Effect of Renin-Angiotensin-Aldosterone System Blockade on the progression of Diabetic Kidney Disease compared to the use of antioxidants and the combined use of both of them

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Abstract: Diabetic kidney disease is a complication that occurs in some people with diabetes. It may progress to kidney failure in some cases. Among the best treatment is the aim to prevent or delay the progression of the disease. Blockade of the renin-angiotensin-aldosterone system (RAAS) can act to prevent the development and progression of diabetic kidney disease (DKD) as well as the antioxidants. It is a proven cornerstone of therapy for the prevention and treatment of diabetic kidney disease (DKD) is the use of the Renin Angiotensin blockades and antioxidants. This work aimed to study the effect of Renin-Angiotensin-Aldosterone system blockade on the progression of the kidney disease in rats compared to the use of the antioxidants and the use of both of them. Fifty white male albino rats were included in this study. The rats were divided into five groups. The first group was the control group, while group two was the diabetic and renal affected group, group three was the diabetic and renal affected group treated with Renin –Angiotensin –Aldosterone system blockade (Captopril). Group four was the diabetic and renal affected group treated with antioxidant alpha lipoic acid, while group five was the diabetic and renal affected group treated with both Renin –Angiotensin –Aldosterone system blockade (Captopril) and the antioxidant alpha lipoic acid. After one month glucose, and albuminuria were measured in urine, as well as 8-hydroxydeoxyguanosine, as an indicator of mitochondrial oxidative stress, and increased renal peroxynitrite formation. While urea and creatinine were measured in blood. Rats of groups II, III, IV, and V were sacrificed and histopathologic slides were made to show the renal tissue. Groups II, III, IV and V all showed albuminuria but group V the diabetic and renal affected group treated with both the renin angiotensin aldosterone blockade captopril with the antioxidant lipoic acid showed least proteinuria followed by group III which was treated with renin angiotensin aldosterone blockade alone then group four treated with the antioxidant lipoic acid alone and lastly group II the diabetic and renal affected non treated group. The results for the urea and creatinine were significantly better in the group treated with both the renin aldosterone system blockade and the antioxidant lipoic acid than the group treated with the renin angiotensin aldosterone blockade alone then comes the group treated with the antioxidant lipoic acid alone and the diabetic and renal affected non treated group. Conclusion: Renin-Angiotensin-Aldosterone system blockade (Captopril) may be more beneficial in delaying the progression of the diabetic kidney disease than the antioxidants while the combined use of both of them may be much more beneficial in delaying the progression of the disease.
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Keywords: ACE: Angiotensin convertase enzyme, ARB: Angiotensin receptor blocker, GFR: Glomerular filtration rate, STZ: Streptozotocin, CKD: Chronic kidney disease, DKD: Diabetic kidney disease, RAAS: Renin angiotensin aldosterone system

1. Introduction

The RAAS is currently the primary therapeutic target for the prevention and treatment of DKD. Angiotensinogen produced in the liver is converted to angiotensin I by renin, which is produced in the juxtaglomerular apparatus of the kidney. Angiotensin I is converted to angiotensin II by ACE. Angiotensin II promotes the release of aldosterone from the adrenal glands (1).

High-quality animal experimental and human studies clearly demonstrate that RAAS over activity plays a central role in the pathogenesis of DKD. Specifically, angiotensin II and aldosterone are each directly implicated in the pathogenesis of DKD (2). Angiotensin II promotes elevations in blood pressure by direct vasoconstriction and by increasing sodium reabsorption in the proximal tubule. Angiotensin II also raises intraglomerular pressure through efferent arteriolar vasoconstriction and directly stimulates tubulointerstitial fibrosis through transforming growth factor-β and other mediators (3). Aldosterone promotes renal sodium retention and elevated blood pressure by increasing sodium reabsorption in the cortical collecting duct and also directly stimulates tubulointerstitial fibrosis. In addition, recent evidence suggests a pathogenic role for renin itself (4). Renin binding to the prorenin receptor can lead to mitogen-activated protein kinase signaling (5).

RAAS can now be blocked by a number of therapeutic interventions, including direct renin inhibitors, ACE inhibitors, ARBs, aldosterone antagonists (spironolactone and eplerenone), and prorenin receptor antagonists (6). These agents reduce glomerulosclerosis, tubulointerstitial fibrosis, albuminuria, and loss of glomerular filtration rate (GFR) in animal experimental models and/or human randomized clinical trials (7).

Many studies suggest that Combined therapy with RAAS blockade may markedly ameliorates diabetic nephropathy, but although dual blockade of the renin-angiotensin-aldosterone system (RAAS) with the combination of an angiotensin-converting enzyme inhibitor (ACE-I) and angiotensin II receptor blocker (ARB) is generally well-established as a treatment for nephropathy, this treatment is not fully effective in some patients (8).

It is to be documented that the oxidative stress may be having a crucial role in the pathogenesis of diabetic nephropathy, but despite the satisfactory results for the use of the antioxidant therapy it showed conflicting results in combating diabetic nephropathy (9).

The treatment of diabetic nephropathy is not sufficient with the Renin –angiotensin blockade and so additional use of the antioxidants may be beneficial with the renin angiotensin blockers (10). They may be beneficial to the mitochondria and the antioxidant enzymes. The antioxidants can correct the oxidative imbalance and improve the oxidative stress-induced renal injury, decreasing albuminuria and fibrosis. They can also diminish the severity of hypertension, and improve the oxidative stress and ameliorate the abnormalities of antioxidant enzyme expressions and activities (11).

2. Material and methods:

Fifty white male albino rats (150 – 170 grams) were included in the present study. They were obtained from Ophthalmology Institute and were housed in wire mesh cages (40 x 25 x 15) at room temperature and maintained on normal chow and had free axis to water.

Rats were divided into five equal groups:

Group I: control group

Group II: Diabetic & renal affected

Group III: Diabetic & renal affected treated by the Renin Angiotensin system blockade Captopril at a dose of 5mg/Kg/day **intraperitoneal** injections for one month (9).

Group IV: Diabetic and renal affected treated with the Alpha lipoic acid 300 antioxidant at a dose of 50mg/day administered orally combined with food rich in alpha lipoic acid as carrots and broccli.

Group V: Diabetic renal affected treated with both the Renin angiotensin blockade captopril and the antioxidant alpha lipoic acid.

Diabetes was induced in groups II, III, IV, and V by intraperitoneal injection of Streptozotocin (STZ) (60 mg/kg).

Sigma Chemical, St. Louis, MO).

Renal affection was induced in groups II, III, IV, and V by injection of gentamycin at a dose of 20mg/Kg bodyweight/day intraperitoneally for ten days which is half the dose needed to induce renal failure **(10).**

Urine samples were collected from groups II, III, IV and V after STZ injection to detect glycosuria using glucose strips (11) to confirm the presence of diabetes, while urine samples were collected at the end of the experiment after one month from the four groups to detect albuminuria and 8-hydroxydeoxyguanosine.

Assay of the 8-hydroxydeoxyguanosine:

The urine samples were centrifuged at 2000 rpm for ten minutes and then the supernatant was measured by ELISA procedure (New 8-OHdG check, Japan institute for the control of aging Fukuroi, Sizuoka). Urine creatinine values were used to correct the daily excretion. Concentration of the urinary 8-OHdG was calculated as ng/mg of creatinine.

Blood samples were collected from groups II and III, IV, and V by the retro-orbital method after 10 days of gentamycin administration to detect urea and creatinine levels to confirm renal affection.

Blood samples were collected again at the end of the experiment from groups II and III, IV and V by the retro-orbital method to detect urea and creatinine levels.

At the end of the experiment rats of the five groups were sacrificed and kidneys were harvested for histopathologic examination as the kidney tissue were fixed in 10% buffered neutral formal saline solution then after fixation tissues were embedded in paraffin. Solid sections were cut at four micro meter and stained with haematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken and the severity of renal lesions were scored.

Statistical analysis:

The data were encoded and entered using the statistical package SPSS version 15. The data were summarized using mean, standard deviation (SD) and range for quantitative variables. comparison between studied groups was done using unpaired t-test. *P* value < 0.05 were considered statistically significant.

3. Results

The obtained results showed that the injected STZ was toxic to the pancreases of the rats and resulted in induction of diabetes in groups II, III, IV, and V. This was confirmed by the glycosuria presented in the four groups after the injection as shown in table (1).

The obtained results also showed that gentamycin was toxic to the renal tissue and resulted in affection of the renal tissue in the rats of groups II, III, IV, and V within 10 days. This was confirmed by the significant elevated levels for the urea and creatinine after the injection compared to control group.

The results showed also that treating the renal affected group with the Renin – Angiotensin blocker (Captopril) resulted in significant improvement in the results for renal function (urea creatinine albuminuria and 8 hydroxydeoxyguanosine) compared to the diabetic and renal affected non treated group ($P < 0.05$).

The results showed also that treating the renal affected group with the the antioxidant alpha lipoic acid resulted in significant improvement in the results for renal function (urea creatinine albuminuria and 8 hydroxydeoxyguanosine) compared to the diabetic and renal affected non treated group ($P < 0.05$).

Combined use of the antioxidant alpha lipoic acid and the Renin – Angiotensin blocker captopril in the diabetic and renal affected group resulted in significant improvement in the results for renal function (urea creatinine albuminuria and 8 hydroxydeoxyguanosine) compared to the diabetic and renal affected non treated group ($P < 0.05$).

The results showed non-significant improvement in the urea and creatinin in the diabetic and renal affected group treated with Renin – Angiotensin blockade captopril alone compared to the diabetic and renal affected group treated with the antioxidant alpha lipoic acid alone.

The results showed significant improvement in the urea and creatinin in the diabetic and renal affected group treated with both the Renin – Angiotensin blockade captopril combined with the antioxidant alpha lipoic acid compared to the diabetic and renal affected group treated with any of them alone ($P < 0.05$).

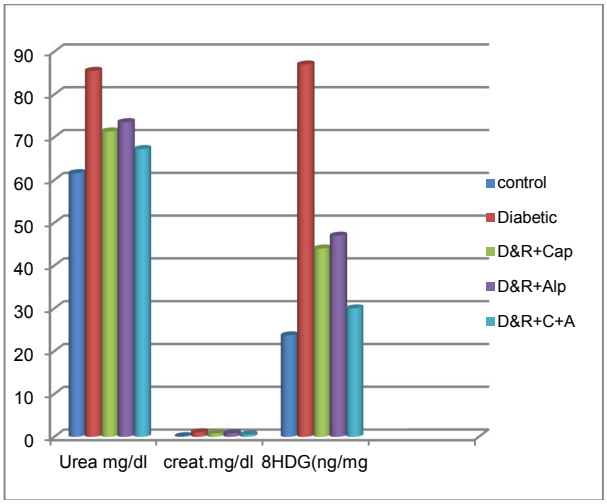
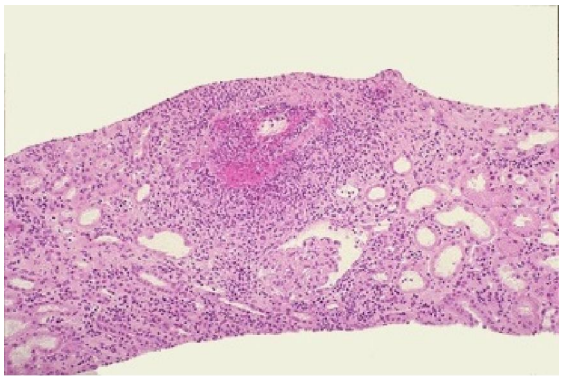


Figure (1): levels for urea, creatinine and 8 hydroxydeoxy guanosine for the control group, diabetic group, diabetic group treated with captopril only, diabetic group treated with alpha lipoic acid only, and diabetic group treated with both captopril and alpha lipoic acid together.

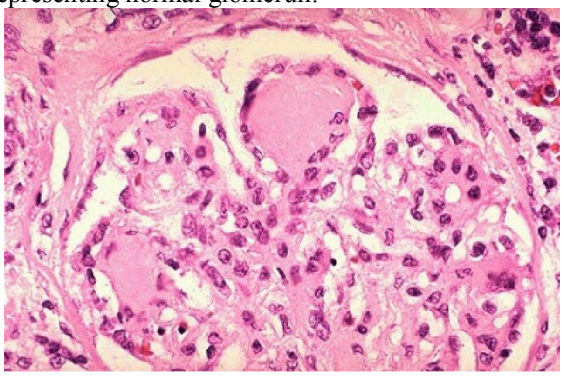
Table (1): Glucose, Urea, creatinine and albuminuria levels for the control group, diabetic & renal affected group, diabetic & renal affected group treated with Renin –Angiotensin blocker Captopril, Diabetic and renal affected group treated with antioxidant alpha lipoic acid and Diabetic and renal affected group treated with both Renin – angiotensin blockade and alpha lipoic acid antioxidant together (Mean ± SD)

	Control group	Diabetic & renal affected group	Diabetic & renal affected group treated with Renin –Angiotensin blocker (Captopril)	Diabetic & renal affected group treated with alpha lipoic acid antioxidant	Diabetic & renal affected group treated with Renin – Angiotensin blocker (Captopril) and alpha lipoic acid antioxidant
Glycusuria	**-**	+ + +	+ + +	+ + +	+ + +
Urea mg/dl	**61.6**	**85.52± 9 ***	**71.36± 8 ***	**73.52± 7 ***	**67.25± 9 ***
Creatinine mg/dl	**0.15**	**1.055± 0.14 ***	**0.75 ± 0.11 ***	**0.83± 0.43 ***	**0.55± 0.23 ***
Albuminuria	**_ _ _**	+ + + +	+ +	+ + +	+
8hydroxydeoxyguanosine ng/mg creatinine	**23.7**	**87**	**44**	**47**	**30**

* Significant (P value < 0.05)

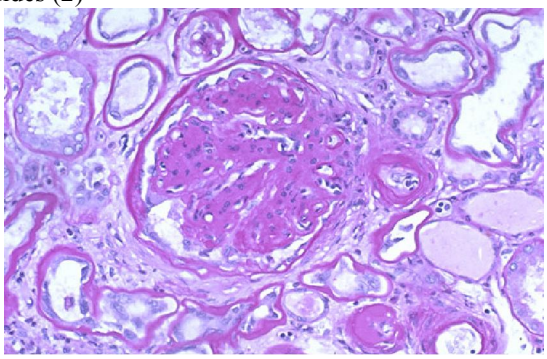


Slide (1): Histologic observation in the kidney tissue of the control group of rats showing normal structure of glomerulus surrounded by the Bowman's capsule, representing normal glomeruli.



(A)

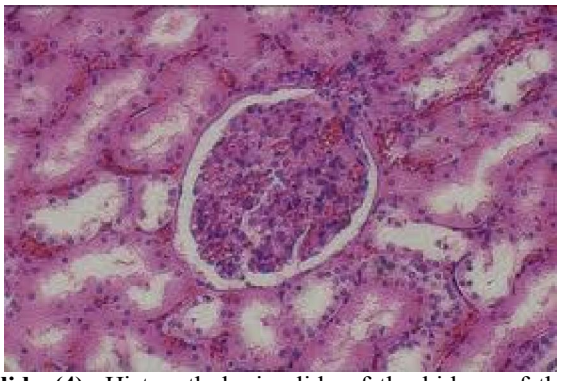
Slides (2)



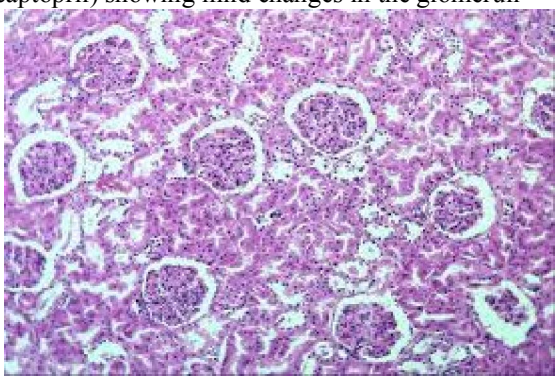
(B)

Slides (3)

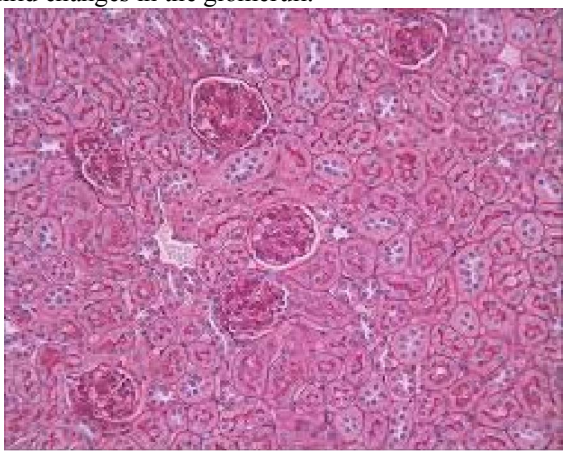
Slides (2,3): Histopathologic slides of the kidney of the rats of group II the diabetic and renal affected group not treated with angiotensin –aldosterone blockade (captopril) showing glomerulosclerosis of diabetes and there are nodules of pink hyaline material form in regions of glomerular capillary loops in the glomerulus. This is due to marked increase in mesangeal matrix from damage as a result of non enzymatic glycosylation of proteins. There are thickening of the vesicles which is consistent with chronic kidney disease with loss of renal function over time.



Slide (4): Histopathologic slide of the kidney of the rats of group III the diabetic and renal affected group treated with Renin- angiotensin –aldosterone blockade (captopril) showing mild changes in the glomeruli



Slide (5): Histopathologic slide of the kidney of the rats of group IV the diabetic and renal affected group treated with the antioxidant alpha lipoic acid showing mild changes in the glomeruli.



Slide (6): Histopathologic slide of the kidney of the rats of group V the diabetic and renal affected group treated with both Renin- angiotensin blockade (captopril) and the antioxidant alpha lipoic acid showing near normal glomeruli.

4. Discussion:

In the pathogenesis of diabetes there are two interwoven, major factors establishing the efficacy of the Renin-angiotensin blocker in reducing and

delaying the onset of diabetic nephropathy as determined by assessing albuminuria progression. these are the direct damage from hyperglycemic states, e.g., increased generation of advanced glycosylation end products (AGEs) and reactive oxygen species (ROS), and hemodynamic modifications, e.g., glomerular hyperfiltration, thrombotic microangiopathy, and shear stress.

The high serum glucose levels lead to the excessive formation of AGEs. Upon interaction with their receptor (RAGE), multiple pathways are initiated resulting in increased activity of growth factors. This, in turn, leads to abnormal expression of extra cellular matrix (ECM) proteins (e.g., multiple types of collagen, fibronectin, laminin, and many others), which causes anomalous polymerization and expansion of the ECM. Of note, excessive transforming growth factor beta one (TGF-beta1) is believed to be the primary cytokine responsible for ECM pathology because it induces the excessive production and deposition of proteins. Very importantly, AGE-related intracellular events also lead to the formation of reactive oxygen species (ROS), which exacerbate the damage.

Hemodynamic modifications—is closely related to the excessive production of AGEs. AGEs lead to perturbed interactions between the cell and matrix and changes in capillary permeability, all of which lead to vascular abnormalities. Among other cascades, AGEs lead to excessive activation of protein kinase C (PKC), which is believed to cause endothelial dysfunction and, importantly, decreased nitric oxide production. This, in turn, results in the loss of the endothelium's vasodilatory effect.

One of ACE's best-known functions is its role in the degradation of bradykinin. There is growing evidence that bradykinin, among other functions, has two effects firstly it acts indirectly as a vasodilator by producing signals leading to increased production of nitric oxide, and secondly it enhances insulin sensitivity on skeletal muscle, most notably during conditions of insulin resistance. Increased production of ACE results in decreased levels of bradykinin, leading to a decrease in nitric oxide production as well as a loss of its effect on insulin sensitivity.

There is an obvious role for the ACE in the activation of angiotensin I to angiotensin II, which allows angiotensin II to interact with its receptor and ultimately cause vasoconstriction. It turns out that hypertension (or more specifically, increased glomerular capillary pressure) greatly contributes to the acceleration of all the complications described above. Hyperglycemia is believed to sensitize target organs to blood pressure-induced damage, "most likely by activation of [RAS] with local production of angiotensin II in the kidney". Multiple kidney cells—most importantly, the podocytes—are not only involved in the activation of the local RAS, but also themselves produce angiotensin II and express angiotensin II receptors.

The STZ was shown to be toxic to the pancreatic tissue of the rats causing them to have diabetes after its injection which was confirmed by the glycosuria.

The gentamycin which was given to the rats was suggested to cause renal affection**.** This was shown in groups II and III in which the urea and creatinine showed significantly higher levels ($P < 0.005$), compared to the control group, as well as albuminuria which was absent in the control group.

Group III which was treated with the RAAS blockade (Captopril) showed significant lower levels in urea and creatinine in comparison to the non treated group ($P < 0.005$). Also this group showed less albuminuria than the non treated group.

The RAAS blockade which was injected was suggested to delay the progression of the renal disease and improve the renal function (13). The present results agreed with the results of (14) as they stated that reduction of blood pressure and proteinuria by blockade of the renin-angiotensin-aldosterone system (RAAS) has been the cornerstone of renoprotective intervention for patients with chronic kidney disease (CKD).They stated that RAS blokade has been shown to reduce proteinuria and the rate of decline of GFR in proteinuric nephropathies, including diabetic nephropathy, (15).

The results of the present study did not agree with the results of **(16)** who stated that the use of renin-angiotensin-aldosterone system (RAAS) blockade to aggressively control blood pressure in young hypertensive patients with autosomal dominant polycystic kidney disease (ADPKD) has no effect on kidney function, although it improves some cardiac and renal factors.

The results of the study agree with the results of (17) whom stated that Patients with hypertension and advanced CKD who receive therapy with ACEIs exhibit a lower risk for long-term dialysis by 6%.

In diabetic nephropathy, the excessive activation of RAAS results in progressive renal damage. RAAS blockade using angiotensin-converting-enzyme inhibitors or angiotensin-receptor blockers may be beneficial in the treatment of diabetic renal disease. Alternative RAAS-blockade strategies include renin inhibition and aldosterone blockade.

In early affection of the renal tissue and in cases of hypertension it is to be beneficial to give the patients RAAS blockade combined with antioxidant to improve the condition of the renal tissue.

The results of the study showed the antioxidant alpha lipoic acid to be beneficial in kidney disease and this was consistant with…. (18) who stated that the

antioxidants as the alpha lipoic acid are commonly used in patients with CKD, and that several studies of antioxidant supplementation, suggest that although they have failed to show any cardiovascular benefit, they may help to protect the progression of CKD (18).

The antioxidants can neutralize free radicals and other reactive oxygen species, as well as metal-binding proteins that sequester iron and copper atoms (which can promote certain oxidative reactions if free).

Antioxidants can neutralize variety of reactive oxygen species and can "recycle" vitamins C and E in the body. In people with diabetes, ALA appears to enhance insulin action and blood vessel circulation, and inhibit protein glycation (a reaction between excess glucose and protein that impairs the protein's function and forms harmful end products in the body).

Some, but not all, studies have found that treating people with Type 2 diabetes with ALA improved blood glucose control and also reduced measures of oxidative stress that may contribute to diabetes complications. In one German study, people with Type 1 and Type 2 diabetes who were given 600 mg per day of ALA for 18 months had lower levels of two markers for diabetic kidney disease than a control group of people with diabetes that did not receive the ALA (19).

References:

1. Phillips, M. I.; Schmidt-Ott, K. M. (1999). "The Discovery of Renin 100 Years Ago". *News in physiological sciences: an international journal of physiology produced jointly by the International Union of Physiological Sciences and the American Physiological Society* 14: 271–274.
2. 2-Wood, J. M.; Gulati, N.; Forgiarini, P.; Fuhrer, W.; Hofbauer, K. G. (1985). "Effects of a specific and long-acting renin inhibitorst". *Hypertension* 7(5): 797–803.
3. 3-Segall, L.; Covic, A.; Goldsmith, D. J. A. (2007). "Direct renin inhibitors: The dawn of a new era, or just a variation on a theme?" *Nephrology Dialysis Transplantation* 22 (9): 2435–2439.
4. 4-Weir MR (September 2007). "Effects of renin-angiotensin system inhibition on end-organ protection: can we do better?". *Clin Ther* 29(9): 1803–24.
5. Castrop H, Höcherl K, Kurtz A, Schweda F, Todorov V, Wagner C (April 2010). "Physiology of kidney renin". *Physiol. Rev.* 90 (2): 607–73.
6. Ferrario, C. M. (2006). "Role of angiotensin II in cardiovascular disease therapeutic implications of more than a century of research". *Journal of the renin-angiotensin-aldosterone system: JRAAS* 7 (1): 3–14.
7. Brown, M. J. (2006). Direct renin inhibition — a new way of targeting the renin system. Journal of Renin-Angiotensin-Aldosterone System, 7(2 suppl), S7-S11.
8. Hsueh, W. A.; Wyne, K. (2011). "Renin-Angiotensin-Aldosterone System in Diabetes and Hypertension". *The Journal of Clinical Hypertension* 13 (4): 224–237.
9. Ruggenenti P., P. Cravedi, and G. Remuzzi, "The RAAS in the pathogenesis and treatment of diabetic nephropathy," Nature Reviews Nephrology, vol. 6, no. 6, pp. 319–330, 2010
10. Huang W., C. Xu, K. W. Kahng, N. A. Noble, W. A. Border, and Y. Huang, "Aldosterone and TGF-β1 synergistically increase PAI-1 and decrease matrix degradation in rat renal mesangial and fibroblast cells," American Journal of Physiology, vol. 294, no. 6, pp. F1287–F1295, 2008.
11. Navaneethan S. D., S. U. Nigwekar, A. R. Sehgal, and G. F. M. Strippoli, "Aldosterone antagonists for preventing the progression of chronic kidney disease: a systematic review and meta-analysis," Clinical Journal of the American Society of Nephrology, vol. 4, no. 3, pp. 542–551, 2009.
12. Moser M, Izzo JL (2003). "Plasma renin measurement in the management of hypertension: the V and R hypothesis". *J Clin Hypertens (Greenwich)* 5 (6): 373–6.
13. Weir, M.; Bush, C.; Anderson, D.; Zhang, J.; Keefe, D.; Satlin, A. (2007). "Antihypertensive efficacy, safety, and tolerability of the oral direct renin inhibitor in patients with hypertension: A pooled analysis". *Journal of the American Society of Hypertension* 1 (4): 264–277.
14. Gao D, Ning N, Niu X, Wei J, Sun P, Hao G Proteinuria, a target for renoprotection in patients with type 2 diabetic nephropathy: lessons from RENAAL. Kidney Int. 2004 Jun; 65(6):2309-20.
15. Fisher, N. D. L.; Hollenberg, N. K. (2005). "Renin Inhibition: What Are the Therapeutic Opportunities?" *Journal of the American Society of Nephrology* 16 (3): 592–599.
16. Webb, D. J.; Manhem, P. J.; Ball, S. G.; Inglis, G.; Leckie, B. J.; Lever, A. F.; Morton, J. J.; Robertson, J. I.; Murray, G. D.; Ménard, J.; Hallett, A.; Jones, D. M.; Szelke, M. (1985). "A study of the renin inhibitor H142 in man". *Journal of hypertension* 3 (6): 653–658.
17. Gradman, A. H., & Kad, R. (2008). Renin inhibition in hypertension. [Review]. J Am Coll Cardiol, 51(5), 519-528.
18. Matter, H.; Scheiper, B.; Steinhagen, H.; Böcskei, Z.; Fleury, V. R.; McCort, G. (2011). "Structure-based design and optimization of potent renin inhibitors on 5- or 7-azaindole-scaffolds". *Bioorganic & Medicinal Chemistry Letters* 21 (18): 5487–5492.
19. Politi, A.; Durdagi, S.; Moutevelis-Minakakis, P.; Kokotos, G.; Mavromoustakos, T. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. N Engl J Med. 2010 Nov 11; 329(20):1456-62.
20. Akahane, K.; Umeyama, H.; Nakagawa, S.; Moriguchi, I.; Hirose, S.; Iizuka, K.; Murakami, K. Proteinuria as a surrogate outcome in CKD: report of a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. Am J Kidney Dis. 2009 Aug; 54(2):205-26.
21. Hsu YH, Liu WH, Chen W, Kuo YC, Hsiao CY, Hung PH, et al. Association of betel nut chewing with chronic kidney disease: a retrospective 7-year study in Taiwan. Nephrology (Carlton). 2011 Nov;16(8):751-7.
22. Hallan I, Coresh J, Astor B *et al*. International comparison of the relationship of chronic kidney disease prevalence and ESRD risk. *J Am Soc Nephrol* 2012; 17: 2275–2284.