D021

SILDENAFIL INDUCED-REVASCULARIZATION OF RAT HINDLIMB INVOLVED ARTERIOGENESIS THROUGH PI3K/-AKT AND ENOS ACTIVATION

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Hypoxia and inflammation play a major role in the revascularization following ischemia. Sildenafil inhibits phosphodiesterase-5, increases intracellular cGMP content and thus induces vasodilation. Sildenafil also induces neovascularization following ischemia but through a pathway remaining incompletely understood. Thus, we investigated the consequences of a long-term sildenafil treatment on post-ischemic revascularization.

The left femoral artery was ligated in sildenafil (25 mg/kg per day)-treated rats. Vascular density and blood flow were evaluated in both legs and expressed as left/right leg (L/R) ratio. After 7 or 21 days, the L/R ratio was 33¡Ó2% and 54¡Ó9%, respectively in control rats. Sildenafil increased significantly the ratio to 47¡Ó4% and 128¡Ó11%, respectively. A neutralizing VEGF antibody significantly decreased vascular density (x0.48-fold) in control rats without affecting density in sildenafil-treated animals. Blood flow and arteriolar density followed the same pattern. In the ischemic leg, HIF1*f*Ñ and VEGF expression level increased in control, not in sidenafil ¡Vtreated rats, suggesting that sildenafil might not preferentially induce angiogenesis. PI3-kinase, Akt and eNOS were activated after 7 days with a down-regulation after 21 days. Sildenafil-induced migration of endothelial cells was prevented by PI3-kinase inhibition with LY294002. Finally, sildenafil-induced rise in blood flow in mesenteric resistance arteries was associated with an increased luminal diameter (outward remodeling or arteriogenesis). This arteriogenesis was also associated with eNOS proteins activation.

Conclusion — Long term sildenafil treatment increased local blood flow and collateral arteries growth independent of VEGF but in association with activation of PI3-kinase, Akt and eNOS which might preferentially activate arteriogenesis.

D022

NATURAL CD4/CD25/FOXP3 REGULATORY T CELLS MODULATE POST-ISCHEMIC INFLAMMATORY RESPONSE: ROLE IN NEOVASCULARIZATION

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CD4+ and CD8+ T lymphocytes control revascularization after vascular occlusion. T cell activation is mediated by two major costimulatory signalings: the B7/CD28 and the CD40-CD40 ligand pathways. Interestingly, CD28 interactions with the structurally related ligands B7-1 and B7-2 are also required for the generation and homeostasis of CD4+CD25+ regulatory T cells (Treg), which actively maintain immunological tolerance to self and nonself antigens. We hypothesized that naturally arising Treg modulate the immuno-inflammatory response to ischemic injury, and subsequently vessel growth.

Ischemia was induced by right femoral artery ligation in CD28-deficient mice (n=10 per group). After 21 days of ischemia, CD28 deficiency showed a profound reduction in Treg number and upregulated post-ischemic inflammatory response and neovascularization. Similarly, injection of splenocytes isolated from CD28-/- mice in Rag1-/- mice with hindlimb ischemia increased angiographic score, foot perfusion, and capillary density by 2.2-, 2.3- and 1.1-fold, respectively, compared to PBS-injected Rag1-/- mice. These effects were associated with enhanced accumulation of CD3-positive T cells and Mac-3 positive macrophages in the ischemic leg of Rag1-/- mice treated with CD28-/- splenocytes. Interestingly, cotransfer of Treg with CD28-/- splenocytes in Rag1-/- mice abrogated activation of neovascularization induced by CD28-/- splenocytes. Inflammatory cells accumulation was also decreased in Rag1-/- transplanted with both Treg and CD28-/- splenocytes compared to mice receiving CD28-/- splenocytes only. In contrast, treatment of C57Bl/6 Wild-Type mice with an anti-CD25 antibody (PC61) markedly reduced endogenous Treg levels in blood and spleen. At day 14 of ischemia, inflammatory response and neovascularization were markedly increased in anti-CD25 treated Wild-Type mice compared to untreated mice. These results provide new insights into the immunoregulation of post-ischemic neovascularization.

D023

CHOP-10 DELETION IMPROVES NEOVASCULARIZATION AND STEM/PROGENITOR CELLS PRO-ANGIOGENIC POTENTIAL IN TYPE I DIABETIC MICE WITH HINDLIMB ISCHEMIA

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Diabetes-induced reactive oxygen species overproduction impairs neovascularization. CHOP 10 is a novel developmentally regulated nuclear protein that emerges as critical transcriptional integrator among pathways regulating differentiation, proliferation and survival. Of interest, CHOP-10 has been shown to trigger oxidative stress-induced β cells apoptosis in the setting of diabetes. Here, we analyzed the role of CHOP-10 in postnatal neovascularization and bone-marrow-derived mononuclear cells (BMC) pro-angiogenic potential in type I diabetic mice with hindlimb ischemia.

Ischemia was induced by right femoral artery ligation in C57/Bl6 animals (WT, n=8), diabetic C57/Bl6 animals (diab WT, n=8, Streptozotocin 40 mg/kg) and diabetic CHOP-10—deficient animals (diab CHOP-10 KO, n=8). Two days after ischemia, CHOP-10 mRNA and protein levels were increased by 7- ($p<0.001$) and 4-fold ($p<0.01$), respectively in ischemic muscle of WT diab compared to WT. Angiographic score, capillary density and foot perfusion were increased by 3.3- ($p<0.01$), 1.8- ($p<0.001$) and 2.2-fold ($p<0.001$)