EUGENE AND MARILYN GLICK EYE INSTITUTE
INDIANA UNIVERSITY School of Medicine

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

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Abstract

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.

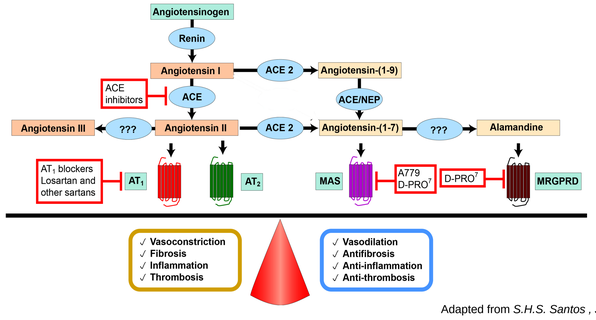
Methods: Study subjects (n=43) were recruited as controls or diabetics with either no DR, mild non-proliferative DR (NPDR), moderate NPDR, severe NPDR or proliferative DR (PDR). Fundus photography and fluorescein angiograms were analyzed using Vessel Generation Analysis (VESGEN) software in a cohort 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=0.0008). By VESGEN analysis, an increase in small vessel density was observed in NPDR subjects when compared with the controls.

Conclusions These data suggest the protective RAS axis within diabetic CACs may help maintain their vasoreparative potential. The VESGEN analysis supports the presence of retinal repair in small vessels. The loss of the protective arm of RAS may predict the progression of DR.

Background

- Endothelial dysfunction is an essential pathological change in the process of diabetic retinopathy
- CACs play a vital role in endothelial repair and new vessel growth by homing to the injured vasculature and providing paracrine factors
- In diabetes with microvascular complications, CD34⁺ CACs are dysfunctional
- The protective renin-angiotensin system (RAS) plays an important role in restoring the function of diabetic CACs



Adapted from *S.H.S. Santos , J.M.O. Andrade Peptides 59 (2014) 34-41*

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)
- CD34⁺ CACs were isolated from the peripheral blood mononuclear cells by using the EasySep™ human CD34 positive selection kit
- RAS gene expression levels were measured by qPCR
- Migration function of CACs was analyzed by measuring their ability to migrate towards CXCL12 using the QCM 5μM 96-well chemotaxis cell migration assay.
- Fundus photography and fluorescein angiograms were analyzed using Vessel Generation Analysis (VESGEN) software in a cohort of subjects

Results

➢ **Characteristics of Control and Diabetic Individuals**

	Control	Diabetes			
Number	13	30			
Gender (M/F)	6/7	13/17			
Age	39±13	60±11			
HbA1C	4.8	8.6±2.0			
Retinopathy	-	25			
		Mild NPDR 7	Moderate NPDR 12	Severe NPDR 3	PDR 3
Neuropathy	-	7			
Nephropathy	-	5			
Hypertension	1	25			
Hypercholesterolemia	1	16			

➢ **Activation of Protective RAS Genes in CACs from Diabetic Individuals with No Diabetic Retinopathy**

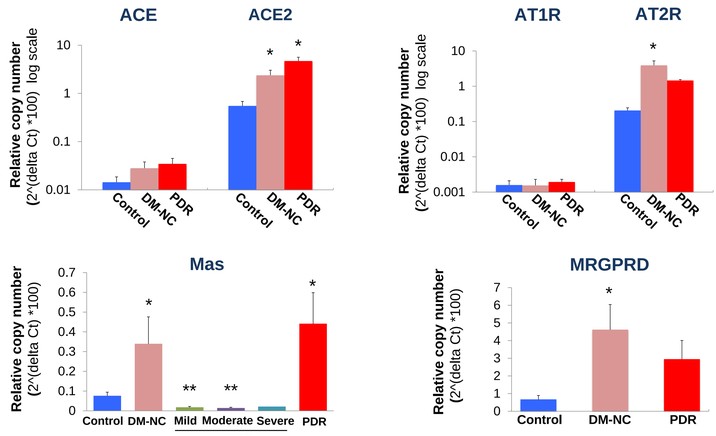


Figure1. mRNA levels of RAS genes within CACs. * P < 0.05 Compared to control; ** P<0.05 Compared to DM-NC

➢ **Ang 1-7 Treatment Enhanced The Migration of Impaired CACs toward CXCL12 in Severe NPDR**

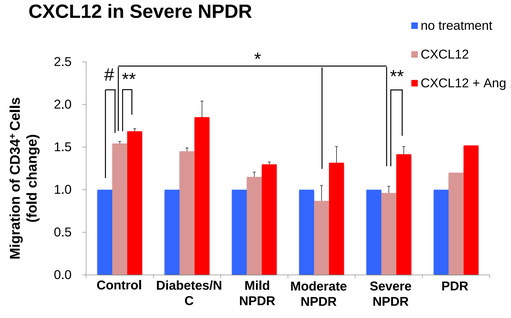


Figure2. Effects of Ang-(1-7) on migration of CD34⁺ cells toward CXCL12. (*p<0.05-compared to control, **p<0.05 compared to CXCL12 group, #p< 0.05 compared to no treatment group)

Results

➢ **Alamandine Improves The Migratory Ability of CACs from Healthy Control**

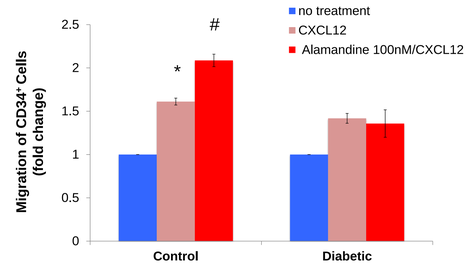
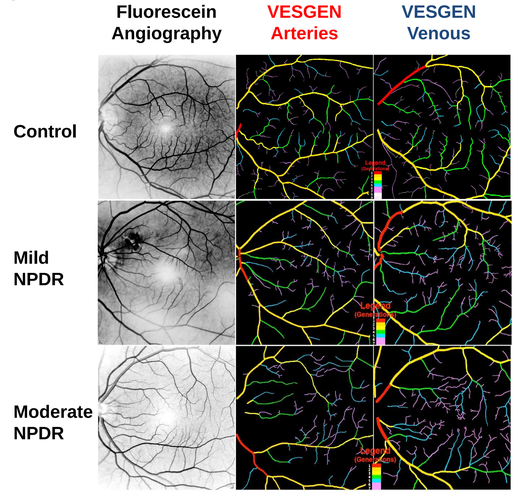


Figure3. Effects of Alamandine on migration of CD34⁺ cells toward CXCL12. *p<0.05 compared to no treatment group; #p<0.05 compared to CXCL12 group.

➢ **Vascular Changes of Retinopathy by Vessel Generation (VESGEN) Analysis**



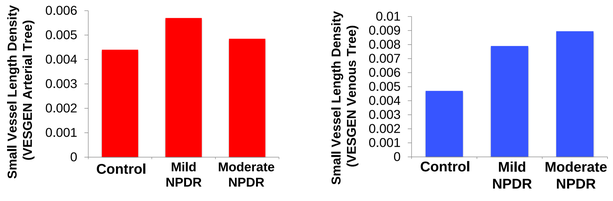


Figure4. Vascular Generation Analysis of Different Stages of Retinopathy.

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.
- The VESGEN analysis helps to interpretate the presence of retinal repair in small vessels.