trophoblast cells occupy the anatomic location of maternal endothelial cells. We have recently shown that fetal pro-thrombotic mutations that reduce thrombomodulin (thbd) function on trophoblast cells synergize with maternal thrombophilia and precipitate fetal loss in factor V leiden mice. Fetal death is caused by a disruption in placental development. Platelet depletion, or elimination of the thrombin receptor Par4 from the mother, allows normal placentation and prevents fetal loss. These observations underscore an important role of fetal trophoblast cells in placental hemostasis, and suggest that Par4 mediated localized activation of maternal platelets at the feto-maternal interface can disrupt placentation and mediate pregnancy complications. In the current work we have addressed the role of platelets and Par4 in a different mouse model of pregnancy disorder, where a complete absence of thbd from fetal trophoblast cells disrupts placentation and causes fetal loss in mothers with normal hemostatic function. In this model the pathogenesis of placental failure requires tissue factor, yet is not associated with increased thrombosis and persists in the absence of fibrinogen. Through genetic studies, we demonstrate that in contrast to fibrinogen deficiency, Par4-deficiency of the mother, or the absence of maternal platelets, restores normal development in 1/3rd of Thbd-null embryos. We find that fetal loss in the majority of Thbd-null embryos continues in the absence of maternal platelets, does not involve complement activation or other immune cell-mediated pathogenesis, and is not ameliorated by eliminating Par1 or Par2 from trophoblast cells. The mechanisms of platelet-dependent and -independent disruption of placental development are under investigation. We conclude that maternal platelets and Par4 contribute to defective placenta formation in thbd-null embryos.

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Contrasting Roles of uPAR and uPA on the Development of AngII-Induced Abdominal Aortic Aneurysms in LDL Receptor-Deficient Mice

Haruhito A Uchida, Lisa A Cassis, Alan Daugherty; Univ of Kentucky, Lexington, KY

Objective: Chronic infusion of angiotensin II (AngII) into hyperlipidemic mice induces abdominal aortic aneurysms (AAAs). Previous studies have demonstrated increased urokinase type plasminogen activator (uPA) expression in AAAs co-localized with macrophages. Furthermore, deficiency of uPA attenuates the development of AngII-induced AAAs in apoE-/- mice. The aim of these studies was to define the role of uPA receptors (uPAR) in the development of AngII-induced AAAs. *Methods and Results:* To determine the role of macrophage expression of uPAR, male LDL receptor -/- mice were irradiated and repopulated with bone marrow derived cells from uPAR +/+ (n=15) or -/- (n=16) mice. Five weeks after transplantation, mice were fed a modified diet (21% wt/wt milk fat; 0.15% wt/wt cholesterol). Mice were infused subsequently with saline or AngII (1,000 ng/kg/min) for 4 weeks. The uPAR genotype of bone marrow-derived cells had no effect on plasma cholesterol concentrations, lipoprotein-cholesterol distributions, or systolic blood pressure. Deficiency of uPAR on donor cells also had no effect on the maximal width of the abdominal aorta or incidence of AngII-induced AAAs (+/+ = 56%, -/- = 47%). To determine whether uPAR deficiency in other cells influenced AAA development, AngII was infused into male LDL receptor -/- that were either wild type (n=21) for uPAR or had a whole body deficiency (n=17). Whole body uPAR deficiency had no effect on any parameter measured, including incidence of AAAs (+/+ = 54%, -/- = 50%). Saline-infused mice had no AAAs. Thus, we were unable to discern any role of uPAR in AngII-induced AAAs. To determine whether we could reproduce the previous study using uPA deficient mice, we infused male LDL receptor -/- mice that were either +/+ or -/- for uPA. Contrary to the protective effect noted in a previous study, uPA deficiency increased the incidence of AAA (+/+ = 63%, -/- = 100%), and greatly augmented death due to aortic rupture (+/+ = 0%, -/- = 66%). *Conclusion:* We were unable to discern any effect of uPAR deficiency on the development of AngII-induced AAAs or atherosclerosis. Contrary to a previous report, uPA deficiency led to augmented vascular pathology during infusion of AngII in LDL receptor -/- mice.

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Increased Sensitivity to Atherosclerosis in ApoE+/- Mice Is Induced by Maternal ApoE Status Rather than Hypercholesterolemia

Fanneke E Alkemade, Adriana C Gittenberger-de Groot, J C VanMunsteren, Shirley Vis, Leiden Univ Med Cntr, Leiden, Netherlands; Louis M Havekes, TNO Quality of Life, Leiden, Netherlands; Ko Willems van Dijk, Marco C DeRuiter; Leiden Univ Med Cntr, Leiden, Netherlands

Apoe$^{+/-}$ mice are relatively insensitive for development of atherosclerosis. However, offspring from *Apoe*$^{-/-}$ mothers (*Apoe*$^{+/-}$ (M$^{-/-}$)) are more susceptible for atherosclerosis than genetically identical offspring from *Apoe*$^{+/+}$ mothers (*Apoe*$^{+/-}$ (M$^{+/+}$)). In utero the *Apoe*$^{+/-}$ (M$^{-/-}$) fetuses are exposed to maternal hypercholesterolemia (HC) and associated risk factors resulting in significantly enhanced late fetal cholesterol levels compared with *Apoe*$^{+/-}$ (M$^{+/+}$) fetuses (2.11 vs 1.54 mmol/l). In addition, a significant increase in loss of endothelial cell volume in the carotid arteries of *Apoe*$^{+/-}$ (M$^{-/-}$) fetuses was observed indicating major cellular damage. Lesion induction in the carotid artery in adult life resulted in severe neointima formation in *Apoe*$^{+/-}$ (M$^{-/-}$) offspring compared with only minor lesions in *Apoe*$^{+/-}$ (M$^{+/+}$) offspring. The *Apoe*$^{-/-}$ phenotype is characterized by HC, high oxidative stress and a pro-inflammatory state. In the present study we investigated fetal programming of atherosclerosis in the *Ldlr* knockout model to elucidate the role of apoE. Maternal HC significantly augmented cholesterol levels in *Ldlr*$^{+/-}$ (M$^{-/-}$) fetuses compared with *Ldlr*$^{+/-}$ (M$^{+/+}$) offspring, 2.86 vs 2.33 mmol/l. Although cholesterol levels in *Ldlr*$^{-/-}$ mothers were almost twice as high as in *Apoe*$^{-/-}$ mothers (35.73 vs 20.13 mmol/l), plasma cholesterol levels in *Ldlr*$^{+/-}$ (M$^{-/-}$) fetuses were not dramatically enhanced compared with *Apoe*$^{+/-}$ (M$^{-/-}$) offspring. In addition, increased maternal and fetal cholesterol levels were not accompanied by damage to the vascular wall in *Ldlr*$^{+/-}$ (M$^{-/-}$) fetuses. Except for endothelial cell activation, no vascular pathology was detected. Prior to cuff-induced neointima formation in the two *Ldlr*$^{+/-}$ groups in adult life, plasma cholesterol in these mice was two times higher than in *Apoe*$^{+/-}$ counterparts (19.29 vs 7.80 mmol/l). However, 4 weeks after collar placement no lesions were found. In conclusion, damage to the fetal vascular wall and priming of adult atherosclerosis as indicated by study of the *Apoe*$^{+/-}$ and *Ldlr*$^{+/-}$ model is not primarily induced by HC and oxidative stress.

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PESDA Microbubble Interactions with Injured Vascular Tissue: The Potential Role of Toll-like Receptors and Aldehyde Modified Proteins in the Initiation and Imaging of Atherosclerosis

Daniel Anderson, Michael Duryee, Feng Xie, Rajeev Anchan, Geoffry Thiele, Lynell Klassen, Thomas R Porter; Univ of Nebraska Med Ctr, Omaha, NE

BACKGROUND: Perfluorocarbon exposed sonicated dextrose albumin (PESDA) microbubbles bind to injured vascular endothelium and can be non-invasively imaged. Toll-like receptors (TLR) are regulators of innate and adaptive immune responses, are expressed in atheromatous lesions, and are involved in cell signaling and initiation and progression of atherosclerosis. TLR's have also been shown to bind malondialdehyde-acetaldehyde (MAA) modified proteins. In this study, we sought to determine whether PESDA and MAA modified PESDA (MAA-PESDA) could be utilized to image pre-atherosclerotic and angioplastied aortic tissue by non-invasive methods. Endothelial inflammatory markers was also evaluated. **METHODS:** Myocardium and abdominal aortic tissues in non-atherosclerotic Sprague-Dawley (SD) rats (n=5) and in 1% cholesterol fed JCR:LA-cp (leptin receptor -/-) atherosclerotic diabetic rats (n=5) were analyzed by: 1) Real-time RT-PCR for complement proteins (C1q, C3, C4a and CR 1), CRP, eNOS, ICAM and IL-6; 2) Immunohistochemistry staining for ICAM and eNOS; and, 3) Western blotting for MAA-modified proteins. PESDA and MAA-PESDA microbubbles were utilized to assess binding to TLR expressing Chinese Hamster Ovary (CHO) cells and to aortic endothelium of SD and JCR rats. Ultrasound, MRI and scanning electron microscopy were utilized to evaluate PESDA binding. **RESULTS & CONCLUSIONS:** Real-time RT-PCR of the aorta from JCR and SD rats, demonstrated an up-regulation in the mRNA from JCR rats of the inflammatory mediators C1q, C3, C4a, CR1, CRP, eNOS, ICAM, and IL-6. In the myocardium of JCR rats, only CRP mRNA was up-regulated, whereas C1q, C4a, CR1, eNOS, and IL-6 were down-regulated. Western blotting of aortas from JCR rats detected the presence of MAA-modified proteins that were not detected in SD rat aortas. PESDA and MAA-PESDA were incubated in the presence of normal human serum and were shown to bind to CHO cells which express TLR2 and TLR4, but not control cells. Therefore, these studies demonstrate that: 1) MAA-modified proteins may play a role in atheromatous plaque development; 2) PESDA and MAA-PESDA bind to TLR's and may serve as surrogate markers of TLR's; and, 3) The MAA-PESDA construct can potentially be used to image early pathophysiologic processes.

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Pressure Drop Coefficient Effectively Distinguishes Between Epicardial and Microvascular Dysfunction

Koustubh D Ashtekar, Abhijit Sinha Roy, Univ of Cincinnati, Cincinnati, OH; Edward Kim, Cincinnati Children's Hosp, Cincinnati, OH; Tarek Helmy, Mohamed Effat, Saeb F Khoury, Eric Schneeberger, Univ of Cincinnati, Cincinnati, OH; William Gottliebson, Cincinnati Children's Hosp, Cincinnati, OH; Rupak K Banerjee; Univ of Cincinnati, Cincinnati, OH

Introduction: An alternative diagnostic index, pressure drop coefficient (CDP) was developed to distinguish severity of epicardial coronary stenosis from microvascular dysfunction. This index is the hemodynamic endpoint that integrates pressure gradient (dp) and square term of hyperemic blood velocity (APV). The ratio of anatomic endpoint and CDP forms the basis of our newly introduced parameter: Lesion flow coefficient (LFC). Hypothesis: We hypothesize that the CDP is an effective diagnostic parameter that distinguishes between mild and severe epicardial stenosis under normal or abnormal microcirculation. Methods: In 7 out of 14 pigs (46±3 kg), polybead microspheres were injected to disrupt microcirculation. In all pigs, angioplasty balloons were inflated to create epicardial blockage. The dp, extracted from the commonly used Fractional flow reserve (FFR) and APV were measured simultaneously by dual sensor tipped guidewire distal to the balloon at peak hyperemia, induced by vasodilator. CDP was calculated as (dp)/(0.525×APV^2) whereas LFC is given by (% area stenosis)/(CDP$_{½}$). Results: For normal microvascular function mean FFR, CDP and LFC were 0.64±0.11, 65±44 and 0.14±0.04, respectively. These changed to 0.61±0.16, 243±96, and 0.08±0.03 after injection of microspheres ($p<0.001$ for all). Compared to the FFR, CDP showed improved capability of distinguishing the status of epicardial and microvascular dysfunction with mild and severe epicardial diseases. Conclusion: CDP increased three-fold for normal to abnormal microcirculation and hence, it could be used to guide the diagnostic procedures to delineate epicardial stenosis from microvascular impairments.