showed a similar marked increase with postnatal age [(fr3HM) = .014 Age [weeks] + .203; $p< 0.001$] that did not differ between diets. The maturation of caffeine elimination does not appear to begin until after birth. Formula feeding appears to accelerate the development of caffeine metabolism but not dextromethorphan. There may be specific ligand(s) unique to infant formula or human breastmilk that modify the developmental regulation of *CYP1A* gene expression and/or activity. Dietary modification of CYP activity may alter drug bioavailability and increase exposure to modified xenobiotics or endogenous metabolites from a very early age.

6

IDENTIFYING THE MOUSE GENE RESPONSIBLE FOR CONGENITAL PROGRESSIVE HYDRONEPHROSIS (CPH).

PD Brophy, J Clarke, RM Raymond Jr. University of Michigan Department of Pediatrics, Ann Arbor, MI.

Obstructive uropathy is the leading cause of chronic renal failure and ESRD in children. Understanding the genetic and molecular control of urinary tract development is critical for making an impact on obstructive uropathy. Presently, little is known about the specific molecular causes of obstructive uropathy.

The congenital progressive hydronephrosis (cph) mouse was described as having a random recessive mutation in the C57Bl/6J strain of mice in 1978 and was crudely mapped to mouse chromosome 15 between the visible markers belted (*bt*) *(now ADAMTS20)* and Caracul (*Ca*) (*now Krt2–6g)*. The *cph* mutant provides an excellent model of obstructive uropathy that closely mimics the process in humans. The renal phenotype of homozygous *cph* mutants is not grossly visible at day 1 of age but can be detected microscopically with abnormalities of the renal papillae indicating onset in-utero. Radiographic analysis demonstrates profound hydronephrosis with obstruction at the level of the UPJ.

We hypothesize that the identification of the *cph* gene and mutation therein, will provide novel insight into the mechanism of development of congenital progressive hydronephrosis and allow identification of important signaling molecules required for both normal and abnormal genitourinary tract development.

Obligate heterozygote mice for *cph*, were mated to the 129Ola strain of mice to generate F1 progeny containing the *cph* mutation. Random F1 animal matings has resulted in the generation of F2 mice homozygous for the *cph* mutation. DNA samples from F2 mice homozygous for the *cph* mutation have been used for analysis. We have obtained multiple *cph* mutants on this F2 background and we have begun linkage analysis using SSLP markers in the candidate region on Chromosome 15. Through cross-over analysis using MIT markers we have narrowed the region of the mutation on Chromosome 15. We have found a marker, D15Mit172, very close to the proposed genomic location of the *cph* gene that is consistently inherited as homozygous for the B6 allele in all affected F2 animals genotyped, giving 100% concordance with this marker and the *cph* phenotype in seven of fifty-one F2 mice to date. This finding leads us to believe that the D15Mit172 marker can be used as a proxy locus for genotyping embryos in the F2 genetic background for in-utero analyses. Simultaneous candidate gene sequencing and Real Time RT-PCR analysis has been performed and narrowed the candidate field. A promising gene has been identified.

7

THE EFFECT OF A CHILD PEDESTRIAN SAFETY STREET PROGRAM ON IMPROVING KNOWLEDGE.

R Kregenow and D Jaffe, Washington University, St Louis, MO.

***Purpose*:** Children between the ages of 5 to 8 years are at risk for pedestrian, motor vehicle, bicycle, stranger, and animal bite injuries. Safety Street was developed to educate children in this age group about safe behaviors using didactic teaching and practical training in a mobile three dimensional Safety Street. The study's goal is to demonstrate the efficacy of this method on improving knowledge in these safety areas.

***Methods*:** A quasi-experimental study was conducted on kindergarten through second grade children from St. Louis region schools. Students were tested using a previously validated testing method. Intervention school children were tested 1 week prior to the Safety Street program arrival, one month later, and 4–6 six months later. Control school children were tested 4–6 months prior to Safety Streets arrival, one-month later, and right before Safety Street's arrival.

***Summary of results*:** There are 371 control subjects and 369 intervention subjects who took test 1 and test 2, with a mean age of 6.4 and 6.8 years respectively. The control school subjects were 47% male (and 53% female) and the intervention school subjects were 53% male (and 47% female). Overall, intervention subjects increased 12% (10.9, 12.6) on test scores compared to an increase of 5% (10.2, 10.7) in the control subjects ($p<0.001$). This difference was also observed when stratified by grade ($p<0.001$). For those students who took test 3 (6 mos. after test 2), there were 342 control subjects and 96 intervention subjects. Overall, the difference between test 1 and test 3 for the intervention subjects was 9.3% (11.3, 12.6) compared to an increase of 5% (10.2,10.9) in the control subjects ($p<0.001$). This difference was also observed when stratified by grade ($p<0.001$). The greatest improvement was shown in kindergarten children and mean scores increased with age.

***Conclusion*:** Using a validated testing method, we were able to demonstrate that the Safety Street program is an effective means of transmitting knowledge about pedestrian, bicycle, animal, and passenger safety to children between the ages of 5 to 8 years. The increase in knowledge is retained after 4 to 6 months.

8

GALECTIN-1 IS LOCATED IN FIBROBLASTS OF NEONATAL MOUSE LUNG AND IS UP-REGULATED BY RETINOIC ACID

Jennifer J Foster, and Jeanne M Snyder, University of Iowa Hospitals and Clinics, Iowa City, Iowa

Background: Our previous work demonstrated that galectin-1 is enriched in the tips of developing secondary alveolar septa in neonatal mouse lung. Thus, we hypothesize that galectin-1 is a regulator and/or marker of alveologenesis, a process that is impaired in infants with bronchopulmonary dysplasia.

Objective: In order to further elucidate the role of galectin-1 in distal lung development, we characterized the localization of galectin-1 in the developing lung and evaluated the effects of retinoic acid, a known stimulator of alveolar formation, on galectin-1 expression.

Design/Methods: The lungs of postnatal day six mice were inflation fixed and immunostained for galectin-1, surfactant protein B (a marker of type II alveolar epithelial cells) and endoglin (an endothelial cell marker). In addition, fibroblasts were isolated from postnatal lung tissue, maintained *in vitro*, then treated with retinoic acid at a 1 micromolar concentration for 24 hours. Immunoblot analysis was used to evaluate the levels of galectin-1 protein in the cultured lung fibroblasts.

Results: The pattern of galectin-1 immunostaining in neonatal mouse lung differs markedly from that of surfactant protein B and endoglin, demonstrating that galectin-1 is localized to neither the type II alveolar cell nor the microvascular endothelium. The galectin-1 stained cells are located in the center of the secondary septal tips, making it likely that the galectin-1 is contained in fibroblasts. Fibroblasts isolated from neonatal mouse lung were found to contain galectin-1 protein, and galectin-1 levels were increased after treatment of the fibroblasts with retinoic acid.

Conclusions: In the neonatal mouse lung, galectin-1 is localized in secondary septal wall fibroblasts, a cell type which is important in the pathogenesis of bronchopulmonary dysplasia. The up-regulation of galectin-1 expression in lung fibroblasts represents a potential mechanism by which retinoic acid stimulates alveologenesis, since galectin-1 has been shown to promote the growth and differentiation of smooth muscle cells and endothelial cells, which are both important components of the developing alveolar wall.

9

EFFECTS OF ERYTHROPOIETIN ON RESPIRATORY EPITHELIAL CELL GROWTH AND FUNCTION.

P DeYoung, A Kapur, M Lichtman, P Kling, and D Carlton, Department of Pediatrics, University of Wisconsin, Madison, WI.

Erythropoietin has a wide variety of nonhematopoietic effects in the body, including the regulation of important enzymatic activity in the pulmonary endothelium. If erythropoietin plays a role in lung vascular cell function, we hypothesized that it might play a similar role in respiratory epithelial cell function. We used the A549 cell, an immortal adenocarcinoma cell line that has features similar to Type II alveolar cells, to test the effect of erythropoietin (50 U/ml) on growth and ion transport activity. Erythropoietin increased total cell protein in monolayers of A549 cells by an average of 30 % (n = 6, control: 47 ± 10 vs erythropoietin 62 ± 3, mean ± SD, $p < 0.05$), an effect that was largely the result of an increase in cell number and not simply cell hypertrophy (n = 6, control: 1.5 million ± 0.4 vs erythropoietin 2.2 million ± 0.4, $p < 0.05$). To see if erythropoietin also modulated ion transport in these cells, we measured the accumulation of ^{22}Na and efflux of ^{36}Cl following a 24 h erythropoietin exposure in monolayer. Total sodium uptake was increased on average by 30% (n = 10, control: 214 ± 128 nmol/mg protein vs erythropoietin 278 ± 169, $p < 0.05$), an effect that was the result predominately of an increase in transport through amiloride-sensitive sodium channels (n = 10, control: 180 ± 122 nmol/mg protein vs erythropoietin 238 ± 159, $p < 0.05$), the sodium transport pathway linked to fluid removal in the distal airspace. In related studies we measured the effect of a 24 h exposure to erythropoietin on the rate of ^{36}Cl efflux from these same cells. Chloride efflux activity is linked to fluid secretion into the lung lumen, an important respiratory epithelial cell function before birth. We found erythropoietin decreased ^{36}Cl efflux on average by nearly 40% (n = 6, control: 3.6 ± 1.1 nmol/mg protein/min vs erythropoietin 2.2 ± 0.9, $p < 0.05$). These results suggest that erythropoietin might play a role in the growth and function of the respiratory epithelium of the distal lung. Because of the influence of erythropoietin on sodium and chloride transport, we speculate that erythropoietin might influence fluid clearance from the alveolar space.

10

INSULIN RESPONSE TO GLUCOSE PREDICTS ENDOTHELIAL DYSFUNCTION IN ADOLESCENCE

Mary M. Duck, Robert P. Hoffman. University of Cincinnati, Cincinnati Oh, Childrens Research Institute and The Ohio State University, Columbus Oh.

Background:Insulin resistance is known to affect endothelial function in adults leading to cardiovascular disease. It is important to assess adolescent changes during puberty and how they relate to carbohydrate and insulin physiology.

Design/Methods: Strain gauge plethysmography and radial arterial tonometry were used to determine the percent change in forearm vascular resistance (mean arterial pressure/forearm blood flow; reactive hyperemia; RH) following 5 min of upper arm vascular occlusion after overnight fast in 14 healthy Caucasians (age 14.3±2.0 years; BMI 19.8±3.0 kg/m^2; mean SD) The frequently sampled intravenous glucose tolerance test, IVGTT, and minimum model were used to assess insulin sensitivity (SI), glucose effectiveness (SG) and acute insulin response to glucose (AIRG).

Results: The RH was negatively correlated with AIRG ($r=-0.74$, $p=0.022$) and tended to positively correlate with log disposition index (AIRGxSI) ($r=0.62$, $p=0.056$) (figure). No relations between RH and SG or SI.

Conclusions: These results demonstrate that Caucasian adolescents with a high acute insulin response have poorer endothelial function. Thus high insulin levels may impair endothelial function and increase risk of cardiovascular disease. The relationship between DI and RH suggest that even in adolescence there is a close relationship between risk for type 2 diabetes and cardiovascular disease.