Atorvastatin Has no Effects on Kidney Tissues of Wistar Albino Rats in the Long-Term Intake: An Electron Microscopic Study

SUMMARY: In this study, we evaluated the ultrastructural findings of kidney with systemic administration of different doses of atorvastatin in a rat model. Statins may have anti-inflammatory effects that would play a role in preventing the cellular damage. The aim of this study was to investigate how atorvastatin could play a role in kidney tissues. Forty adult male Wistar albino rats (200–250 g) were randomly divided into 4 groups of ten rats each (A1, A2, A3 and Control). Three different doses of atorvastatin were used to determine the effects on kidney tissues during 90 day period. The kidneys of A1 (0.1-mg group), A2 (0.5-mg group) and A3 (1-mg group) group were excised and the tissues were examined after the 90 days by transmission electron microscopy. Despite increasing the dose of atorvastatin intake, the histological structures of atorvastatin groups were appeared normal in the same period. In conclusion, long-term use of atorvastatin was not found to have an adverse effect on kidney tissue.

KEY WORDS: Kidney; Wistar-albino rat; Statins; Atorvastatin; Ultrastructural.

INTRODUCTION

Experimental evidence suggest that lipids are important of progressive renal disease. In recent years, the inhibitors of 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase, so called “statins”, have demonstrated beneficial effects in the different models of progressive renal failure. Statins have been used frequently in experimental studies (Zhang et al., 2006; Sabbatini et al., 2004; Gianella et al., 2007; Agarwal, 2007; Gueler et al., 2002; Oda & Keane, 1999; Chen et al., 2008). Statins, or 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, are used clinically for their cholesterol-lowering properties (Sacks et al., 1996). The roles of statins in coronary artery disease are well reported. Some studies have focused on the mechanisms involved in the anti-inflammatory effects of statins (Dunzendorfer et al., 1997; Wong et al., 2001; Patel & Corbett, 2003; Patel & Corbett, 2004). Due to their favorable effects on lipid levels and cardiovascular outcome, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors have been commonly used after kidney transplant. Recent studies have demonstrated that statins have pleiotropic effects unrelated to lipid levels including reduced inflammation, improved endothelial function, and improved insulin sensitivity (Goicoechea et al., 2006; Collins et al., 2006; Yoshimura et al., 1998). The incidence of chronic renal diseases is increasing worldwide, and there is a great need to identify therapies capable of arresting or reducing disease progression. The current treatment of chronic nephropathies is limited to angiotensin converting enzyme inhibitors and angiotensin receptor blockers, but there is growing clinical and experimental evidence that statins could play a therapeutic role. In experimental studies,
beneficial effects of atorvastatin on chronic allograft nephropathy (Zhang et al.), improving ischemic acute renal failure in aging rats (Sabbatini et al.), preventing kidney inflammation and in fibrosis in stroke-prone rats (Gianella et al.; Guerler et al.) have been shown in vivo animals models. Except for atorvastatin in statins, lovastatin ameliorated the extent of glomerular injury in 5/6 nephrectomy Sprague-Dawley rats (Kasiske et al., 1988), obese Zucker rats (O’Donnell et al., 1993), Dahl-sensitive rats (O’Donnell et al., 1992), guinea pigs and promycin aminonucleo-side model of the nephrotic syndrome (Harris et al., 1990). Simvastatin suppressed the cell proliferation in rats with anti-Thy-1.1 nephritis (Yoshimura et al.).

In light of these informations, we aimed to evaluate atorvastatin effects on rats without any kidney disease in ultrastructural level.

MATERIAL AND METHOD

Animals. Adult male albino Wistar rats weighing 250 to 300 g were used in this experimental study. All rats were given standard rat chow and tap water ad libitum. This study was carried out according to the principles of the Declaration of Helsinki.

Experimental Conditions. On the first study day, the rats were randomly divided into 4 groups: the atorvastatin 0.1, 0.5, 1.0-mg groups and the control group. All the rats were housed in the same room, which was 7 m3 in size, and were kept at 20°C (±2°C) under a 12-hour light/dark regimen in stainless steel cages.

Atorvastatin Preparation and Administration. During 90 day period, the atorvastatin groups received 0.1, 0.5 and 1.0 mg/kg/d of atorvastatin dissolved in 2 ml methyl cellulse solution and the control group received 2 ml of methyl cellulose solution alone. Dosages of atorvastatin were administered orally in 5ml per kg of body weight of each rat.

Procedure. The rats were euthanized using ether anesthesia. The kidneys were excised and fixed in 2.5% phosphate-buffered glutaraldehyde for histopathologic evaluation. Samples of semithin cross-sections of the lung tissues were prepared with an ultramicrotome, stained with toluidine blue, and examined using a photomicroscope. After the selection of appropriate specimens, the tissues, which were stained with uranyl citrate and lead acetate, were screened by transmission electron microscopy (TEM) (Carl Zeiss Libra 120 EFTEM, Germany).

RESULTS

Fourty adult male albino Wistar rats were divided into 4 groups of 10 rats each. All rats survived the 90-day study period. Groups were identified as A1 (0.1-mg group), A2 (0.5-mg group), A3 (1-mg group) and Control.

Physiological effects of Atorvastatin. During 90-day study period, all dosages of atorvastatin had no effect on food or fluid intake, and body weight.

Atorvastatin 0.1, 0.5 and 1.0-mg Groups. Electron microscopy showed normal histologic appearance of the foot processes or villous transformation of podocytes in the glomeruli of Wisstar albino rat’s given all atorvastatin groups. The podocytes showed no degenerative changes, including extensive villous transformations, cell shape abnormalities, and, notably, focal detachment of the foot processes. Strikingly, no podocyte detachment was found in the glomeruli of the atorvastatin given rats, which indicates preserved cell interactions with the underlying glomerular basement membrane (Figs. B, C and D). As a result, no significant differences were observed between the different doses of atorvastatin during long-term administration. When the thin sections of microphotographs of group A1, A2 and A3 compared with the control group showed a normal histologic structure in any of the atorvastatin groups. None of the atorvastatin groups had pathologies such as interstitial cell infiltration, fibrosis, vascular congestion and hemorrhage (Figs. B, C and D). These data suggest that atorvastatin has no devastating effect on kidney morphology and does not lead to any kidney ailment in the long-term intake. Besides well known benefits of statins on the cardiovascular system and chronic kidney diseases, the absence of any adverse effect of atorvastatin on kidney tissue is a clinically significant finding.

Control Group. The thin section of microphotograph of the control group showed that glomerular podocytes cells, glomerular filtration, capillary endothelium, proximal and distal tubules, mitochondrions and mikrovilli in apical, and, notably, focal detachment of the foot processes. In the thin sections of microphotographs of group A1, A2 and A3 compared with the control group showed a normal histologic structure in any of the atorvastatin groups. None of the atorvastatin groups had pathologies such as interstitial cell infiltration, fibrosis, vascular congestion and hemorrhage (Figs. B, C and D). These data suggest that atorvastatin has no devastating effect on kidney morphology and does not lead to any kidney ailment in the long-term intake. Besides well known benefits of statins on the cardiovascular system and chronic kidney diseases, the absence of any adverse effect of atorvastatin on kidney tissue is a clinically significant finding.

DISCUSSION

In this experimental study, we aimed to put forward long-term intake of atorvastatin and changes in kidney tissue in ultrastructural level. According to our findings, we determined that atorvastatin had no effects on kidney tissue in the long-term. Considering a broad range of use of statins...
in humans for preventing disorders, this is a very important finding for experimental and human studies to be carried out.

Statins lower serum cholesterol levels and may therefore be expected to reduce lipid deposits in the kidney. Nevertheless, the exact mechanism by which statins protect against renal damage is unclear. Statins inhibit the rate-limiting enzyme (HMG-CoA reductase) in cholesterol synthesis, but inhibition of this enzyme also leads to downstream inhibition of the synthesis of the isoprenoids farnesyl pyrophosphate and geranyl pyrophosphate (Epstein & Campese, 2005; Blancco-Colio et al., 2003). These isoprenoids normally attach to intracellular signaling proteins to facilitate a variety of cellular responses, including gene expression, membrane trafficking, cell proliferation and migration, and programmed cell death. By blocking isoprenoid synthesis, statins produ-
ce an array of antiinflammatory and vascular effects that are independent of cholesterol reduction. For example, stem cells govern numerous ischemic and degenerative disorders, and recent investigations have shown that inflammation may play a role in modulating stem cell function (Romagnani et al., 2007). Similarly, impaired endothelial progenitor cell function is characteristic of vascular injury, and statin therapy may improve the regenerative capacity of progenitor cells (Walter et al., 2004).

It has been generally accepted that hyperlipidemia is involved in the pathophysiological mechanisms that accelerate progression of renal failure by lowering LDL cholesterol level and triglycerides by statins may be beneficial for the kidney. Lipid deposition can directly damage the glomular basement membrane. It can also stimulate mesangial cell activation and proliferation, a process similar to smooth muscle cell proliferation in the evolution of atherosclerotic plaque (Kasiske et al., 1990; Agarwal & Curley, 2005). Mesangial cells then release chemokines that recruit monocytes to the mesangium, where they are transformed into resident macrophages that secrete proinflammatory and profibrotic mediators capable of augmenting the proliferative process (Rovin & Tan, 1993; Pai et al., 1995; Guijarro et al., 1995). The macrophages also ingest lipids to become foam cells, which are commonly detected at early stages of glomerulonephritis. Each of these cell types is capable of producing reactive oxygen species that oxidize LDL. Oxidized LDL, in turn, can cause further monocyte recruitment, endothelial dysfunction, and mesangial cell cytotoxicity (Guijarro et al.; Kamanna et al., 1996; Tashiro et al., 1999). Analogous pathophysiological mechanisms have been proposed to operate in glomerulosclerosis and atherosclerosis (Diamond, 1991). In diseases ranging from glomerulonephritis and hypertension, renal damage frequently leads to cellular proliferation mainly mesangial (O'Donnell et al., 1993) and epithelial cells (Vrtovsnik et al., 1997), that triggers a chronic mechanism which can cause permanent loss of nephrons. Statins inhibit proliferation, and this effect could be of therapeutic value in kidney diseases. They also promote apoptosis that mediates the resolution of glomerular hypercellularity and glomerular scarring in experimental mesangial proliferative nephritis (Buemi et al., 1999). Buemi et al. (2000) observed that fluvastatin lowered proteinuria in IgA nephropathy.

In the studies of ischemia reperfusion, the results of their study demonstrated that in aging rats, pretreatment with atorvastatin mitigates the course of ischemic acute renal failure by blunting the more pronounced negative response to renal ischemia through a mechanism independent of cholesterol levels. Their data, moreover, suggested that the partial inhibition of Rho, the activation of ENOS, and the higher availability of NO after statin treatment play a major role in this protection, obtained in rats free of age-related nephropathy. Their data have important clinical implications in treating elderly patients with ischemic ARF and in preventing renal dysfunction due to renal hypoperfusion (Sabbatini et al.). Furthermore, Guerler et al. observed that statin treatment reduced damage in the S3 segment of proximal tubules in the outer medullary stripe, the area that is most susceptible to hypoperfusion and hypoxia. Untreated animals demonstrated typical changes in the S3 segment, namely loss of the brush border, destruction of epithelial cells, naked basement membranes, and tubular obstruction. Statin treatment clearly reduced the morphologic damage. Inflammatory reactions attributable to ischemia-reperfusion injury are characterized by leukocyte infiltration. They determined that a statin ameliorated post-ischemic acute renal failure. According to the results, inflammatory mechanisms were significantly affected, supporting the hypothesis that the statin exerted direct antiinflammatory effects in vivo. Cell infiltration, ICAM-1 and iNOS upregulation, matrix molecule expression, MAPK kinase ERK1/2 activation, and transcription factor activation were all reduced.

In chronic kidney diseases, the results of Fasset et al. suggested that the effectiveness and safety of atorvastatin and establish its effects on oxidative stress and inflammation in patients with chronic kidney disease. The hypothesis was that atorvastatin 10 mg would significantly slow the rate of decline of kidney function (eGFR) in subjects with chronic kidney disease (Fasset et al., 2008). In addition, a recent reanalysis of the Treating to New Targets (TNT) study revealed that atorvastatin improved GFR in 10,001 patients with chronic kidney disease. This effect was significantly greater in patients on high-dosage atorvastatin compared with those on low-dosage therapy (Shepherd et al., 2007). Beneficial effects of statins in various kinds of glomerulonephritis have been largely reported in diabetic nephropathy (Casey et al., 2005), CsA nephrotoxicity (Kandoussi et al., 2002), unilateral ureteral obstruction rats (Mizuguchi et al., 2004); however, studies of its protection on CAN after kidney transplantation have not been fully explored. Zhang et al. presented that atorvastatin could prevent the progression of disease in the established rat model of chronic allograft nephropathy with F344 (Fisher kidneys) to Lewis rat recipients allograft kidney transplantation. The F344 (Fisher kidneys) to Lewis rat recipients model is a common model of chronic rejection between rat strains. The histological changes of this model are of great similarity with human kidney transplantation allografts.

A study showed that statin use was associated with reduced mortality rates in a large cohort of kidney transplant...
Recipients (Wiesbauer et al., 2008). Previous trials have consistently demonstrated that statins reduce cardiovascular morbidity and mortality in the general population; however, in renal patients especially in renal transplant recipients the beneficial effects of statins are less well established. Welten et al. (2008) reported that statin use was associated with improved recovery from acute kidney injury after major surgery and had a beneficial effect on long-term survival.

It is worthwhile to mention that statins reduce mortality in uremic patients (Seliger et al., 2008). Previous trials have cautiously. Pravastatin is excreted in 50% by the kidney and should be avoided in renal insufficiency (Sica & Gehr, 2002).

In conclusion, we have shown that long-term atorvastatin intake in Wistar albino-rats without any kidney diseases has no effects on kidney morphology in ultrastructural level. In light of these experimental and human studies, we consider that these data can play an important role in the treatment of cardiovascular and immunological disorders in humans.

REFERENCE


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Received: 24-09-2010 Accepted: 19-11-2010