Published in final edited form as:

Clin Exp Pharmacol Physiol. 2009 August ; 36(8): 750–755. doi:10.1111/j.1440-1681.2009.05172.x.

ROLE OF ACTIVATED INTRARENAL REACTIVE OXYGEN SPECIES AND RENIN–ANGIOTENSIN SYSTEM IN IgA NEPHROPATHY MODEL MICE

Naro Ohashi, Akemi Katsurada, Kayoko Miyata, Ryousuke Satou, Toshie Saito, Maki Urushihara, and Hiroyuki Kobori

Department of Physiology and Hypertension and Renal Center of Excellence, Tulane University Health Sciences Center, New Orleans, Louisiana, USA

SUMMARY

1. Using HIGA (high IgA of ddY) mice as an IgA nephropathy model and BALB/c mice as controls, we demonstrated that reactive oxygen species (ROS) and the renin–angiotensin system (RAS) were activated in kidneys of HIGA mice. However, it was difficult to establish an association between renal damage and changes in ROS and the RAS. Therefore, the present study was performed to determine whether renal injury is associated with changes in ROS and the RAS in HIGA mice.
2. Male HIGA mice were divided into four groups of 10 each: (i) untreated mice (HIGA + null); (ii) mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day; HIGA + OLM); (iii) mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day; HIGA + Tempol); and (iv) mice treated with RAS-independent antihypertensive drugs (30 mg/kg per day hydralazine, 0.6 mg/kg per day reserpine and 12 mg/kg per day hydrochlorothiazide; HIGA + HRH). Mice were treated for 5 weeks.
3. Systolic blood pressure decreased significantly in the HIGA + OLM and HIGA + HRH groups, but not in the HIGA + Tempol group, compared with HIGA + null mice. The expression of two ROS markers (4-hydroxy-2-nonenal and heme oxygenase-1) and angiotensin II as a marker of the RAS decreased significantly in HIGA + OLM and HIGA + Tempol mice, but not in HIGA + HRH mice, compared with HIGA + null mice. As a marker of renal damage, mesangial matrix expansion and the desmin-positive area decreased significantly in the HIGA + OLM and HIGA + Tempol groups, but not in HIGA + HRH group, compared with the HIGA + null group.
4. These data suggest that intrarenal ROS and RAS activation play a pivotal role in the development of IgA nephropathy model mice, from the early phase, independent of blood pressure.

Keywords

high IgA of ddY (HIGA) mice; IgA nephropathy; reactive oxygen species; renin-angiotensin system

INTRODUCTION

Immunoglobulin A (IgA) nephropathy is defined as the predominant deposition of IgA in the glomerular mesangium.¹ Worldwide, IgA nephropathy is recognized as the most common primary glomerulopathy.^{2–4} In previous clinical studies, we demonstrated that the immunoreactivity of intrarenal angiotensinogen (AGT) and angiotensin (Ang) II is significantly higher in kidneys of IgA nephropathy patients than in surgically resected normal kidneys.^{5,6} Further, we showed that immunoreactivity for intrarenal heme oxygenase (HO)-1 and 4-hydroxy-2-nonenal (4-HNE), markers of oxidative stress, is significantly higher in IgA nephropathy patients than in normal controls.⁵ In addition, a recent animal study in which ddY mice with high serum IgA levels (HIGA mice) were used as IgA nephropathy model mice and BALB/c mice were used as control mice revealed that reactive oxygen species (ROS) and the renin–angiotensin system (RAS) were activated in kidneys of HIGA mice.⁷ However, it was difficult in that study to establish an association between renal damage and changes in ROS and the RAS. Therefore, the present interventional study was performed to test the hypothesis that ROS and RAS activation play an important role in the development of IgA nephropathy in HIGA mice.

METHODS

Experimental design

The present experimental protocol conformed to the guidelines of the Animal Care and Use Committee of Tulane University. Male HIGA mice ($n = 40$; 21 weeks of age), which were used as IgA nephropathy model mice, were purchased from Japan SLC (Hamamatsu, Japan). Mice were subdivided into four groups of 10 mice each: (i) untreated mice (HIGA + null); (ii) mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day; Daiichi Sankyo, Tokyo, Japan; HIGA + OLM);⁸ (iii) mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day; Sigma-Aldrich, St Louis, MO, USA; HIGA + Tempol);⁹ and (iv) mice treated with RAS-independent anti-hypertensive drugs (30 mg/kg per day hydralazine (MP Biomedicals, Irvine, CA, USA), 0.6 mg/kg per day reserpine (MP Biomedicals) and 12 mg/kg per day hydrochlorothiazide (MP Biomedicals); HIGA + HRH).¹⁰ All mice were maintained in a temperature-controlled room under a 12 h light–dark cycle, with water available *ad libitum*. All drugs were administered via the drinking water. Mice were harvested at the age of 25 weeks. Systolic blood pressure (SBP) was measured once a week in conscious, restrained mice over the duration of the 5 week study (age 21–25 weeks) with the tail-cuff method.

Western blot analysis

Proteins were extracted from the kidneys and western blot analysis for renal 4-HNE was performed as described previously, using an infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA).^{7,11} A mouse anti-4-HNE monoclonal antibody (Japan Institute for the Control of Ageing, Fukuroi, Japan) was used. A mouse anti-GAPDH monoclonal antibody was purchased from Sigma (St Louis, MO, USA). The accumulation of 4-HNE determined in the western blot analysis was normalized against that of GAPDH.

Evaluation of glomerular lesions

Kidney tissue samples were fixed in zinc-saturated formalin and embedded in paraffin. Tissue sections (3 μ m) were stained with periodic acid-Schiff (PAS; Mass Histology Services, Worcester, MA, USA) for histopathological evaluation. The percentage of the intraglomerular area occupied by mesangial matrix was estimated in 20 glomeruli from each mouse and assigned values as follows: 0, 0–5%; 1, 5–10%; 2, 10–20%; 3, > 20%. Mean values were calculated as described previously.¹²

Immunohistochemical analysis

The IgA-positive area in the glomeruli was examined by immunohistochemistry with a commercially available antibody against IgA (Dakocytomation, Glostrup, Denmark), as described previously.^{5,7,10} Immunohistochemistry was performed using a robotic system (Autostainer; Dakocytomation) and was counterstained with haematoxylin. The fraction of the glomeruli in which IgA was present was determined using the Image-Pro Plus software (Media Cybernetics, Bethesda, MD, USA). For each glomerulus, the IgA-positive area (brown) was automatically calculated by the software and this area was, in turn, divided by the total area of the glomerulus. Twenty glomeruli were examined for each mouse and the average percentage of IgA-positive area was determined for each mouse. The ratio was calculated relative to the HIGA + null group. Similarly, desmin expression in the glomeruli was examined using a commercially available mouse anti-human desmin monoclonal antibody (Dakocytomation). The glomerular area exhibiting desmin expression (red) was determined using the Image Pro Plus software (Media Cybernetics). For each mouse, 20 glomeruli were examined and mean percentages of the affected lesions were calculated.

The intensity of AngII immunoreactivity was examined by performing immunohistochemistry using a commercially available rabbit anti-AngII serum (Phoenix Pharmaceuticals, Burlingame, CA, USA), as described previously.^{5,7} For each mouse, the tubules in 20 consecutive microscopic fields were examined and the intensity of AngII immunoreactivity (brown) was calculated and averaged. The ratio was calculated relative to that determined for the HIGA + null group. Similarly, the intensity of HO-1 immunoreactivity (brown) was examined by performing immunohistochemistry using a rabbit anti-human HO-1 polyclonal antibody (Stressgen, Ann Arbor, MI, USA).⁵ All quantitative analyses were performed in a blinded manner to avoid any bias.

Statistical analysis

Statistical analyses were conducted by performing analysis of variance (ANOVA) using Statview (Abacus Concepts, Berkeley, CA, USA). All data are presented as the mean±SEM. $P < 0.05$ was considered significant.

RESULTS

IgA deposition in mesangial areas

After harvesting kidneys from 25-week-old mice, the IgA deposition in the mesangial areas was quantified to confirm that the IgA nephropathy model had been established in these mice. No significant differences were observed among the four groups (Fig. 1). This indicates that the IgA nephropathy model had been established to the same degree in all four groups.

Systolic blood pressure

Systolic blood pressure was measured once a week for 5 weeks using the tail-cuff method. In the HIGA + null group, SBP increased gradually and was significantly higher in mice at 25 weeks of age than in mice at 21 weeks of age. However, olmesartan treatment suppressed the increase in SBP. In the HIGA + OLM group, from 24 weeks of age, SBP decreased significantly compared with SBP in the HIGA + null group. In addition, HRH treatment remarkably reduced the SBP; from 22 weeks of age, the SBP in the HIGA + HRH group decreased significantly compared with that in the HIGA + null group. In the HIGA + Tempol group, SBP did not increase and there were no significant differences in SBP in mice at 21 and 25 weeks of age (Fig. 2).

Intrarenal ROS activation

The accumulation of renal 4-HNE was evaluated in mice at 25 weeks of age by performing western blot. 4-Hydroxy-2-nonenal is a major aldehydic product of lipid peroxidation that has been suggested to play an important role in tissue toxicity associated with lipid peroxidation.¹³ The accumulation of renal 4-HNE was significantly lower in the HIGA + OLM and HIGA + Tempol groups compared with that in the HIGA + null group. Conversely, there was no significant difference in 4-HNE accumulation between the HIGA + null and HIGA + HRH groups (Fig. 3).

In addition to the accumulation of 4-HNE, we estimated the immunoreactivity of HO-1, which is another ROS marker. Heme oxygenase-1 is an inducible form of HO, which is a rate-limiting enzyme functioning in heme catabolism. Activated HO-1 is considered to function as an anti-oxidant and anti-inflammatory defence mechanism via the degradation of cellular heme (pro-oxidant) and via elevation of biliverdin (anti-oxidant) concentrations.¹⁴

Immunohistochemistry for HO-1, performed on tissues from HIGA + null group mice, revealed that this marker was distributed in both the proximal and distal tubules of the kidneys, but was not distributed in the glomeruli. The intensity of HO-1 staining in the tubular cells was significantly lower in the HIGA + OLM and HIGA + Tempol groups than in the HIGA + null group. Conversely, no significant differences were noted between the HIGA + null and HIGA + HRH groups (Fig. 4).

Intrarenal RAS activation

Next, we investigated the activation of the intrarenal RAS. The AngII immunoreactivity signals were distributed in the proximal and distal tubular cells of mice in the HIGA + null group at 25 weeks of age. However, olmesartan or tempol treatment significantly decreased the immunoreactivity signals obtained for AngII. In the HIGA + OLM and HIGA + Tempol groups, staining was largely limited to the distal tubular cells and some proximal tubular cells also exhibited weak positive signals for AngII. Significant differences were noted in the HIGA + OLM and HIGA + Tempol groups compared with the HIGA + null group with regard to AngII immunoreactivity signals, whereas no significant differences were observed between the HIGA + null and HIGA + HRH groups (Fig. 5).

Renal damage

The extent of mesangial matrix expansion was determined by performing PAS staining in mice at 25 weeks of age. The extent of mesangial matrix expansion was significantly ameliorated in the HIGA + OLM and HIGA + Tempol groups compared with the HIGA + null group. However, there was no significant difference between the HIGA + null and HIGA + HRH groups (Fig. 6a,c). Desmin immunoreactivity was also estimated in mice at 25 weeks of age. The results revealed that this marker was expressed in the mesangial cells and podocytes of the HIGA + null group, consistent with previous reports on mouse models.¹⁵ The immunoreactivity signals obtained for desmin were significantly lower in the HIGA + OLM and HIGA + Tempol groups compared with the HIGA + null group. Conversely, no significant differences were noted between the HIGA + null and HIGA + HRH groups (Fig. 6b,d).

DISCUSSION

The HIGA mice are an inbred strain with the high serum IgA levels of ddY mice, which are an outbred strain with IgA nephropathy-like characteristics.¹⁶ The HIGA mice exhibit high serum IgA levels from the time they are young.¹⁷ Although these mice do not develop haematuria, which is one of the characteristics of IgA nephropathy,¹⁶ IgA deposition in the mesangial areas and an increase in the number of mesangial cells can be seen in the same way as in IgA nephropathy patients. These changes are first visible at approximately 25 weeks of

age and are completed by approximately 40 weeks of age.^{16,17} Therefore, 25-week-old HIGA mice were used in the present study as a model of the early phase of IgA nephropathy and BALB/c mice were used as the control, as described previously (data not shown).⁷

Using these animal models, we quantified IgA deposition in the mesangial areas. No significant differences were observed among the four HIGA groups, although IgA deposition was significantly greater in the HIGA + null group than in BALB/c mice. Systolic blood pressure in BALB/c mice did not increase during the 5 week study period. Conversely, SBP in the HIGA + null group increased gradually over the 5 week study period (but not in the other HIGA groups). That is, SBP in mice at 25 weeks of age was decreased significantly in the HIGA + OLM group and more markedly in the HIGA + HRH group than in the HIGA + null group; no significant differences were noted between the HIGA + null and HIGA + Tempol groups. Although immunoreactivity for HO-1 in BALB/c mice tended to decrease compared with that in the HIGA + null group, the differences did not reach statistical significance. However, accumulation of renal 4-HNE, expression of renal AngII, the extent of mesangial matrix expansion as determined by PAS staining and desmin-immunoreactive areas in BALB/c mice were significantly decreased compared with the HIGA + null group. In addition, we found that all these four parameters were significantly decreased in the HIGA + OLM and HIGA + Tempol groups, but not in the HIGA + HRH group, compared with the HIGA + null group. Although urinary protein excretion in the HIGA + null group was significantly increased compared with that in BALB/c mice, there were no differences between the HIGA + null and HIGA + OLM or HIGA + Tempol groups (data not shown). Because 25-week-old HIGA mice serve as a model for the very early phase of IgA nephropathy, as described previously,^{16,17} the increased proteinuria in the four HIGA groups was not so remarkable. Therefore, it may be difficult to detect differences in urinary protein excretion in the different groups. In addition, Pearson and Spearman single regression analysis indicated that there was a significant positive correlation between the extent of mesangial matrix expansion and the accumulation of 4-HNE ($r = 0.41$; $P < 0.01$) and AngII immunoreactivity ($r = 0.41$; $P < 0.01$) in all samples, including those from BALB/c mice. These data suggest that the renal damage observed was correlated with ROS and activation of the RAS.

The mechanisms underlying the development of IgA nephropathy are gradually being clarified. When IgA immune complexes and polymeric IgA are deposited in mesangial cells, immune mediators such as complements are activated and the mesangial cells are transformed. Platelet-derived growth factor, interleukin-6 and monocyte colony-stimulating factor, which are secreted by transformed mesangial cells, function in an autocrine as well as a paracrine manner in these cells, resulting in cell proliferation and mesangial matrix accumulation. In addition to mesangial cell injuries, podocyte injuries develop, involving detachment of the podocytes from the glomerular capillary and effacement of the foot processes.¹ Desmin expression is known to be increased in mesangial cells and podocytes in mice with renal damage.¹⁵ The increased mesangial matrix expansion and desmin immunoreactivity signals in the HIGA + null group in the present study are consistent with the patterns expected on the basis of the developmental course of IgA nephropathy described above. Moreover, it has been reported that when proximal tubular epithelial cells are cultured with conditioned culture medium from human mesangial cells activated with IgA, intra-tubular AngII production is upregulated.¹⁸ This report coincides with our findings that intrarenal AngII mainly in the tubules was upregulated in the IgA nephropathy model mice.

It has been reported that ROS are associated with some renal diseases. In diabetic nephropathy, hyperglycaemia is the primary determinant of the initiation and progression of renal injury and it not only generates more reactive oxygen metabolites, but also attenuates anti-oxidant mechanisms via non-enzymatic glycation of anti-oxidant enzymes.¹⁹ It has been reported that in renal biopsy specimens obtained from patients with IgA nephropathy and non-IgA mesangial

proliferative glomerulonephritis, both the protein and mRNA expression of the copper and zinc forms of superoxide dismutase (SOD) were lower in moderately or severely damaged tissues than in normal or mildly damaged tissues.²⁰ These authors also demonstrated that reduced levels of SOD activity may suppress the superoxide scavenging reaction and thus render the tissue more vulnerable to oxidative stress.²⁰

There is also increasing *in vitro* and *in vivo* evidence that the RAS is activated by ROS. In studies *in vitro*, Hsieh *et al.*²¹ demonstrated that the stimulatory action of high glucose levels on angiotensinogen (AGT) gene expression in immortalized renal proximal tubular cells is mediated, at least in part, via ROS generation and subsequent activation of p38 mitogen-activated protein kinase. Vidotti *et al.*²² reported that in primary rat mesangial cells, mRNA levels for AGT and angiotensin-converting enzyme were significantly elevated by 72 h high-glucose exposure and that the mRNA levels of prorenin and cathepsin B were significantly increased by 24 h high glucose exposure. In studies *in vivo*, we demonstrated that the intrarenal ROS–RAS axis plays a pivotal role in the progression of the diabetic nephropathy model as follows: after an increment of urinary 8-isoprostane, the major urinary metabolite of F₂ isoprostanes (formed non-enzymatically by the action of superoxide radicals on arachidonic acid and is used as a marker of oxidative stress),²³ intrarenal AGT and AngII were increased in Zucker diabetic fatty (ZDF) obese rats compared with controls and, as a result of the increase in intrarenal ROS and RAS, glomerular and tubulointerstitial damage was caused in ZDF obese rats.²⁴ Moreover, we revealed that urinary excretion of thiobarbituric-acid reactive substances, markers of lipid peroxidation, and kidney AGT were increased significantly in a Dahl salt-sensitive hypertensive rat + high-salt group and that tempol treatment prevented this upregulation.⁹ In the present study, we have shown that both ROS and the RAS are activated, resulting in renal damage in HIGA mice. In addition, tempol administration suppressed ROS production and activation of the RAS, ameliorating renal damage. These results suggest that the RAS, activated by ROS, plays a pivotal role in the development of IgA nephropathy in mice, which coincides data from previous studies.

Izuhara *et al.*²⁵ reported, with special reference to olmesartan, that angiotensin receptor antagonists scavenge hydroxyl radicals, reduce the expression of nicotinamide adenine dinucleotide phosphate oxidase and inhibit pentosidine formation. In animal studies, olmesartan has been shown to inhibit 4-HNE accumulation in the glomeruli and upregulation of p22 phox and p47 phox mRNA expression in the renal cortex in Zucker obese rats,²⁶ as well as to suppress the up-regulation of malondialdehyde, acrolein and 4-HNE expression in subtotaly nephrectomized rats.²⁷ Further, olmesartan inhibits albumin oxidation in haemodialysis patients²⁸ and reduces plasma concentrations of 8-isoprostane 15 (S)-8-iso-prostaglandin-F_{2α} in type 2 diabetes patients.²⁹ In the present study, olmesartan suppressed not only ROS production and activation of the RAS, but also suppressed renal damage. It is possible that olmesartan exerts anti-oxidant as well as AngII inhibitory effects, as indicated by results from previous studies.

The fact that HRH treatment neither inhibited ROS production and activation of the RAS nor exerted renoprotective effects, despite a marked decrease in blood pressure, suggests that olmesartan and tempol exert renoprotective effects via suppression of ROS production and activation of the RAS, independent of blood pressure.

In conclusion, the present data suggest that activated ROS and RAS play a pivotal role in the development of IgA nephropathy in mice, from the early phase, in a manner that is independent of blood pressure. The present study established a novel foundation to treat IgA nephropathy patients with ROS inhibitors and/or RAS blockade to retard the development and progression of IgA nephropathy.

Acknowledgments

This study was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK072408). Olmesartan was kindly provided from Daiichi Sankyo (Tokyo, Japan). The authors acknowledge critical reviews and valuable comments of Drs Romer A Gonzalez-Villalobos and L Gabriel Navar (Tulane University).

References

1. Barratt J, Feehally J. IgA nephropathy. *J Am Soc Nephrol* 2005;16:2088–97. [PubMed: 15930092]
2. Nationwide and long-term survey of primary glomerulonephritis in Japan as observed in 850 biopsied cases. Research Group on Progressive Chronic Renal Disease. *Nephron* 1999;82:205–13. [PubMed: 10395992]
3. Briganti EM, Dowling J, Finlay M, et al. The incidence of biopsy-proven glomerulonephritis in Australia. *Nephrol Dial Transplant* 2001;16:1364–7. [PubMed: 11427626]
4. Simon P, Ramee MP, Boulahrouz R, et al. Epidemiologic data of primary glomerular diseases in western France. *Kidney Int* 2004;66:905–8. [PubMed: 15327379]
5. Kobori H, Katsurada A, Ozawa Y, et al. Enhanced intrarenal oxidative stress and angiotensinogen in IgA nephropathy patients. *Biochem Biophys Res Commun* 2007;358:156–63. [PubMed: 17482564]
6. Kobori H, Ozawa Y, Suzuki Y, et al. Young Scholars Award Lecture: Intratubular angiotensinogen in hypertension and kidney diseases. *Am J Hypertens* 2006;19:541–50. [PubMed: 16647630]
7. Ohashi N, Katsurada A, Miyata K, et al. Activation of reactive oxygen species and the renin–angiotensin system in IgA nephropathy model mice. *Clin Exp Pharmacol Physiol* 2009;36:509–15. [PubMed: 19673933]
8. Kikuchi Y, Yamada M, Imakiire T, et al. A Rho-kinase inhibitor, fasudil, prevents development of diabetes and nephropathy in insulin-resistant diabetic rats. *J Endocrinol* 2007;192:595–603. [PubMed: 17332527]
9. Kobori H, Nishiyama A. Effects of tempol on renal angiotensinogen production in Dahl salt-sensitive rats. *Biochem Biophys Res Commun* 2004;315:746–50. [PubMed: 14975764]
10. Kobori H, Ozawa Y, Suzuki Y, Nishiyama A. Enhanced intrarenal angiotensinogen contributes to early renal injury in spontaneously hypertensive rats. *J Am Soc Nephrol* 2005;16:2073–80. [PubMed: 15888567]
11. Kobori H, Ozawa Y, Satou R, et al. Kidney-specific enhancement of ANG II stimulates endogenous intrarenal angiotensinogen in gene-targeted mice. *Am J Physiol Renal Physiol* 2007;293:F938–45. [PubMed: 17634399]
12. Ohashi N, Yamamoto T, Huang Y, et al. Intrarenal RAS activity and urinary angiotensinogen excretion in anti-thymocyte serum nephritis rats. *Am J Physiol Renal Physiol* 2008;295:F1512–18. [PubMed: 18784264]
13. Ji C, Kozak KR, Marnett LJ. IkappaB kinase, a molecular target for inhibition by hydroxy-2-nonenal. *J Biol Chem* 2001;276:18 223–8.
14. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 1987;235:1043–6. [PubMed: 3029864]
15. Barisoni L, Madaio MP, Eraso M, Gasser DL, Nelson PJ. The *kd/kd* mouse is a model of collapsing glomerulopathy. *J Am Soc Nephrol* 2005;16:2847–51. [PubMed: 16120817]
16. Miyawaki S, Muso E, Takeuchi E, et al. Selective breeding for high serum IgA levels from noninbred ddY mice: Isolation of a strain with an early onset of glomerular IgA deposition. *Nephron* 1997;76:201–7. [PubMed: 9200412]
17. Muso E, Yoshida H, Takeuchi E, et al. Enhanced production of glomerular extracellular matrix in a new mouse strain of high serum IgA ddY mice. *Kidney Int* 1996;50:1946–57. [PubMed: 8943478]
18. Chan LY, Leung JC, Tang SC, Choy CB, Lai KN. Tubular expression of angiotensin II receptors and their regulation in IgA nephropathy. *J Am Soc Nephrol* 2005;16:2306–17. [PubMed: 15930094]
19. Wolff SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. *Free Radic Biol Med* 1991;10:339–52. [PubMed: 1855674]
20. Kashem A, Endoh M, Yamauchi F, et al. Superoxide dismutase activity in human glomerulonephritis. *Am J Kidney Dis* 1996;28:14–22. [PubMed: 8712210]

21. Hsieh TJ, Fustier P, Wei CC, et al. Reactive oxygen species blockade and action of insulin on expression of angiotensinogen gene in proximal tubular cells. *J Endocrinol* 2004;183:535–50. [PubMed: 15590980]
22. Vidotti DB, Casarini DE, Cristovam PC, Leite CA, Schor N, Boim MA. High glucose concentration stimulates intracellular renin activity and angiotensin II generation in rat mesangial cells. *Am J Physiol Renal Physiol* 2004;286:F1039–45. [PubMed: 14722017]
23. Patrono C, FitzGerald GA. Isoprostanes: Potential markers of oxidant stress in atherothrombotic disease. *Arterioscler Thromb Vasc Biol* 1997;17:2309–15. [PubMed: 9409197]
24. Miyata K, Ohashi N, Suzaki Y, Katsurada A, Kobori H. Sequential activation of the reactive oxygen species/angiotensinogen/renin–angiotensin system axis in renal injury of type 2 diabetic rats. *Clin Exp Pharmacol Physiol* 2008;35:922–7. [PubMed: 18430060]
25. Izuhara Y, Nangaku M, Inagi R, et al. Renoprotective properties of angiotensin receptor blockers beyond blood pressure lowering. *J Am Soc Nephrol* 2005;16:3631–41. [PubMed: 16236804]
26. Namikoshi T, Tomita N, Satoh M, et al. Olmesartan ameliorates reno-vascular injury and oxidative stress in Zucker obese rats enhanced by dietary protein. *Am J Hypertens* 2007;20:1085–91. [PubMed: 17903692]
27. Takahashi T, Konta T, Takasaki S, Ichikawa K, Takeishi Y, Kubota I. An angiotensin II type-I receptor blocker, olmesartan medoxomil, attenuates lipid peroxidation in renal injury induced by subtotal nephrectomy. *Clin Exp Nephrol* 2007;11:202–8. [PubMed: 17891346]
28. Kadowaki D, Anraku M, Tasaki Y, et al. Effect of olmesartan on oxidative stress in hemodialysis patients. *Hypertens Res* 2007;30:395–402. [PubMed: 17587751]
29. Fliser D, Wagner KK, Loos A, Tsikas D, Haller H. Chronic angiotensin II receptor blockade reduces (intra) renal vascular resistance in patients with type 2 diabetes. *J Am Soc Nephrol* 2005;16:1135–40. [PubMed: 15716329]

Fig. 1.

Immunoglobulin A (IgA) deposition in mesangial areas in HIGA (high IgA of ddY) mice after harvesting of tissues from mice at 25 weeks of age. HIGA + null, untreated mice; HIGA + OLM, mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day); HIGA + Tempol, mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day); HIGA + HRH, mice treated with renin–angiotensin system-independent antihypertensive drugs (30 mg/kg per day hydralazine, 0.6 mg/kg per day reserpine and 12 mg/kg per day hydrochlorothiazide). (a) Representative photomicrographs of IgA deposition in mesangial areas in the different groups; IgA deposition was detected in mesangial areas and no significant differences were observed between the four groups (original magnification $\times 400$). (b) Semiquantitative analysis of IgA deposition in mesangial areas in the different groups. Twenty glomeruli were examined for each mouse and an average percentage IgA-positive area was obtained for each mouse. The ratio of IgA-positive areas was calculated relative to the HIGA + null group.

Fig. 2.

Systolic blood pressure (SBP) in HIGA (high IgA of ddY) mice from 21 to 25 weeks of age. (●) untreated mice (HIGA + null); (■) mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day; HIGA + OLM); (▲) mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day; HIGA + Tempol); (◆) mice treated with RAS-independent antihypertensive drugs (30 mg/kg per day hydralazine, 0.6 mg/kg per day reserpine and 12 mg/kg per day hydrochlorothiazide; HIGA + HRH). Data are the mean ± SEM. **P* < 0.05, ****P* < 0.001, *****P* < 0.0001 compared with the HIGA + null group; †††*P* < 0.001, ††††*P* < 0.0001 compared with 21-week-old HIGA + null mice; §§*P* < 0.01 compared with 21-week-old HIGA + Tempol mice; ‡*P* < 0.05, ‡‡‡*P* < 0.001 compared with 21-week-old HIGA + HRH mice.

Fig. 3.

Accumulation of intrarenal 4-hydroxy-2-nonenal (4-HNE) determined by western blot in HIGA (high IgA of ddY) mice after harvesting of tissues from mice at 25 weeks of age. HIGA + null, untreated mice; HIGA + OLM, mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day); HIGA + Tempol, mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day); HIGA + HRH, mice treated with renin-angiotensin system-independent antihypertensive drugs (30 mg/kg per day hydralazine, 0.6 mg/kg per day reserpine and 12 mg/kg per day hydrochlorothiazide). (a) Accumulation of 4-HNE in the HIGA + OLM and HIGA + Tempol groups was significantly decreased compared with HIGA + null mice. Conversely, there were no significant differences between HIGA + null and HIGA + HRH groups. Accumulation of 4-HNE was normalized against

glyceraldehyde-3-phosphate dehydrogenase (GAPDH) protein levels, determined as an internal control. (b) Densitometric ratios of 4-HNE bands against GAPDH bands were calculated relative to HIGA + mice. Data are the mean $\pm$ SEM. * P < 0.05 compared with HIGA + null mice.

Fig. 4.

Intrarenal heme oxygenase (HO)-1 protein levels in HIGA (high IgA of ddY) mice after harvesting of tissues from mice at 25 weeks of age. HIGA + null, untreated mice; HIGA + OLM, mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day); HIGA + Tempol, mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day); HIGA + HRH, mice treated with renin-angiotensin system-independent antihypertensive drugs (30 mg/kg per day hydralazine, 0.6 mg/kg per day reserpine and 12 mg/kg per day hydrochlorothiazide). (a) Representative photomicrographs of immunoreactivity for HO-1 in the different groups. In HIGA + null mice, HO-1 immunoreactivity was noted in both proximal and distal tubules, but not in glomeruli. Immunoreactivity for HO-1 was significantly decreased in HIGA + OLM and HIGA + Tempol groups compared with HIGA + null mice. However, there were no significant differences between HIGA + null and HIGA + HRH mice. (Original magnification $\times 200$.) (b) Semiquantitative analysis of the intensity of intrarenal HO-1 immunoreactivity in the different groups. Twenty tubulointerstitial lesions were examined for each mouse and an average intensity of HO-1 immunoreactivity was obtained for each mouse. The ratio was calculated relative to intensity in HIGA + null mice. Data are the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ compared with HIGA + null mice.

Fig. 5.

Intrarenal angiotensin (Ang) II protein levels in HIGA (high IgA of ddY) mice after harvesting of tissues from mice at 25 weeks of age. HIGA + null, untreated mice; HIGA + OLM, mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day); HIGA + Tempol, mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day); HIGA + HRH, mice treated with renin–angiotensin system-independent antihypertensive drugs (30 mg/kg per day hydralazine, 0.6 mg/kg per day reserpine and 12 mg/kg per day hydrochlorothiazide). (a) Representative photomicrographs of AngII immunoreactivity in the different groups. In HIGA + null mice, AngII immunoreactivity was noted in the proximal and distal tubules. Immunoreactivity for AngII in the HIGA + OLM and HIGA + Tempol groups was significantly decreased compared with that in HIGA + null mice; AngII immunoreactivity was largely limited to the distal tubules, although some proximal tubular cells also exhibited weak positive signals. Conversely, there were no significant differences between HIGA + null and HIGA + HRH mice. (Original magnification $\times 200$.) (b) Semi-quantitative analysis of the intensity of intrarenal AngII immunoreactivity in the different groups. Twenty tubulointerstitial lesions were examined for each mouse and an average intensity of AngII immunoreactivity was obtained for each mouse. The ratio was calculated relative to that in HIGA + null mice. Data are the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ compared with HIGA + null mice.

Fig. 6.

Levels of renal damage in HIGA (high IgA of ddY) mice after harvesting of tissues from mice at 25 weeks of age. HIGA + null, untreated mice; HIGA + OLM, mice treated with the angiotensin AT₁ receptor antagonist olmesartan (5 mg/kg per day); HIGA + Tempol, mice treated with the superoxide dismutase mimetic tempol (50 mg/kg per day); HIGA + HRH, mice treated with renin–angiotensin system-independent antihypertensive drugs (30 mg/kg per day hydralazine, 0.6 mg/kg per day reserpine and 12 mg/kg per day hydrochlorothiazide). (a,b) Representative photomicrographs of periodic acid-Schiff (PAS) staining (a) and desmin immunoreactivity (b) in the different groups. Mesangial matrix expansion (a) and desmin immunoreactivity (b) were significantly decreased in the HIGA + OLM and HIGA + Tempol groups compared with the HIGA + null group. Conversely, there were no significant differences between the HIGA + null and HIGA + HRH groups. (Original magnification $\times 400$.) (c,d) Semiquantitative analysis of mesangial matrix expansion determined by PAS staining (c) and the desmin-positive area (d) in the different groups. The percentage of the intraglomerular area occupied by mesangial matrix was assigned the following scores: 0, 0–5%; 1, 5–10%; 2, 10–20%; 3, > 20%. For both mesangial matrix expansion and desmin-positive area, 20 glomeruli were examined for each mouse. Data are the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ compared with HIGA + null mice.