**Impact of The Protective Renin-Angiotensin System (RAS) on The Vasoreparative Function of CD34+ CACs in Diabetic Retinopathy**

**Purpose:** In diabetes, the impaired vasoreparative function of circulating angiogenic cells (CACs) is believed to contribute to the progression of diabetic retinopathy (DR). Accumulating evidence suggests that the protective arm of renin-angiotensin system (RAS) “ACE2/Angiotensin-(1-7)/Mas” plays an important role in restoring the function of diabetic CACs. We examined the protective RAS in CACs in diabetic individuals with different stages of retinopathy.

**Methods:** Study subjects (n=43) were recruited as controls or diabetics with either no DR, mild non-proliferative DR (NPDR), moderate NPDR, severe NPDR or proliferative DR (PDR). Fundus photography and fluorescein angiograms were analyzed using Vessel Generation Analysis (VESEG) software in a cohort of subjects. CD34+ CACs were isolated from peripheral blood of diabetics and control subjects. RAS gene expression in CACs were measured by qPCR. The vasoreparative function of CACs was assessed by their migration ability toward CXCL12 using the QCM 5μM 96-well chemotaxis cell migration assay.

**Results:** ACE2 gene is a key enzyme converting the deleterious Angiotensin II to the beneficial Angiotensin-(1-7). ACE2 expression in CACs from diabetic subjects without DR was increased compared to controls, suggestive of compensation (p=0.0437). The expression of Mas (Angiotensin-(1-7) receptor) in CACs was also increased in diabetics without DR, while being reduced in NPDR compared to controls (p=0.0002). This indicates a possible loss of compensation of the protective RAS at this stage of DR. The presence of even mild NPDR was associated with CD34+ CAC migratory dysfunction. When pretreating CACs of DR subjects with Angiotensin-(1-7) migratory ability to CXCL12 was restored (p=0.0088). By VESEG analysis, an increase in small vessel density was observed in NPDR subjects when compared with controls. These data suggest protective role of CD34+ CACs in diabetic individuals that may help maintain their vasoreparative potential. The VESEG analysis supports the presence of retinal repair in small vessels. The loss of the protective arm of RAS may predict the progression of DR.

**Conclusions:** The protective RAS axis is activated within CACs from diabetic patients with no microvascular complications. However, a possible loss of compensation of the protective RAS is observed at the stage of NPDR. CACs from the diabetic patients with moderate and severe NPDR have decreased migration toward CXCL12 compared with healthy subjects. Angiotensin 1-7 treatment improved the migration function of CACs from severe NPDR.

**VESEG analysis helps to interpret the presence of retinal repair in small vessels.**

**Abstract**

**Purpose:** To examine the role of CACs in diabetic retinopathy (DR) progression. To investigate the effects of protective RAS genes ACE2 and Mas in CACs from diabetic patients with different stages of retinopathy.

**Methods:** Study subjects (n=43) were recruited as controls or diabetics with either no DR, mild non-proliferative DR (NPDR), moderate NPDR, severe NPDR or proliferative DR (PDR). Fundus photography and fluorescein angiograms were analyzed using Vessel Generation Analysis (VESEG) software in a cohort of subjects. CD34+ CACs were isolated from peripheral blood of diabetics and control subjects. RAS gene expression in CACs were measured by qPCR. The vasoreparative function of CACs was assessed by their migration ability toward CXCL12 using the QCM 5μM 96-well chemotaxis cell migration assay.

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