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Role of Tissue Renin-angiotensin System and the Chymase/angiotensin-(1-12) Axis in the Pathogenesis of Diabetic Retinopathy

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Abstract

Diabetic retinopathy (DR) is a major diabetes complication and the leading cause for vision loss and blindness in the adult human population. Diabetes, being an endocrinological disorder dysregulates a number of hormonal systems including the renin angiotensin system (RAS), which thereby may damage both vascular and neuronal cells in the retina. Angiotensin II (Ang II), an active component of the RAS is increased in diabetic retina, and may play a significant role in neurovascular damage leading to the progression of DR. In this review article, we highlight the role of Ang II in the pathogenesis of retinal damage in diabetes and discuss a newly identified mechanism involving tissue chymase and angiotensin-(1-12) [Ang-(1-12)] pathways. We also discuss the therapeutic effects of potential RAS inhibitors targeting blockade of cellular Ang II formation to prevent/protect the retinal damage. Thus, a better understanding of Ang II formation pathways in the diabetic retina will elucidate early molecular mechanism of vision loss. These concepts may provide a novel strategy for preventing and/or treating diabetic retinopathy, a leading cause of blindness worldwide.

Keywords

Angiotensin II; chymase; diabetic retinopathy; neurodegeneration; oxidative stress; renin-angiotensin system; retina

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CONFLICT OF INTEREST

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1. INTRODUCTION

Diabetic retinopathy (DR) is the most common microvascular complication in diabetic subjects and is the leading cause of visual loss and blindness among working adults worldwide. DR develops within 20 years in most patients with Type 1 diabetes and about half of Type 2 diabetes after the onset of diabetes. Globally, the epidemic of diabetes is increasing rapidly and based on the most recent estimate, 415 million have some form of diabetes, suggesting a growing concern of DR [1]. According to the World Health Organization, the prevalence of DR is about 30% among diabetic subjects and the number of people at risk of vision loss is predicted to be double in the next 30 years [2]. The clinical signs of the disease in the retina include blood vessels swelling, leakage, and neovascularization which lead to severe vitreous hemorrhage and/or retinal detachment compromising vision loss. Before any clinical signs of vascular damage, neuronal components of the retina have been found to be compromised with retinal function deficit, suggesting neurodegeneration might be an initiating factor leading to vascular damage in DR [3, 4]. Cellular, molecular and biochemical analyses of diabetic retina revealed that neurovascular damage is caused by a number of mechanisms including oxidative stress, apoptosis, neurodegeneration and inflammation that lead to DR [5, 6].

The prevalence and incidence of DR increase with diabetic duration, and worsen with poor control of hyperglycemia and blood pressure (hypertension) according to two well-known clinical trials: Diabetes, Control and Complications Trial (DCCT) [7], and the UK Prospective Diabetes Study (UKPDS) [8]. Hypertension has been widely recognized as a potential risk factor for the damage of vasculature in diabetic induced complications including retinopathy and nephropathy. In diabetes, the components of the renin angiotensin system (RAS) are found to be increased both systemically and locally in several tissues including the retina. Angiotensin II (Ang II), the principal component of the RAS regulates blood pressure, body fluid, electrolytes balance and influences homeostasis both systemically and at cellular levels [9]. However, elevated Ang II levels in diabetic retina plays a pathogenic role in activating inflammation, oxidative stress, neurodegeneration and endothelial dysfunction, which may lead to DR [10, 11].

Past studies had concluded that the biochemical mechanisms involved in Ang II formation consisted of a linear sequential processing of the angiotensinogen substrate into angiotensin I (Ang I) by renin which was then acted upon by angiotensin converting enzyme (ACE) to generate Ang II. The singularity of this biotransformation process was first challenged by Ferrario and collaborators with the discovery of an alternate processing of Ang I into angiotensin-(1-7) [Ang-(1-7)] which acted as a potent endogenous Ang II antagonist [12]. The more recent characterization of a shorter form of the Ang II-forming substrate angiotensin-(1-12) [Ang-(1-12)] has now demonstrated a more complex and distinctly discriminative pathway for the generation of the active Ang II and Ang-(1-7) hormones both upstream and downstream of Ang I [13]. Studies demonstrating a direct formation of Ang II from Ang-(1-12) by chymase brought to the forefront past studies which implicated chymase rather than ACE as the major enzyme accounting for processing Ang I into Ang II as depicted in Fig. (1) [14].

Chymase, another RAS enzyme primarily expressed in various tissues and cells converts Ang I into Ang II at 20-fold higher rate than ACE [15]. Ang II is the major effector peptide which acts through RAS receptors, type 1 (AT1R) and type 2 (AT2R). Binding of Ang II to AT1R activates various downstream cellular signaling pathways which subsequently stimulates the cellular factors [16–19]. The functional and biological effect mediated through AT2R is still unclear. However, activation of the AT2R has been documented to oppose the AT1R-mediated actions. Ang II is further cleaved by ACE2 into Ang-(1-7). Ang-(1-7) is a protective molecule which opposes the pathological effects of Ang II *via* its Mas receptor (MasR).

2. ROLE OF RAS COMPONENTS IN DIABETIC RETINOPATHY

Clinical and experimental studies have suggested that abnormalities of the RAS may play a significant role in the progression of the diabetic retinopathy, presumably through local changes in the blood flow and the production of Ang II primarily by ACE. Besides circulating RAS, this system is also present locally in various eye tissues including retina and occurs independently at the tissue level [20, 21]. Components of RAS are most abundantly expressed both in the neuronal as well as vascular cells of the retina. RAS genes expressions have also been detected in the various eye tissues [22, 23]. Rodents studies suggest that components of RAS including prorenin, angiotensinogen, Ang II, ACE, ACE2, AT1R are increased in the retina with diabetes [24–27]. An increased level of Ang II and other RAS substances have been detected in vitreous pool of proliferative diabetic retinopathy patients compared to non-diabetic subjects [28]. Further, it has been found that the RAS components are produced locally in the tissues and not filtered from the circulation [22, 26]. Numerous studies have shown that RAS plays a pivotal role in the progression of retinal disease, presumably through local changes in RAS level by increasing the Ang II production [24, 29, 30]. They suggested that these local RAS factors, especially Ang II formation are the major source of pathophysiological action in damaging both neuronal and vascular components of the diabetic retina.

3. RAS AND VASCULAR DAMAGE IN DIABETIC RETINA

Retinal microvascular cells play important roles in the regulation of capillary tone and retinal homeostasis [31, 32]. Ang II impacts the retinal microvasculature by damaging both pericytes and endothelial cells [33]. Ang II causes a decrease in pericyte viability by increasing apoptosis which thereby uncouples them from the vasculature to initiate the generation of DR [33–35]. In addition, Ang II induced several biochemical abnormalities in vitreous of humans with proliferative diabetic retinopathy including increased vascular endothelial growth factor (VEGF), deposition of advanced glycation end products (AGEs), matrix metalloproteinase-9 (MMPs) and collagen level [36–40]. AGEs (an important mediator of diabetes-related vascular injury) is produced and deposited as a result of prolonged hyperglycemia. In animal model, it has been observed that exogenous administration of AGEs *in vivo* promotes atherosclerosis, whereas chemical degradation of AGEs or inhibition of AGE formation decreases microvascular and macrovascular diabetic complications.

In diabetic patients, these vasoactive factors (VEGF, AGEs, MMPs and collagen level) are induced and have been shown to be associated with retinopathy. Studies in diabetic rodents have indicated that ACE inhibitors and AT1R blockers reduced retinal microvascular damage with reductions in vascular leakage, decreased formation of acellular capillaries, and decreased expression of angiogenic factors such as VEGF [41–43]. Retinal leukostasis and the upregulation of adhesion molecules are also reduced with ACE inhibition and AT1R blockade [41, 44, 45]. Thus, increased levels of Ang II is critical for vascular damage in diabetic retina. New approaches to combat Ang II formation or blocking its receptors at an early disease stage may prevent the damages of retinal cells and vessels; thereby may ameliorate the progression of DR.

4. RAS AND NEURONAL DAMAGE IN THE DIABETIC RETINA

A large number of studies show that several prominent neurotrophic factors (such as brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), VEGF, *Pigment epithelium-derived factor* (*PEDF*), apoptotic markers (Caspase-3/9) and oxidative stress parameters glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase have been altered in the retina early after the onset of diabetes [46–52]. These altered factors play a significant role in damaging neuronal cells in the retina that causes the microvascular damage later in DR. In the neural retina, ganglion and Muller cells have been found to express Ang II, AT1R and AT2R [53, 54]. AT1R is expressed in synaptic vesicles in the inner retina which is critical for neurotransmission [54]. Few studies suggest that diabetes induced Ang II activates NADPH oxidase *via* AT1R stimulation and produces reactive oxygen species, subsequently inducing retinal ganglion cell impairment and death in the diabetic retina [55–58]. AT2R is expressed mostly in the neuronal component of the retina compared to vasculature both in human and rodents model of diabetic retina [30, 53]. In addition, an increased expression level of AT2R was found in diabetic retina [59]. However, the role of AT2R is not yet fully understood in diabetic retina, but some studies suggest its beneficial effects towards cell survival and regeneration in neuronal tissues [60, 61].

Numerous studies reported that an increased level of Ang II is implicated not only in vascular but also in neuronal damage in diabetic retina. Previously, an increased level of AT1R is reported in diabetic retina which resulted in the impaired neuronal function and the AT1R blocker telmisartan suppressed the impaired retinal function [54, 62]. In animal model of glaucoma, AT1R blocker treatment resulted in neuroprotection against retinal ganglion cell loss [63]. In our recent study beneficial effects towards reduction of neurotrophic factors and oxidative stress in the diabetic rat retina were achieved with telmisartan, a drug possessing strong AT1R affinity [64]. The ameliorated level of oxidative stress by the drug may induce an increase in the level of neurotrophic factors and thereby decrease apoptosis in the diabetic retina. In addition, other studies suggested beneficial effects of telmisartan in several brain diseases including the inhibition of cognitive function in the hippocampus of hypertensive rats, lowering the activity of sympathetic nervous system, and in the suppression of impaired retinal function in diabetes [62, 65, 66]. In diabetic animals, ACE inhibition and AT1R blockade have also been reported to attenuate the deficits in retinal function, indicating benefits to neuronal cells. Another study showed that AT1R blockade

reduced diabetes-related changes in the electroretinogram and attenuated the level of the synaptic vesicle protein, synaptophysin, showing the effect on retinal neurons in diabetes [62, 67]. In addition, AT1R antagonists reduced the degeneration of dopaminergic neurons [68]. Thus, ameliorating the Ang II level or signaling may protect retinal neurons and thereby retinal function in diabetic retinopathy.

5. ACE2/ANG-(1-7)/MAS RECEPTOR AXIS IN DR

Several studies showed the upregulation of ACE2/Ang-(1-7)/MasR axis which contribute to protective roles associated with complications in diabetic eyes [27, 69–71]. However, the impact of the vasoprotective axis of the ACE2/Ang-(1-7)/MasR in diabetic patient with DR remains poorly understood. Studies suggest that the protective effect of Ang-(1-7) signaling is at least in part mediated *via* stimulation of nitric oxide production and decreased production of reaction oxygen species, and increased in endothelial nitric oxide synthase [27]. Recent study shows that diabetic microvascular complications can be prevented and reversed by overexpressing the ACE2 level in diabetic rodent retina [72]. Another study reported that the inflammatory response induced by lipopolysaccharide in retinal pigment epithelium (RPE) cells were decreased with diminazene aceturate (DIZE, an ACE2 activator) treatment [73]. They also found that DIZE reduced the expression of Ang II and AT1R, whereas it increased the Ang-(1-7) level in RPE cells. Thus, strategies to enhance the ACE2/Ang-(1-7)/MasR axis in diabetic eye could be a novel therapeutic approach in DR prevention.

6. CLINICAL TRIALS TO BLOCK ANG II OR ITS RECEPTOR IN DIABETIC RETINOPATHY

Studies from clinical and experimental models suggest that the RAS is activated in diabetic retinopathy and its blockade might protect retinal damage [74–80]. Several clinical trials have been done on hypertensive diabetic people (type 1 and type 2 diabetes) to investigate the beneficial effects of antihypertensive drugs (ACE inhibitors and/or ARBs blockers) on DR reduction. We have summarized the results of major clinical trials in Table 1.

These clinical trials show modest benefits of ACE inhibitors and/or ARBs treatments on reduction of DR in hypertensive diabetic people. Further, these clinical trials data indicate that a significant number of diabetic people were unbenefited from the ACE inhibitor and ARB therapy, which suggests that the non-ACE-dependent pathway might also be responsible for the progression of DR. Although, the Ang II receptor antagonist drugs are successfully included in the management of diabetic complications, dismissal of adverse effects of these drugs exposes treated patients to progression of the disease process. Also the ACE drugs have limited efficacy to cross the cell membrane to inhibit the intracellular Ang II formation. Thus, the therapeutic approaches to prevent the progression of DR in diabetic people using the ACE inhibitor require further exploration. Our recent studies show that the chymase/Ang-(1-12) axis is primarily responsible for the generation of cellular Ang II rather than the ACE [81, 82]. These findings further suggest that the combination therapy (chymase and ACE inhibitions), compared to ACE inhibition alone, might be more beneficial to prevent the DR progression in diabetic people.

7. FORMATION OF ANG II BY TISSUE SPECIFIC CHYMASE

Chymase (EC 3.4.21.39, also known as mast cell protease) is a member of the serine class of proteases which are abundantly present in mast cells co-existing with tryptases and carboxypeptidase A [83, 84]. In non-rodent mammals only α-form of the chymase gene is found which selectively hydrolyzes the Phe^{8}-His^{9} bond of the angiotensin substrates to generate directly Ang II from both Ang-(1-12) and Ang I [85]. However, rodents have several isoforms of β-chymase in addition to the α-form [83, 86]. One of the β-chymase (rat mast cell protease-1, RMCP-1) shows a strong preference to hydrolyze the Tyr^{4}-Ile^{5} bond, also known as Ang II-destroying β-chymase [87]. Chymases are expressed in various tissues and cells, and the enzyme has been reported to convert Ang I into Ang II at much higher rate compared to ACE [15]. Thus, the classical concept of Ang II formation from Ang I by ACE both in circulation and locally in various organs is amended with the new concept that the intracellular Ang II generation primarily occurs by chymase rather than ACE.

RAS was originally thought to be in a linear pathway which sequentially processes the angiotensinogen to generate biologically active Ang peptides. Researchers believed Ang I as the sole substrate for Ang II production. This concept was changed after the discovery of a new member of the RAS family [Ang-(1-12)] [13]. The amino acid sequence of this novel RAS peptide [Ang-(1-12)] is similar to the sequence of Ang I plus -Val^{11}-Ile^{12} (in human) and -Leu^{11}-Try^{12} (in rodent) position of the C-terminus. Compared to Ang I, higher concentration of Ang-(1-12) has been detected in hypertensive rat and diseased human tissues [88, 89].

8. CURRENT CONCEPT OF THE ANG-(1-12)/ CHYMASE AXIS

The Ang II peptide [Ang-(1-8)] is the principal component of RAS which regulates various physiological processes and influences the homeostasis both systemically and at cellular levels. A growing number of studies suggest that Ang-(1-12) plays a significant role in Ang II-formation both at the tissue level and in the circulation [85, 90–93]. Our studies suggest that Ang-(1-12) might serve as an alternate pathway for the generation of Ang II, a pathway that may be of relevance in situations of suppressed renin activity or secretion, as well as functioning as an intracellular precursor for the formation of Ang II [92, 93]. Chymase plays a crucial role in the pathogenesis of organ specific tissue damage, including atherosclerosis, adverse cardiac remodeling, and arrhythmias [85, 94]. Our studies show that human chymase converts Ang-(1-12) and Ang I directly into Ang II with greater efficiency and selectivity than the ACE [85, 92]. More recently Ahmad *et al.,* (2016) demonstrated that chymase has a higher affinity for the Ang-(1-12) compared to Ang I to generate Ang II product in both human and rodents [81].

9. POSSIBLE ROLE OF CHYMASE/ANGIOTENSIN-(1-12) AXIS IN DIABETIC RETINOPATHY

Several recent studies suggest that chymase and RAS components are upregulated in several diseases including diabetes and hypertension in various tissues [85, 92, 95, 96]. Our initial study shows a higher chymase activity in vitreous fluid of diabetic patients compared to non-

diabetic subjects [97]. At present, we do not know whether Ang-(1-12) level is also increased along with chymase enzyme and responsible for increased synthesis of Ang II in diabetic retina and vitreous fluids *via* this non-canonical pathway as depicted in the Fig. (2). The enzyme responsible for the generation of Ang-(1-12) has not yet been identified, but ubiquitous distribution of the Ang-(1-12) has been detected in rodent tissues and diseased human heart [13, 92]. Growing number of studies support a role of a local RAS in retinal damage and progression of retinopathy. Recent studies by our group suggest that intracellular Ang II generation may occur primarily by chymase rather than ACE [81, 92]. Moreover, the affinity of chymase to generate intracellular Ang II is several folds higher than that of ACE in circulation. Addition of AGEs in the cultured human aortic vascular smooth muscle cells (VSMCs) induced a marked expression of chymase mRNA and protein in a time and dose-dependent manner [38]. Studies also show fibrosis and apoptosis in the macular region of the eye after chymase injected into the monkey vitreous [98]. High levels of chymase activity have been detected in the vitreous humor of patients with idiopathic macular hole [95, 96, 98]. Chymase activity was also detected in the anterior uveal tract, choroid and sclera in dog eyes and in the anterior uveal tract of monkey eyes [99]. Thus, cellular Ang II formation by chymase might also be a source for pathophysiological actions and the progression of retinopathy in diabetic subjects. However, no confirmatory studies have been done to show whether the increase in Ang II level in the diabetic eye was due to ACE or chymase. The angiotensin peptides are unable to cross the blood retinal barriers into the circulation unless there is damage in the blood vessels and leakage of blood in the retina (hypertensive retinopathy). Studies show that cellular formation of Ang II *via* intracellular chymase pathway is independent of ACE and not affected by inhibition of Ang II production by ACE inhibitors as these agents do not penetrate beyond the cell membrane.

10. NEW THERAPEUTIC APPROACH TO BLOCK CELLULAR ANG II FORMATION

Overall, this review article clearly describes the non-canonical pathway of Ang II formation *via* chymase and from Ang I upstream precursor [Ang-(1-12)], and its importance in progression of retinopathy in diabetic subjects. There is a dire need for new approaches in the management of diabetic retinopathy since current non-surgical approaches using ACE inhibitors and AT1R blockers have relatively partial success in prevention and progression of retinopathy in diabetic subjects. Expanding knowledge of the biochemical pathways including RAS offers an opportunity for novel therapies with the recognition of each new component responsible for diabetic retinopathy. Chymase-mediated Ang II-forming peptide at cellular levels from the novel Ang-(1-12) substrate has generated a new clinical concept of the role of RAS in the pathogenesis and progression of diseases. This working paradigm may resolve the previous observations that Ang II formation in cells is not prevented or partially prevented by ACE inhibitors and Ang II receptors blockers (ARBs). These advances explain the dual role of the RAS as circulating hormonal and tissue-specific system wherein the expression of angiotensin substrate serves not only autocrine/paracrine but also intracrine functions. Research focus on the intracrine synthesis of Ang II produced from a chymase-mediated cleavage of Ang-(1-12) and/or Ang I in the retina of either hypertensive-diabetic human or diabetic rodent models are subjects of further investigation. Selective

chymase inhibitors will be helpful to understand the mechanisms of retinopathy progression and also to develop the early treatment. We have evidence that the intracellular formation of Ang II *via* chymase pathway is independent and not affected by inhibition of Ang II production by ACE or ARBs [12, 100]. However, further research is needed in this regard to investigate the beneficial effects of chymase inhibition over ACE in the treatment/prevention of retinopathy and other eye diseases. Furthermore, studies are also warranted to determine whether systemic reduction of blood pressure or cellular blockage of Ang II would improve neurovascular pathology in diabetic retinopathy.

CONCLUSION

Numerous studies suggest an increased level of Ang II in the diabetic retina which may play a major role in neurovascular damage that leads to DR. Previously, Ang I was considered as the sole source of Ang II formation from sequential cleavage of angiotensinogen protein by renin to Ang I and further by ACE/chymase into Ang II. However, our recent studies in human and rodent tissues showed that Ang-(1-12), an Ang I-upstream precursor was the preferred substrate for intracellular Ang II formation *via* chymase rather than ACE. Consistent with studies reported on tissue specific increased expression and activity of chymase and Ang II content under pathological conditions, our initial studies also show an increased activity of chymase in the diabetic retina which might be responsible for the retinal damage and DR progression. Moreover, few clinical trials of ACE and ARBs inhibitors showed modest reduction of DR while a significant number of DR patients did not get benefit, suggesting that non-ACE dependent pathway might also be responsible for the progression of DR. Thus, further research is needed to investigate the potential alternate chymase-Ang II pathway and the beneficial effects of chymase inhibition over ACE and/or ARBs in the treatment/prevention of diabetic retinopathy.

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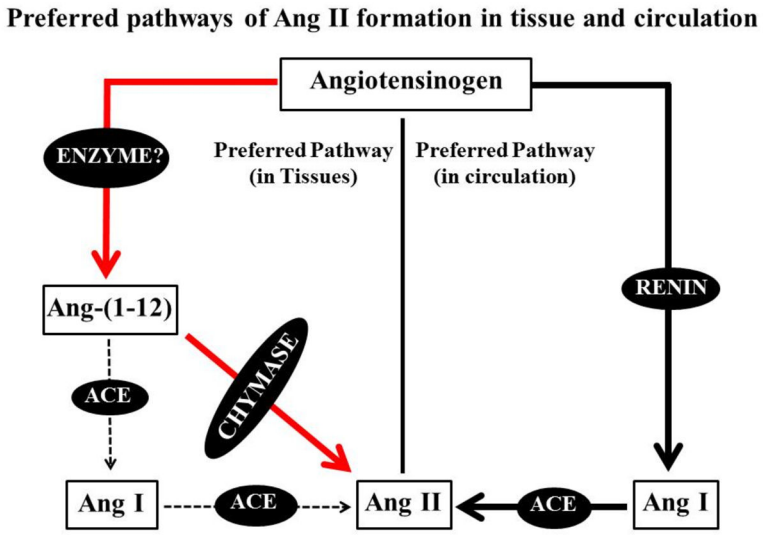

Fig. 1.

The preferred pathways of Ang II formation in tissues and circulation. In tissues, Ang II formation is primarily mediated *via* chymase and Ang-(1-12) [red solid lines] and is independent of renin whereas, Ang II formation in circulation *via* renin and ACE (black solid lines). Also in tissues, ACE is not preferred pathway to generate Ang II from Ang-(1-12)/Ang I (black dash lines).

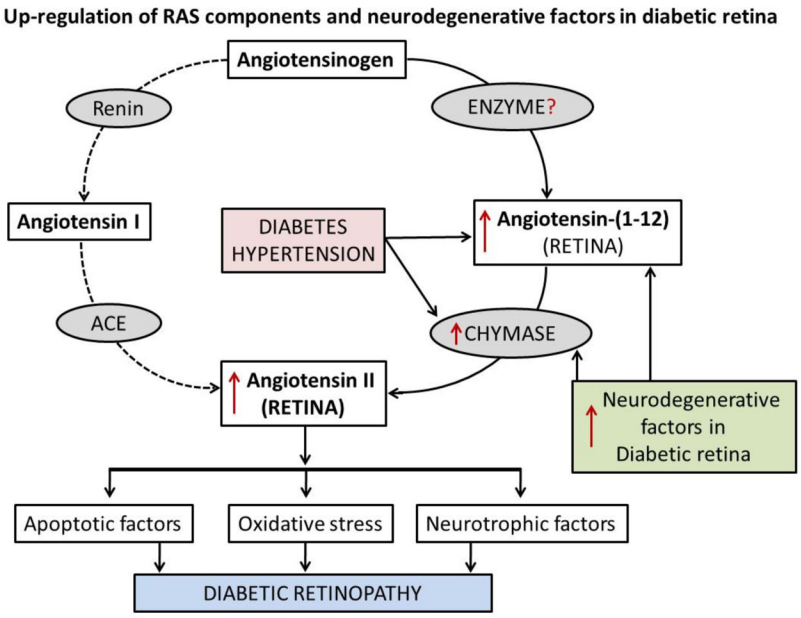

Fig. 2.

Possible pathways for the upregulation of RAS components and retinal degenerative factors in diabetic retinopathy. Progression of diabetic retinopathy *via* classical RAS pathway (dotted lines) and alternate RAS pathway of increased Ang II formation from Ang-(1-12) by chymase, influences retinal damaging factors in the diabetic retina.

Table 1

List of antihypertensive (ACE, ARBs and diuretic) drugs in clinical trials on DR.

Trials Name	Group	DR risk factor	References
EUCLID Trial			
Lisinopril (ACE inhibitor)	Insulin dependent	50% reduction	#74
DIRECT Trial			
Candesartan (AT1R blocker)	Type 1 diabetes	18% (reduction)	#75
	Type 2 diabetes	34% (reduction)	#76
RASS (5 year follow-up)			
Enalapril (ACE inhibitor)	Type 1 diabetes	65% (reduction)	#77,78
Losartan (AT1R blocker)	Type 1 diabetes	70% (reduction)	#77,78
ABCD Trial			
On blood pressure medications	Hypertensive + Type 2 diabetes	No progression of DR	#79
ADVANCE Trial			
Perindopril (ACE inhibitor) + Indapamide (antihypertensive/diuretic drug)	Hypertensive + Diabetes	Reduced cardiovascular mortality but no effect on retinopathy risk.	#80

(EUCLID: The EURODIAB Controlled trial of Lisinopril in Insulin dependent Diabetes; DIRECTEUCLID: Diabetic Retinopathy Candesartan Trial; RASS: The Renin-Angiotensin System Study; ABCD: The Appropriate Blood Pressure Control in Diabetes; ADVANCE: The Action in Diabetes and Vascular Disease Controlled Evaluation).