

75

VASCULAR ENDOTHELIAL GROWTH FACTOR SIGNALING BLOCKADE AMELIORATES DIABETIC ALBUMINURIA IN MICE: EFFECTS ON THE KIDNEY PODOCYTE. S. Chen, A. Wang, P. Pyagay, Y.S. Kanwar, F.N. Ziyadeh, Northwestern University, Chicago, IL; Philadelphia, PA.

To investigate how the vascular endothelial growth factor (VEGF) system participates in the pathogenesis of diabetic kidney disease, type 2 diabetic db/db and control db/m mice were treated intraperitoneally with vehicle or 2 mg/kg of a pan-VEGF receptor tyrosine kinase inhibitor, SU5416, twice a week for 8 weeks. Efficacy of SU5416 treatment in the kidney was verified by the inhibition of VEGF receptor 1 phosphorylation. Glomerular VEGF immunostaining, normally increased in diabetes, was unaffected by SU5416. The primary end point of albuminuria increased ≈fourfold in the diabetic db/db mice but was significantly ameliorated (almost completely prevented) by SU5416. Correlates of albuminuria were then investigated. Diabetic thickening of the glomerular basement membrane (GBM) was prevented in the SU5416-treated db/db mice, concurrent with the amelioration of albuminuria, whereas diabetic mesangial matrix expansion remained unchanged by treatment. The density of open slit pores between podocyte foot processes, a marker that tracks well with diabetic albuminuria, was decreased in db/db diabetes but was partly increased toward normal by SU5416. Finally, podocyte-based nephrin protein, which correlates inversely with albuminuria and whose mutation is the genetic basis of Finnish congenital nephrotic syndrome, was decreased in the db/db mice by immunofluorescence but was significantly restored by SU5416. Paradoxically, the same nephrin protein measured by Western blotting was increased in diabetes, pointing toward a possible dysregulation of nephrin trafficking. Diabetic albuminuria is partially a function of VEGF receptor signaling overactivity. VEGF signaling was found to affect a number of podocyte-driven manifestations, such as GBM thickening, slit pore density, and nephrin quantity, all of which are associated with the extent of diabetic albuminuria. By impeding these pathophysiologic processes, VEGF receptor inhibition by SU5416 might become a useful adjunct to antialbuminuria therapy in diabetic nephropathy.

76

ATTENUATION OF A RODENT MODEL OF PULMONARY HYPERTENSION: A NOVEL ROLE FOR SORAFENIB, A MULTIKINASE INHIBITOR. A.A. Desai, L. Moreno, M.M. Gombert-Maitland, M. Maitland, K. Collins, S. Sammani, S. Ma, A.N. Husain, Y. Liu, L. Sam, R.M. Lang, M.J. Ratain, Y.A. Lussier, J. Garcia, The University of Chicago, Chicago, IL.

Drawing from new drug discovery studies is the observation that severe pulmonary hypertension (PH) and cancer pathophysiology share common signal transduction pathways leading to abnormal smooth muscle and endothelial cell (EC) interactions and angioproliferative vasculopathy. Sorafenib (Sor), a chemotherapeutic agent in clinical trials for the treatment of renal cell cancer, is an inhibitor of multiple kinases, including Raf-1 kinase, MAPK, VEGFR-2, and VEGFR-3, genes implicated in angiogenesis, proliferation, and the inhibition of apoptosis. We therefore tested the hypothesis that Sor will attenuate the development of PH using an established rodent model of the disease. We performed two 3-week hypoxia (FiO₂ 10%) and SU5416 (a selective VEGFR-2 inhibitor known to dramatically augment hypoxia-induced PH) studies to induce PH in Dahl salt-sensitive rats (SS). Rat groups were normoxia/vehicle (Norm), hypoxia/vehicle (H), H-Su, hypoxia/sorafenib (H-Sor), and hypoxia/sorafenib/SU5416 (H-Su-Sor). Except for Norm, all rats were kept in hypoxia, whereas the H-Su group received SU5416 at day 1 (20 mg/kg, sc) and Sor was gavaged daily (2.5 mg/kg). Echocardiography, pulmonary artery pressures (PAPs), right ventricular pressures (RVPs), and lung gene microarray analyses were assessed at 3 weeks. Our results showed that H-Su rats developed severe PH compared with Norm, rats in the H alone group had mildly elevated pressures compared with Norm, and no changes were seen in pressures, weights, or remodeling in the H-Sor or H-Su-Sor groups compared with Norm. The H-Su-Sor rats showed significant reductions in PAP (56%), RVP (55%), and RV hypertrophy (52%). Gene expression profiling data were compared with Norm using GCRMA normalization in R and SAM (> .639, MFC > 1.7). With false discovery rates (FDRs) of 5.1% and 0.7%, respectively, 356 and 293 genes were up- or down-regulated. Forty-seven of the 356 H genes were recapitulated from previous H studies in the rodent model. In addition, 45 genes were differentially expressed between H-Su and H-Su-Sor (FDR 12%), with ECM, cytoskeleton, and angiogenesis gene ontologies, and 81 genes were changed in the H and H-Su groups but not in the H-Su-Sor group. These studies suggest Sor as a powerful novel treatment in PH.

77

THE GREEN TEA POLYPHENOL (-)-EPIGALLOCATHECHIN-3-GALLATE REDUCES INFLAMMATORY RESPONSE TO LIPOPOLYSACCHARIDE AND INDUCES APOPTOSIS IN HUMAN PERIPHERAL BLOOD MONOCYTES. G.W. Dryden, H.H. Qazzaz, R. Fernandez-Bortan, C.J. McClain, M.W. Linder, University of Louisville, Louisville, KY.

Introduction: Increasing attention has focused on the therapeutic potential of green tea polyphenols owing to the anti-inflammatory, proapoptotic, and multiple other effects they have produced in a variety of mammalian cell lines and animal models of disease. (-)-Epigallocatechin-3-gallate (EGCG), a potent anti-inflammatory compound, makes up 40% of the polyphenolic fraction of green tea. EGCG has also proven to be a potent inhibitor of nuclear factor κB (NF-κB), a key regulatory factor in the control of intestinal inflammation. We investigated whether the anti-inflammatory effects of EGCG also apply to human peripheral blood monocytes. **Aim:** To evaluate the ability of EGCG to inhibit the production of proinflammatory cytokines and to induce apoptosis in human PBMCs *in vitro* in the presence or absence of lipopolysaccharide (LPS). **Methods:** PBMCs were preincubated for 1 hour in the presence of increasing concentrations of EGCG (0–125 ng/mL) prior to incubation for 16 hours with 10 ng/mL LPS. The anti-inflammatory effect of EGCG was measured by the reduction of TNF-α production, as well as other inflammation-related cytokines, in LPS-stimulated cells. The effect of EGCG on apoptosis of LPS-activated PBMCs was measured by annexin V-FITC/PI (propidium iodide) staining. UV light exposure was used as a positive control for apoptosis studies. **Results:** In the absence of EGCG, LPS increased TNF-α production from a mean of 186 pg/mL to 503 pg/mL. Pretreatment of cells with increasing concentrations of EGCG (5–125 ng/mL) proportionally reduced TNF-α production by 20%, 33%, 38%, and 45% at 5, 25, 50, and 125 ng/mL EGCG, respectively. Reductions in TNF-α production were not caused by loss of viability of cultured cells, as assessed by mitochondrial reduction of MTT. An additional indicator of viability, interferon-γ production, was unaffected by the administration of EGCG until the highest concentration was reached. EGCG did increase apoptosis in a concentration-dependent fashion from 1.5% in untreated cells to 55% at the highest tested concentration. **Discussion:** This study demonstrates the ability of EGCG to induce anti-inflammatory effects on human PBMCs and the ability of EGCG to induce apoptosis in LPS-stimulated human PBMCs. In light of the fact that tissue macrophages constitute a significant source of proinflammatory cytokines in inflammatory bowel disease (IBD), we will proceed to investigate the effects of EGCG *in vitro* and *in vivo* on peripheral blood monocytes and mucosal tissue macrophages from human subjects with IBD.

Work supported by K23 DK073750-02 (G.W.D.) and K23 AA014235 (M.W.L.).

EGCG Effects on Cytokine Production

EGCG (ng/μL)	(-)/LPS	(+)/LPS	MTT
<i>TNF-α Production (pg/mL)</i>			
0	187	504	100
5	186	339	98
25	172	342	95
50	128	332	93
125	65	111	92
<i>IFN-γ Production (pg/mL)</i>			
0	12	13	100
5	11	14	98
25	8	12	95
50	12	10	93
125	6	7	92

78

GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR PRODUCTION BY HUMAN KERATINOCYTES IN THE PRESENCE OF PIMECROLIUMS. M. Frieri, A. Capetandes, Nassau University Medical Center, East Meadow, NY.

Rationale: Granulocyte-macrophage colony-stimulating factor (GM-CSF) is overproduced by human keratinocytes (HKs) in chronic lesions of atopic dermatitis (AD) (Boguniewicz M. JACI 2006;117 Suppl:475–80). Increased concentration of GM-CSF could explain persistent inflammation with activation of dendritic cells. Additionally, *Staphylococcus aureus* exacerbates chronic lesions in AD by secreting superantigens, stimulating marked activation of T cells, macrophages, and eosinophils. **Methods:** Confluent monolayers of HKs (ATCC CRL 2309) were stimulated with a cytomix composed of 100 mg/mL staphylococcal enterotoxin B and 10 U/mL rhIL-1β as determined by dose-response studies. HKs were stimulated with cytomix ± 10⁻⁶ to 10⁻¹⁰ M pimecrolimus (dosing range determined by dose-response studies) for 24 hours in serum-free media supplemented with insulin-transferrin-selenium (SFM) using standard cell culture conditions. The conditioned media was assayed for GM-CSF secretion by ELISA at the end of 24 hours of incubation. The HKs were analyzed for cell viability and proliferation using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] incorporation assay. The GM-CSF data (pg/mL) were normalized to the MTT absorption data (A550 nm). Parametric data are expressed below as the fold change mean ± 2 SD of normalized GM-CSF secretion by HKs incubated with cytomix plus drug relative to control (cytomix without drug) and analyzed by the two-tailed t-test (α = 0.05). Power for all studies was > 0.6. **Results:** HKs showed > 95% viability by the MTT incorporation test in the presence of SFM. HK viability decreased to 50 to 70% in the presence of cytomix. The cytomix stimulated normalized GM-CSF secretion over SFM by 2.0 ± 0.9-fold. 10⁻⁶ to 10⁻¹⁰ M pimecrolimus did not improve the cell viability relative to cytomix; 10⁻⁶ and 10⁻⁷ M pimecrolimus in the presence of cytomix decreased normalized GM-CSF secretion by 2.0 ± 0.4 and 4.2 ± 3.0-fold, respectively, relative to cytomix alone (p < .05); 10⁻⁸ to 10⁻¹⁰ M pimecrolimus showed no statistical differences in the percent change in normalized GM-CSF secretion relative to cytomix alone. **Conclusion:** 10⁻⁶ and 10⁻⁷ M pimecrolimus inhibited cytomix-induced increased normalized GM-CSF secretion by HKs in SFM.

Funding: Novartis Pharmaceuticals Corporation.

79

PRIMARY AND SECONDARY PREVENTION OF GESTATIONAL DIABETES BY METFORMIN THERAPY DURING PREGNANCY IN WOMEN WITH POLYCYSTIC OVARY SYNDROME. C.J. Glueck, J. Pranikoff, D. Aregawi, P. Wang, Jewish Hospital of Cincinnati, Cincinnati, OH.

We prospectively assessed whether a metformin diet safely provided primary and secondary prevention of gestational diabetes (GD) in 142 nondiabetic women with polycystic ovary syndrome (PCOS) who had at least one livebirth pregnancy. Women with BMI < 25 kg/m² or ≥ 25 kg/m² were instructed in 2,000 or 1,500 calorie/d, respectively, high-protein (26%), low-carbohydrate (44%), low-fat (30%) diets, with P/S ratio = 2/1. Metformin, targeted to 2 to 2.55 g/d, was given preconception and through pregnancy. On metformin, GD developed in 12 of 171 (7.0%) pregnancies in the 142 women. Of the 142 women, 46 had 1 or more previous livebirth pregnancies (n = 62) without metformin, developing GD in 19 (30.6%). Subsequently, on metformin, these 46 women had 49 livebirth pregnancies, developing GD in 6 (12.2%), McNemar's S = 11.3, p = .0008. Fifteen women without metformin had 19 GD pregnancies. In their subsequent pregnancies on metformin, 10 of these 15 women had 11 pregnancies without GD and 5 of the 15 women had 5 pregnancies with GD. Metformin appears to protect against GD and, speculatively, later type 2 diabetes in PCOS, by reducing insulin resistance and protecting pancreatic beta cells' reserve during pregnancy, when both insulin resistance and insulin secretion are increased. Metformin during pregnancy in women with PCOS may provide primary and secondary prevention against GD.

80

ENDOTHELIAL SIGNALING BY SIMVASTATIN IS MEDIATED BY INTEGRIN β4. J.R. Jacobson, W. Chen, J.N. Garcia, University of Chicago, Chicago, IL.

The statins, a class of HMG CoA-reductase inhibitors, are used clinically for their ability to lower serum cholesterol levels and reduce the morbidity and mortality associated with coronary artery disease. However, not all of their clinical benefits can be attributed to their lipid-lowering properties. We have previously reported the direct effects of simvastatin on endothelial cells (ECs), including the up-regulation of integrin β4 gene and protein expression. Whereas the role of integrins has been described with respect to a variety of pathways, including both Rho GTPase and MAP kinase signaling, the role of integrin β4 in EC signaling is poorly characterized. In this study, human pulmonary artery ECs treated with simvastatin (5 μM, 16 hours) demonstrated a significant increase in both cytosolic Rac activation (Rac-GTP) and integrin β4 protein expression (95% and 50%, respectively). Compared with controls,