19. Makiishi T, Araki S, Koya D, Maeda S, Kashiwagi A, Haneda M. C-106T polymorphism of AKR1B1 is associated with diabetic nephropathy and erythrocyte aldose reductase content in Japanese subjects with type 2 diabetes mellitus. *Am J Kidney Dis* 2003; 42: 943–951
20. Wang Y, Ng MC, Lee SC *et al*. Phenotypic heterogeneity and associations of two aldose reductase gene polymorphisms with nephropathy and retinopathy in type 2 diabetes. *Diabetes Care* 2003; 26: 2410–2415
21. Zhao HL, Tong PC, Lai FM, Tomlinson B, Chan JC. Association of glomerulopathy with the 5'-end polymorphism of the aldose reductase gene and renal insufficiency in type 2 diabetic patients. *Diabetes* 2004; 53: 2984–2991
22. Lajer M, Tarnow L, Fleckner J *et al*. Association of aldose reductase gene Z+2 polymorphism with reduced susceptibility to diabetic nephropathy in Caucasian type 1 diabetic patients. *Diabet Med* 2004; 21: 867–873
23. Vardarli I, Baier LJ, Hanson RL *et al*. Gene for susceptibility to diabetic nephropathy in type 2 diabetes maps to 18q22.3–23. *Kidney Int* 2002; 62: 2176–2183
24. Bowden DW, Colicigno CJ, Langefeld CD *et al*. A genome scan for diabetic nephropathy in African Americans. *Kidney Int* 2004; 66: 1517–1526
25. Halama N, Yard-Breedijk A, Vardarli I *et al*. The Kruppel-like zinc-finger gene ZNF236 is alternatively spliced and excluded as susceptibility gene for diabetic nephropathy. *Genomics* 2003; 82: 406–411
26. Janssen B, Hohenadel D, Brinkkoetter P *et al*. Carnosine as a protective factor in diabetic nephropathy: association with a leucine repeat of the carnosinase gene CNDP1. *Diabetes* 2005; 54: 2320–2327
27. Freedman BI, Hicks PJ, Sale MM, Bowden DW. Association analysis of the carnosinase gene in type 2 diabetic nephropathy [abstract]. *J Am Soc Nephrol* 2005; *in press*
28. Shimazaki A, Kawamura Y, Kanazawa A *et al*. Genetic variations in the gene encoding ELMO1 are associated with susceptibility to diabetic nephropathy. *Diabetes* 2005; 54: 1171–1178
29. Moczulski DK, Rogus JJ, Antonellis A, Warram JH, Krolewski AS. Major susceptibility locus for nephropathy in type 1 diabetes on chromosome 3q: results of novel discordant sib-pair analysis. *Diabetes* 1998; 47: 1164–1169
30. Chistiakov DA, Savost'anov KV, Shestakova MV *et al*. Confirmation of a susceptibility locus for diabetic nephropathy on chromosome 3q23–q24 by association study in Russian type 1 diabetic patients. *Diabetes Res Clin Pract* 2004; 66: 79–86
31. Liu Y, Burdon KP, Langefeld CD *et al*. P-selectin gene haplotype associations with albuminuria in the Diabetes Heart Study. *Kidney Int* 2005; 68: 741–746
32. DeWan AT, Arnett DK, Atwood LD *et al*. A genome scan for renal function among hypertensives: the HyperGEN study. *Am J Hum Genet* 2001; 68: 136–144
33. Hunt SC, Coon H, Hasstedt SJ *et al*. Linkage of serum creatinine and glomerular filtration rate to chromosome 2 in Utah pedigrees. *Am J Hypertens* 2004; 17: 511–515
34. Fox CS, Yang Q, Guo CY *et al*. Genome-wide linkage analysis to urinary microalbuminuria in a community-based sample: the Framingham Heart Study. *Kidney Int* 2005; 67: 70–74
35. Fox CS, Yang Q, Cupples LA *et al*. Genomewide linkage analysis to serum creatinine, GFR, and creatinine clearance in a community-based population: the Framingham Heart Study. *J Am Soc Nephrol* 2004; 15: 2457–2461
36. Freedman BI, Beck SR, Rich SS *et al*. A genome-wide scan for urinary albumin excretion in hypertensive families. *Hypertension* 2003; 42: 291–296
37. Freedman BI, Bowden DW, Rich SS *et al*. A genome scan for all-cause end-stage renal disease in African Americans. *Nephrol Dial Transplant* 2005; 20: 712–718
38. Fogarty DG, Rich SS, Hanna L, Warram JH, Krolewski AS. Urinary albumin excretion in families with type 2 diabetes is heritable and genetically correlated to blood pressure. *Kidney Int* 2000; 57: 250–257
39. Freedman BI, Langefeld CD, Lohman KK *et al*. Relationship between albuminuria and cardiovascular disease in type 2 diabetes. *J Am Soc Nephrol* 2005; 16: 2156–2161
40. Knowler WC, Coresh J, Elston RC *et al*. The Family Investigation of Nephropathy and Diabetes (FIND): design and methods. *J Diabetes Complications* 2005; 19: 1–9

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Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases

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Abstract

Angiotensin II (AngII) participates in the pathogenesis of renal diseases, through the regulation of two key processes inflammation and fibrosis. AT_1 and AT_2 are the main receptors of AngII. AT_1 mediates most of the actions of AngII. This receptor regulates

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the expression of profibrotic factors, such as connective tissue growth factor (CTGF). The Smad signalling pathway and the Rho/Rho kinase system are two novel mechanisms involved in AngII-induced matrix regulation recently described. The role of AT_2 receptors in renal pathophysiological processes is not fully elucidated. Experimental data suggest that AT_2 receptors through activation of nuclear factor-κB participate in renal inflammatory cell recruitment. Studies in animal models of kidney injury have shown that the combined blockade of both AT_1 and AT_2 receptors, as well as the inhibition of the NF-κB pathway are necessary to stop the inflammatory process fully. On the whole, these data highlight the complex signalling systems activated by AngII and suggest novel potential targets to block fibrosis and inflammation in renal diseases.

Keywords: angiotensin II; fibrosis; inflammation; proinflammatory cytokines; growth factors

Introduction

Angiotensin II (AngII), the main peptide of the renin–angiotensin system (RAS), is involved in the pathogenesis of renal diseases [1,2]. This peptide acts through its binding to two specific receptors, AT_1 and AT_2 [2]. AT_1 is responsible for most of the pathophysiological actions of AngII. By promoting proliferation, inflammation and fibrosis, AngII contributes to chronic diseases, such as hypertension, atherosclerosis, cardiac hypertrophy and renal injury, The role of the AT_2 receptor is not completely defined. AT_2 is involved in cell growth inhibition and inflammatory cell recruitment in the kidney [2–4]. We will review here the information regarding the novel mechanisms involved in the fibrotic and inflammatory response caused by AngII.

AngII regulates fibrosis via the AT1 receptor: role of CTGF and the Smad signalling pathway

AngII via AT_1 regulates extracellular matrix (ECM) accumulation mediated by the endogenous production of profibrotic growth factors, such as transforming growth factor-β (TGF-β). Angiotensin-converting enzyme (ACE) inhibitors and AT_1 antagonists decrease tissue expression of TGF-β and fibrosis; furthermore, blockade of TGF-β diminishes AngII-induced ECM production [2,3]. Although TGF-β is one of the main regulators of fibrosis, therapeutic strategies blocking TGF-β actions have not afforded the expected beneficial effects probably because of its anti-inflammatory properties [5]. This is one of the reasons why novel antifibrotic targets are under active investigation. Connective tissue growth factor (CTGF) is a novel profibrotic factor that is upregulated in different human kidney diseases and contributes to renal fibrosis and tubuloepithelial transdifferentiation [6]. In models of renal injury, ACE inhibitors and AT_1 antagonists diminished CTGF upregulation and fibrosis [7,8]. We have also demonstrated that the blockade of CTGF, by an antisense CTGF oligonucleotide, diminished AngII-induced fibrosis, shown by diminution of fibronectin production [7,9]. These results suggest that CTGF could be a novel antifibrotic target (Figure 1).

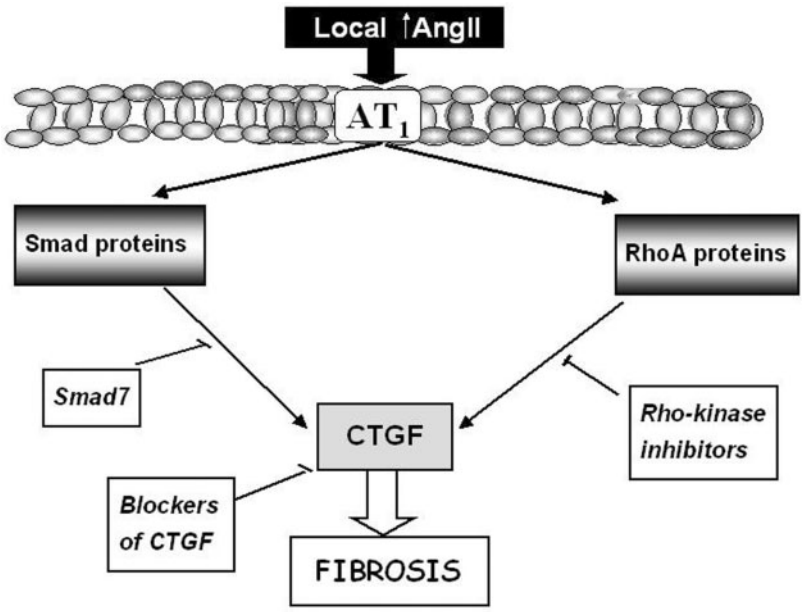

Fig. 1. Potential novel therapeutic strategies to block AngII-induced fibrosis. AngII via AT_1 activates the Smad signalling system and the Rho/Rho kinase pathway that upregulates CTGF production and fibrosis.

The Smad proteins are essential components of the intracellular signalling pathways, acting as transcription factors of TGF-β-mediated responses, including fibrosis [10]. We have recently shown that AngII via AT_1 activates the Smad signalling system, independently of TGF-β [11]. In vascular smooth muscle cells (VSMCs), AngII caused a rapid and direct activation of Smad2 phosphorylation, nuclear translocation of phosphorylated Smad2 and Smad4, increased DNA-binding activity and Smad-dependent gene transcription. In AngII-infused rats, aortic Smad overexpression was associated with CTGF induction and occurred before ECM accumulation [11]. Smad7 may function as a general negative regulator of TGF-β receptor signalling [12]. Transient transfection with Smad7, which interferes with receptor-mediated activation of Smad2 and Smad3, diminished CTGF, fibronectin and type 1 procollagen upregulation caused by AngII [11]. Moreover, Smad7 overexpression blocks TGF-β-induced ECM production and renal fibrosis [13–15]. AT_1 blockade diminishes Smad pathway activation and fibrosis in the model of renal injury caused by unilateral ureteral obstruction (UUO), in myocardial infarction in rats and in the aorta of AngII-infused rats [11,16,17]. These data indicate that Smad signalling could be a common mechanism of AngII-mediated fibrosis in cardiovascular and renal diseases and that the blockade of Smad activation could be another important anti-fibrotic target (Figure 1).

The small G protein Rho and AngII responses

The AT_1 are G-coupled receptors and activate small G proteins, including RhoA and the Rho kinase system [18]. The Rho/Rho kinase signalling pathway participates in the development of fibrotic lesions in several tissues including the kidney. In different experimental models, such as hypertensive glomerulosclerosis, UUO, nephrectomized spontaneously hypertensive rats and L-NAME-treated rats, administration of Rho kinase inhibitors diminished glomerular and tubulointerstitial injury, inflammation and fibrosis, and downregulated smooth muscle α-actin gene overexpression, as well as the TGF-β and ECM proteins [19–24]. In rats infused with AngII, we have shown that the Rho kinase inhibitor Y-27632 diminished tubular damage, the number of inflammatory cells, and renal overexpression of CTGF and proinflammatory parameters [25]. All these data suggest that Rho kinase inhibitors could be novel targets for renal therapy (Figure 1).

AngII and the inflammatory response: role of AngII receptors and the NF-κB pathway

AngII contributes to the recruitment of infiltrating cells into the kidney; AngII causes the adhesion of circulating cells to endothelial and mesangial cells, and the migration of inflammatory cells into the kidney. This process is mediated by upregulation of adhesion molecules, cytokines and chemokines [1].

ACE inhibitors have been shown to diminish inflammatory cell infiltration and inflammatory markers in many animal models of renal injury [1]. AngII via AT_1 receptors upregulates many proinflammatory genes, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1), through the activation of several intracellular signalling systems, including the nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK) cascade, Rho proteins and redox pathways [1]. Some experimental data suggest that AT_2 receptors are involved in the inflammatory cell recruitment in the kidney. Only AT_2, but not AT_1, antagonists diminished the number of inflammatory cells in different animal models, including systemic infusion of AngII and UUO [3,4,8,26,27]. We have recently demonstrated that combined treatment with AT_1 and AT_2 antagonists blocked the inflammatory response, and lowered the number of infiltrating cells and the overexpression of proinflammatory genes to control levels in those models [8,27]. In the UUO model, AT_2 blockade diminished tumour necrosis factor-α (TNF-α) and RANTES overexpression, and the simultaneous blockade of both receptors abolished MCP-1 gene upregulation [8]. NF-κB activation has been described in kidney diseases [1]. *In vivo*, AngII activates the renal NF-κB pathway that was partially diminished by AT_1 or AT_2 antagonists alone, and was abolished by combination of both receptor antagonists or ACE inhibition [4,8,27]. In mesangial cells, NF-κB activation was mediated by both AT_1 and AT_2 receptors [4]; in tubuloepithelial cells, this was mainly by AT_1 [4], while in endothelial cells it was via AT_2 [28]. In the UUO model, we have found that blockade of renal NF-κB activation by treatment with two different NF-κB inhibitors, pyrrolidine dithiocarbamate (PDTC) and parthenolide, diminished the inflammatory cell infiltration and downregulated gene expression of several proinflammatory factors [8]. In spontaneously hypertensive rats, NF-κB inhibition attenuated renal interstitial inflammation and hypertension [29]. These data suggest that in some experimental renal diseases, the blockade of AngII generation by an ACE inhibitor or by combined blockade of both AT_1 and AT_2 receptors, as well as by the inhibition of the NF-κB pathway, is necessary to stop the inflammatory process fully (Figure 2).

In human kidney diseases, the activated renal renin–angiotensin system has been described. In diabetic nephropathy, elevated AngII generation did correlate with the presence of inflammatory cell infiltration, the activation of NF-κB and proinflammatory gene overexpression [30]. These observations emphasize the importance of treatments that block the AngII-induced inflammatory process in human renal diseases.

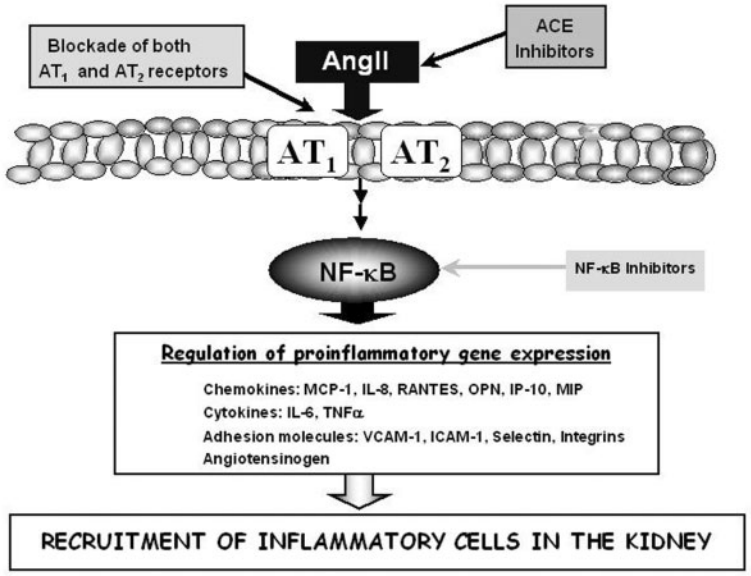

Fig. 2. Effect of pharmacological blockade of the renin–angiotensin system in renal inflammation. The blockade of both AT_1 and AT_2 receptors, or ACE inhibition as well as the inhibition of the NF-κB pathway block the inflammatory response through the regulation of NF-κB and proinflammatory genes.

Conclusion

Drugs that modulate the renin–angiotensin system, such as ACE inhibitors and AT_1 antagonists, have demonstrated protective renal effects and can ameliorate fibrosis. Current strategies in clinical practice combine treatments with ACE inhibitors and AT_1 blockers, due to their potential additive beneficial effects [31]. However, these treatments did not cause regression of renal damage, suggesting that novel approaches are needed. The data presented here highlight potential interesting candidates for antifibrotic treatments, including CTGF, the Smad signalling system and the Rho/Rho kinase pathway. Future studies are necessary to evaluate their potential beneficial effects fully in kidney diseases.

The Ang receptor subtype, AT_1 or AT_2, involved in the inflammatory response in the kidney is not completely elucidated. Our results show that the blockade of both AT_1 and AT_2 receptors is necessary to stop the inflammatory process completely, at least in experimental models. The inhibition of the NF-κB pathway also prevents inflammation and experimental renal damage. All these experimental studies provide a rationale to investigate further the involvement of the AT_2/NF-κB pathway in the inflammatory response in kidney diseases. These results could have potential clinical consequences in the treatment of severe human nephritis.

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References

1. Ruiz-Ortega M, Lorenzo O, Suzuki Y *et al*. Proinflammatory actions of angiotensin II. *Curr Opin Nephrol Hypertens* 2001; 10: 321–329
2. Ruiz-Ortega M, Ruperez M, Esteban V, Egido J. Molecular mechanisms of angiotensin II-induced vascular injury. *Curr Hypertens Rep* 2003; 5: 73–79
3. Wolf G, Ziyadeh FN, Thaiss F *et al*. Angiontensin II stimulates expression of the chemokine RANTES in rat glomerular endothelial cells. Role of the angiotensin type 2 receptor. *J Clin Invest* 1997; 100: 1047–1058
4. Ruiz-Ortega M, Lorenzo O, Ruperez M *et al*. Systemic infusion of angiotensin II into normal rats activates nuclear factor κ-B and AP-1 in the kidney. Role of AT1 and AT2 receptors. *Am J Pathol* 2001; 158: 1743–1756
5. Grainger DJ. Transforming growth factor beta and atherosclerosis: so far, so good for the protective cytokine hypothesis. *Arterioscler Thromb Vasc Biol* 2004; 24: 399–404
6. Perbal B. CCN proteins: multifunctional signalling regulators. *Lancet* 2004; 363: 62–64
7. Ruperez M, Lorenzo O, Blanco-Colio LM *et al*. The connective tissue growth factor is a mediator of angiotensin II-induced fibrosis. *Circulation* 2003; 108: 1499–1509
8. Esteban V, Lorenzo O, Ruperez M *et al*. Angiotensin II, via AT1 and AT2 receptors and NF-kappaB pathway, regulates the inflammatory response in unilateral ureteral obstruction. *J Am Soc Nephrol*. 2004; 15: 1514–1529
9. Ruperez M, Ruiz-Ortega M, Esteban V *et al*. Angiotensin II increases connective tissue growth factor in the kidney. *Am J Pathol* 2003; 163: 1937–1947
10. Massague J, Chen YG. Controlling TGF-signaling. *Genes Dev* 2000; 4: 627–644
11. Rodriguez-Vita J, Sanchez-Lopez E, Esteban V, Ruperez M, Egido J, Ruiz-Ortega M. Angiotensin II activates the Smad pathway in vascular smooth muscle cells by a transforming

growth factor-beta-independent mechanism. *Circulation* 2005; 111: 2509–2517
12. Nakao A, Okumura K, Ogawa H. Smad7: a new key player in TGF-beta-associated disease. *Trends Mol Med* 2002; 8: 361–363
13. Li JH, Zhu HJ, Huang XR *et al*. Smad7 inhibits fibrotic effect of TGF-beta on renal tubular epithelial cells by blocking Smad2 activation. *J Am Soc Nephrol* 2002; 13: 1464–1472
14. Lan HY, Mu W, Tomita N *et al*. Inhibition of renal fibrosis by gene transfer of inducible Smad7 using ultrasound-microbubble system in rat UUO model. *J Am Soc Nephrol* 2003; 14: 1535–1548
15. Chen R, Huang C, Morinelli TA, Trojanowska M, Paul RV. Blockade of the effects of TGF-beta1 on mesangial cells by overexpression of Smad7. *J Am Soc Nephrol* 2002; 13: 887–893
16. Hao J, Wang B, Jones SC, Jassal DS, Dixon IM. Interaction between angiotensin II and Smad proteins in fibroblasts in failing heart and *in vitro*. *Am J Physiol* 2000; 279: H3020–H3030
17. Wamsley-Davis A, Padda R, Truong LD *et al*. AT1A-mediated activation of kidney JNK1 and SMAD2 in obstructive uropathy: preservation of kidney tissue mass using Candesartan. *Am J Physiol* 2004; 287: F474–F480
18. Yamakawa T, Tanaka S, Numaguchi K *et al*. Involvement of Rho-kinase in angiotensin II-induced hypertrophy of rat vascular smooth muscle cells. *Hypertension* 2000; 35: 313–318
19. Nagatoya K, Moriyama T, Kawada N *et al*. Y-27632 prevents tubulointerstitial fibrosis in mouse kidneys with unilateral ureteral obstruction. *Kidney Int* 2002; 61: 1684–1695
20. Kanda T, Wakino S, Hayashi K *et al*. Effect of fasudil on Rho-kinase and nephropathy in subtotally nephrectomized spontaneously hypertensive rats. *Kidney Int* 2003; 64: 2009–2019
21. Satoh S, Yamaguchi T, Hitomi A *et al*. Fasudil attenuates interstitial fibrosis in rat kidneys with unilateral ureteral obstruction. *Eur J Pharmacol* 2002; 455: 169–114
22. Teraishi K, Kurata H, Nakajima A *et al*. Preventive effect of Y-27632, a selective Rho-kinase inhibitor, on ischemia/reperfusion-induced acute renal failure in rats. *Eur J Pharmacol* 2004; 505: 205–211
23. Nishikimi T, Akimoto K, Wang X *et al*. Fasudil, a Rho-kinase inhibitor, attenuates glomerulosclerosis in Dahl salt-sensitive rats. *Hypertensenion* 2004; 22: 1787–1796
24. Kataoka C, Egashira K, Inoue S *et al*. Important role of Rho-kinase in the pathogenesis of cardiovascular inflammation and remodeling induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension* 2002; 39: 245–250
25. Rupérez M, Sánchez-López E, Blanco-Colio LM *et al*. The Rho-kinase pathway regulates AngII-induced renal damage *Kidney Int* 2005; 68 [Supp 99]
26. Morrissey JJ, Klahr S. Differential effects of ACE and AT1 receptor inhibition on chemoattractant and adhesion molecule synthesis. *Am J Physiol* 1998; 274: F580–F586
27. Esteban V, Ruperez M, Vita JR *et al*. Effect of simultaneous blockade of AT1 and AT2 receptors on the NFkappaB pathway and renal inflammatory response. *Kidney Int* 2003; 86: S33–S38
28. Wolf G, Wenzel U, Burns KD, Harris RC, Stahl RA, Thaiss F. Angiotensin II activates nuclear transcription factor-κB through AT1 and AT2 receptors. *Kidney Int* 2002; 61: 1986–1995
29. Rodriguez-Iturbe B, Ferrebuz A, Varegas V *et al*. Early and sustained inhibition of nuclear factor kappa B prevents hypertension in spontaneously hypertensive rats. *J Pharmacol Exp Ther* 2005; 315: 51–77
30. Mezzano S, Aros C, Droguett A *et al*. NF-kappaB activation and overexpression of regulated genes in human diabetic nephropathy. *Nephrol Dial Transplant* 2004; 19: 2505–2512
31. Wolf G, Ritz E. Combination therapy with ACE inhibitors and angiotensin II receptor blockers to halt progression of chronic renal disease: pathophysiology and indications. *Kidney Int* 2005; 67: 799–812

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Hypertensive myocardial fibrosis

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Keywords: fibrosis; hypertension; left ventricular hypertrophy

A variety of cardiac structural and functional changes, such as increased left ventricular mass (LVM), left atrial and aortic root enlargement, LV dysfunction, impairment of coronary reserve and prolonged ventricular repolarization, have been described in patients with long-standing arterial hypertension [1,2]. However, subtle modifications in LV structure and geometry may occur also in the early phases of the natural history of essential hypertension [3]. Among these manifestations of target organ damage, most attention has been devoted to LV hypertrophy (LVH), because the prevalence of this phenotype is relatively high and is associated with an increased risk of cardiovascular morbidity and mortality [4,5].

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