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Atorvastatin improves pathological changes in the aged kidney by upregulating peroxisome proliferator-activated receptor expression and reducing matrix metalloproteinase-9 and transforming growth factor-β1 levels

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ABSTRACT

Objective: To investigate the effects of atorvastatin (AVT) on renal function and renal pathological changes in the aged rat and explore their possible mechanisms.
Methods: Twenty-month-old, normal female Wistar rats were divided into three groups: group A (n = 8) was fed high-dose AVT (10 mg/kg/d); group B (n = 8) was fed low-dose AVT (1 mg/kg/d); and group C (controls, n = 8) received the same volume of normal saline; 3-month-old, normal female Wistar rats served as young normal controls (n = 8). All rats were sacrificed following a 4-month treatment period. Serum creatinine and blood lipid levels were measured. The glomerular sclerosis index and tubulointerstitial lesions were determined using renal periodic acid–Schiff-stained paraffin sections. The mRNA and protein expressions of matrix metalloproteinases (MMP)-9 and -2, tissue inhibitors of metalloproteinase (TIMP)-1 and -2, transforming growth factor-β1 (TGF-β1), and peroxisome proliferator-activated receptors (PPARs) were examined using reverse transcription polymerase chain reactions and Western blots, respectively.
Results: Serum lipid (including serum cholesterol and serum triglycerides) levels in aged rats were significantly higher than those in young rats ($p < 0.05$). Compared to the aged control group, high-dose AVT was associated with significantly lower serum total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels in aged rats ($p < 0.05$); low-dose AVT was associated only with lower serum LDL-C levels ($p < 0.05$). Renal morphological changes in aged rats included focal glomerulosclerosis, infiltration of inflammatory cells, and arteriole sclerosis. Improved renal pathology was observed in aged, AVT-treated rats, and included a decreased glomerular sclerosis index and tubulointerstitial lesion score, especially in those receiving high-dose AVT. Additionally, renal artery wall thickening, luminal narrowing, and arteriolosclerosis were significantly less severe in aged rats receiving high-dose AVT. Upregulated expression of MMP-9 and TGF-β1 was observed in the renal tissue of aged rats. AVT treatment was associated with a reversal of these phenomena and upregulated expression of TIMP-1, $PPAR_{\alpha}$, $PPAR_{\beta}$, and $PPAR_{\gamma}$ in aged rats.
Conclusion: AVT improved the renal pathology of aged rats. These effects may have been induced by the lowering of blood lipids, maintaining the MMP/TIMP balance, and downregulating the expression of TGF-β1. AVT may reduce the levels of MMP-9 and TGF-β in aged rats by upregulating the expression of PPARs.

1. Introduction

A new census in China showed that individuals over the age of 60 years account for 13.3% of the population. The elderly population of the country is expected to increase to 374 million by 2040, accounting for 24.8% of the total population, including nearly 100 million people over 80 years old (Wang, 2006). In China, the incidence of chronic kidney disease (CKD) in those over 60 years is 36%–37%. Recent studies showed that, between 1995 and 2005, half of the maintenance haemodialysis patients with end-stage renal failure were over the age of 65 years (Weinstein and Anderson, 2010).

Aging is an independent risk factor for chronic kidney disease. However, the mechanism of kidney aging remains unclear, and effective therapies are lacking. There have been some potentially significant

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Table 1
The primer sequences for the target genes.

Gene	Primer sequence	Gene	Primer sequence
TIMP-1	5′-AGACCACCTTATACCAGCG-3′ 5′-CGGAGGAAAGGTAAACAGTG-3′	PPAR α	5′-TGCATGTCCGTGGAGACCGTCAC-3′ 5′-ACTCGGTCTTCTTGATGACC-3′
MMP-9	5′-CTTAGATCATTCTTCAGTGCC-3′ 5′-GATCCACCTTCTGAGACTTCA-3′	PPAR β	5′-AGCACATCTACAATGCCTACCT-3′ 5′-TCTTGGCGAACTCGGTGA-3′
TGF-β_1	5′-CTTCAGCTCCACAGAGAAGAACTGC-3′ 5′-CACGATCATGTTGGACAACTGCTCC-3′	PPAR γ	5′-ACTCCCATTCCTTTGACATC-3′ 5′-TCCCCACAGACTCGGCACTC-3′
GAPDH	5′-CCATGGAGAAGGCTGGGG-3′ 5′-CAAAGTTGTCATGGATGACC-3′		

MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase; TGF-β1, transforming growth factor-β1; PPAR, peroxisome proliferator-activated receptor.

therapeutic interventions proposed for the aging kidney, but most have serious possible adverse effects. For example, telomerase (Sitaram et al., 2010), the enzyme that maintains telomere length, is oncogenic in clinical practice. However, the indirect enhancement of telomeres, using atorvastatin (AVT), upregulates the expression of telomere repeat-binding factor, an important protein for telomere capping, and prevents senescence in some cells (Spyridopoulos et al., 2004).

Statins limit cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Both basic research and clinical trials have proven that statins have significant renoprotective effects through cholesterol-lowering and cholesterol-independent pathways (Athyros et al., 2004; Bianchi et al., 2003). Haendeler (Haendeler et al., 2004) also found that statins can delay the senescence process of endothelial cells by reducing overproduction of intracellular oxygen free radicals and inhibiting nuclear expression of telomerase reverse transcriptase. Although few studies have examined the effects of statins on the aging kidney, some (Yano et al., 2007) have shown that the pleiotropic anti-inflammatory effects of statins can be induced by peroxisome proliferator-activated receptors (PPARs). PPARs are members of a large nuclear receptor superfamily and are expressed in some tissues, including the kidney. In this study, we explored the effects of different AVT, one of the most widely used statins in clinical practice, doses and long-term treatment on the aging kidney, and explored the possible mechanism of this effect.

2. Materials and methods

AVT (Lipitor™) was purchased from Pfizer Pharmaceuticals (New York, NY, USA). The primary antibodies (anti-tissue inhibitor of metalloproteinase [TIMP]-1, anti-matrix metalloproteinase [MMP]-9, anti-transforming growth factor [TGF]-β1, anti-PPARα, anti-PPARβ, and anti-PPARγ) and secondary antibodies (Goat-anti-rabbit IgG or rabbit-anti-sheep IgG labelled with horseradish peroxidise) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

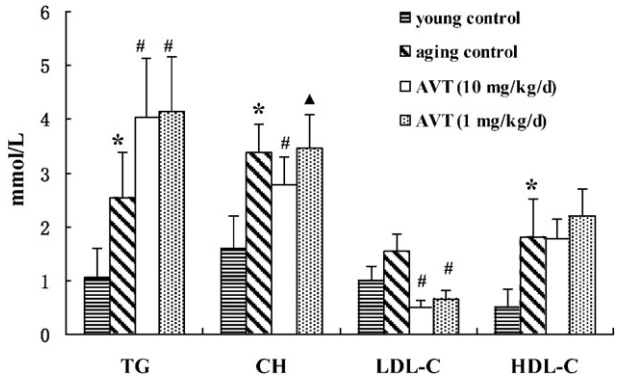

Fig. 1. Serum lipoprotein levels in rats (mean ± SD, mmol/L, n = 8). TG, triglyceride; CH, cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; AVT, atorvastatin. *p < 0.05 compared with the young controls; #p < 0.05, compared with the aged controls; ▲p < 0.05, compared with the 10 mg/kg/d AVT group.

2.1. Animal studies

20-month-old, normal female Wistar rats (Beijing Weitong Lihua, Beijing, China), representing aged animals (Bridges et al., 2014), were randomly assigned to 3 groups (n = 8, each): group A rats were fed high-dose AVT (10 mg/kg/d); group B rats were fed low-dose AVT (1 mg/kg/d); and group C rats (aged controls) received the same volume of normal saline. These animals received the drug (oral gavage) for four months; three-month-old, normal female Wistar rats served as young normal controls. All animals were allowed free access to food and water. Throughout the experiment, the animals were maintained on a 12-h light/dark cycle (lights on at 6:00 a.m.) at 22 °C. All rats were sacrificed for testing after four months of treatment. The experiments were approved by the animal care and use committee of Chinese PLA General Hospital.

2.2. Biochemical analyses

At the end of the experiment, each rat was anaesthetized with 2% pentobarbital (40 mg/kg, intraperitoneally). A 3 mL blood sample was taken from each animal, and serum was recovered following room-temperature centrifugation at 1500 rpm. Serum creatinine (Scr), blood urea nitrogen (BUN), and serum lipid (cholesterol[CH], triglycerides[TG], low-density lipoprotein-cholesterol[LDL-C], high-density lipoprotein-cholesterol[HDL-C]) levels were determined using an automated biochemical analyser at the Department of Biochemistry, Chinese PLA General Hospital.

2.3. Histological analyses

After anaesthesia, an abdominal midline incision was performed and both kidneys were removed. The lower part of each animal's right kidney was fixed in phosphate-buffered saline (PBS), containing 10% formalin, and the remaining part was immediately stored in liquid nitrogen. The kidney tissues were fixed, dehydrated, and embedded in paraffin, and the paraffin sections were stained with periodic acid-Schiff (PAS) reagent. The glomerular and mesangial areas were measured using image analysis software (Image Pro-Plus 5.1, Media Cybernetics, Rockville, MD, USA). The degree of glomerular sclerosis was expressed as the mean glomerular sclerosis index: sclerotic

Table 2
Effects of atorvastatin on renal function in aged rats (mean ± SD, n = 8).

Group	Scr (μmol/L)	BUN (mmol/L)
Young control	41.3 ± 11.3	10.0 ± 2.9
Aging control	39.6 ± 9.2	7.6 ± 0.5
AVT, (10 mg/kg/d)	38.9 ± 9.4	8.4 ± 1.4
AVT, (1 mg/kg/d)	32.4 ± 3.0	7.9 ± 0.7

Scr, serum creatinine; BUN, blood urea nitrogen; AVT, atorvastatin.