Atorvastatin prevents glomerulosclerosis and renal endothelial dysfunction in hypercholesterolaemic rabbits

Sandra Vázquez-Pérez, Paloma Aragoncillo, Natalia de las Heras, Josefa Navarro-Cid, Eva Cediel, David Sanz-Rosa, Luis Miguel Ruilope, Cristina Díaz, Gonzalo Hernández, Vicente Lahera and Victoria Cachofeiro

Physiology Department, School of Medicine, Universidad Complutense, Pathology Department, Unit II, Hospital Clínico San Carlos, Hypertension Unit, Hospital 12 de Octubre, Madrid and Parke Davis R&D Department, Barcelona, Spain

Abstract

Background. Numerous studies have shown that elevated plasma cholesterol can exacerbate renal disease. However, the effect of lipids on renal structure and vascular function in normal kidneys is less well established. Therefore, the aim of this study was to evaluate the impact of hypercholesterolaemia on glomerular structure and vascular reactivity of segmental arteries in rabbits. In addition, we also studied whether or not atorvastatin can prevent these structural and vascular alterations in hypercholesterolaemic rabbits.

Methods. Male New Zealand rabbits were fed either a normal rabbit chow or a diet containing 1% cholesterol and treated or not with atorvastatin (1 mg/kg/day) for 12 weeks. Dose–response curves to acetylated choline (10⁻⁹–10⁻⁴ mol/l) and sodium nitroprusside (10⁻⁹–10⁻⁴ mol/l) were studied in segmental arteries in the presence or absence of the thromboxane A₂/PGH₂ receptor antagonist ifedroban (10⁻³ mol/l). Glomerular size and structure were also evaluated.

Results. Compared with control animals, hypercholesterolaemic rabbits presented glomerular hypertrophy and several types of injuries (capillary collapse, hyalinosis and alterations of Bowman’s capsule), suggesting diffuse glomerulosclerosis. Segmental arteries also showed relaxing responses to acetylcholine and sodium nitroprusside which were lower than and similar to, respectively, those of control animals. The presence of ifedroban improved the acetylcholine response only in hypercholesterolaemic rabbits. Atorvastatin treatment prevented vascular and most glomerular changes associated with hypercholesterolaemia even in the presence of very high cholesterol levels.

Conclusions. Atorvastatin exerts a protective effect on renal damage associated with hypercholesterolaemia even in the presence of deleterious levels of plasma cholesterol.

Keywords: atorvastatin; endothelial function; glomerular hypertrophy; glomerulosclerosis; hypercholesterolaemia

Introduction

Complex interrelationships exist between hyperlipidaemia and progression of renal injury. Several pieces of evidence indicate that hyperlipidaemia is a common feature in renal failure, showing an association between hyperlipidaemia and degree of glomerular injury [1–3]. Cardiovascular diseases, including atherosclerosis, are important clinical complications in long-term haemodialysis patients [4,5]. In addition, HMG-CoA reductase inhibitors reduce the severity of glomerular injury and preserve renal function in several experimental models of renal disease [6–8]. All these data suggest that lipids are important modulators of progressive renal disease. However, there is less information on how hypercholesterolaemia can affect glomerular structure and renal endothelial function in normal animals. Therefore, the aim of this study was to evaluate the impact of high plasma cholesterol levels on glomerular structure and vascular reactivity in renal arteries. In addition, we studied whether or not atorvastatin administration can prevent these renal changes.

Materials and methods

Thirty-two male New Zealand rabbits (Granja Cunicular San Bernardo, Spain; 2207 ± 36 g, body weight) were fed either a normal rabbit chow or a diet containing 1% cholesterol (UAR, Panlab, Spain) for 12 weeks with free access to tap water. Rabbits from each diet group were treated with atorvastatin (1 mg/kg/day) given in the food for the same period. At the end of the experiment, blood samples were collected in tubes containing ethylenediamine tetra-acetic acid (EDTA, 10⁻⁷ mol/l) through...
a catheter inserted in the ear artery of awake rabbits. Plasma cholesterol levels were measured employing a commercial kit (Boehringer-Mannheim, Mannheim, Germany). All experimental procedures were approved by the Animal Care and Use Committee of Universidad Complutense, according to the guidelines for ethical care of experimental animals of the European Community.

Vascular reactivity

After taking blood samples, the left kidney was removed from anaesthetized animals. The superior segmental artery was isolated gently using a Zeiss OPMI 99 stereomicroscope, cut into segments (~2 mm in length and 500 μm internal diameter) and mounted in an isometric microvessel myograph (Multimyograph, model 610M, Denmark) filled with oxygenated Kreb’s solution (95% O₂-5% CO₂, 37°C). Segments were attached to a force transducer and to a micromanipulator (Mitutoyo, Japan) for adjustment of muscle length. Tension was recorded continuously on a polygraph (4006, Letica, Spain) for the rest of the experiment. Renal vessels were set at L₁₀₀, which is the internal circumference the vessel would have had under a transmural pressure of 100 mmHg. After equilibration, vessels were contracted with 120 mmol/l K⁺ for 30 min and rinsed with standard Kreb’s buffer. The dose–response curves to phenylephrine (10⁻⁹–10⁻³ mol/l) as well as to acetylcholine (10⁻⁹–10⁻⁴ mol/l) and sodium nitroprusside (10⁻⁸–10⁻⁴ mol/l) were evaluated in rings pre-contracted with a submaximal dose of phenylephrine (10⁻⁶ mol/l) in the presence or absence of the thromboxane A₂ (TXA₂)/PGH₂ receptor antagonist ifetrotab (10⁻⁵ mol/l).

Histological analysis

Kidney samples were fixed in 15% phosphate-buffered formaldehyde, embedded in paraffin and cut into 3–4 μm sections. These were stained with haematoxylin–eosin, periodic acid–Schiff (PAS) and Masson’s trichrome. The area of the glomerular tuft was calculated with a Leica Q 500I W image analyser (Leica, Spain), as previously described [9]. Briefly, all microscopic images of the sections were recorded on videotape with a videocamera, and the histological sections were digitalized, segmented-coloured, and traced for calculations of the area. To determine glomerular size, the cross-sectional area enclosed by the glomerular tuft was corrected by eliminating capillary lumina and urinary spaces within the glomerular tuft. A minimum of 20 well-preserved glomeruli in each specimen was examined. The percentage of sclerosed glomeruli was determined by counting the number of obsolete glomeruli within a total of 200 glomeruli in Masson-stained preparations.

Drugs

Atorvastatin and ifetrotab were kindly supplied by Parke Davis SL (Spain) and Bristol Myers Squibb (Spain), respectively. All other drugs used for vascular reactivity experiments were purchased from Sigma Chemical Co. (St Louis, MO). Concentrations are expressed as final molar concentration in the organ chamber. Chemicals for morphological studies were purchased from Merck (Darmstadt, Germany).

Calculations and statistical analysis

Relaxing responses are expressed as the percentage reduction of the phenylephrine-pre-constricted state. All results are expressed as mean ± SEM of n = 8 rabbits, except data for histological analysis (n = 6 rabbits). Single variable comparisons were made using a one-way analysis of variance; all other data were analysed by two-way analysis of variance for multiple comparisons, followed by a Newman–Keuls test if differences were noted (P < 0.05).

Results

Rabbits fed a diet enriched with cholesterol showed higher plasma levels of cholesterol (48.2 ± 3.1 vs 1.4 ± 0.1 mmol/l) than animals fed a control diet. Atorvastatin treatment significantly attenuated this increase in plasma cholesterol (31.3 ± 2.3 mmol/l) but did not modify cholesterol levels in control animals (1.3 ± 0.2 mmol/l). No differences were observed in body weight increase among any group at the end of the experiment.

Contracting responses to KCl or phenylephrine were similar in both diet groups, and atorvastatin was not able to modify them (data not shown). Vascular segmental rings from hypercholesterolaemic rabbits shifted the dose–response relaxation to acetylcholine to the right as compared with rings from control animals (Figure 1). Incubation of segmental arteries with ifetrotab did not modify dose-related relaxations to acetylcholine in control rabbits but increased this response in hypercholesterolaemic rabbits (maximal response 94.9 ± 0.92 vs 67.7 ± 3.8% of phenylephrine contraction, P < 0.05). Atorvastatin treatment was able to prevent the reduction in acetylcholine relaxation in rings from animals fed a cholesterol-enriched diet (Figure 1) but did not modify the response in control animals (data not shown). Addition of ifetrotab to the bath did not improve acetylcholine-induced relaxation further in any group of atorvastatin-treated rabbits (data not shown). Neither treatment nor diet was able to modify sodium nitroprusside relaxation (data not shown).

Kidneys from hypercholesterolaemic rabbits showed glomerular hypertrophy (8163.7 ± 524 vs 5245.4 ± 184 μm², P < 0.05) as compared with kidneys from control animals. They also exhibited several types of injuries including global or segmental collapse of the capillaries, hyalinosis, adhesion of the tuft to Bowman’s capsule by synchia and Bowman’s capsule fibrosis. This suggests a diffuse glomerulosclerosis. In addition, the kidneys presented entrapment of foamy macrophages in glomeruli and tubular segments. A minimum sclerosed glomeruli number was found (4–11%). Atorvastatin treatment prevented glomerular hypertrophy (5628.2 ± 235 μm²). This HMG CoA reductase inhibitor also prevented the majority of glomerular morphological changes induced by high cholesterol levels, as well as reduced lipid content and sclerosed glomeruli number (3–6%). Atorvastatin
treatment did not modify glomerular size in control rabbits (5193.2 ± 190 μm²).

Discussion

The present study shows that in rabbits the HMG CoA reductase inhibitor atorvastatin prevents glomerular hypertrophy as well as the glomerular lesions associated with high cholesterol levels. In addition, atorvastatin treatment was able to defend against the reduction in acetylcholine relaxation observed in hypercholesterolaemic rabbits. All these beneficial effects exerted by atorvastatin on glomerular damage and endothelial function were associated with a reduction although not a normalization of plasma levels (Fig. 2).

The results show that hypercholesterolaemia is associated with glomerular damage and hypertrophy, supporting the potential role of lipids in renal injury. In fact, clinical and experimental studies have shown that hyperlipidaemia is an important modulator of progressive renal disease [1–3]. It has been demonstrated that glomerular hypertrophy precedes and accompanies sclerosis development in different human renal diseases, suggesting that it plays a crucial role in the pathogenesis of glomerulosclerosis and therefore in impairment of renal function [10,11]. The increased glomerular size observed in hypercholesterolaemic rabbits seems to occur as a result of increases in both mesangial cell number and extracellular matrix deposits, since hypercellularity and an increase in collagen fibres were observed. Accumulation of plasma components such as macrophages and low-density lipoprotein (LDL), as well as production of cytokine and reactive oxygen species could be some of the mechanisms underlying glomerular injury [4,12,13]. The histological abnormalities observed in hypercholesterolaemic rabbits produce focal and diffuse glomerulosclerosis. However, not many sclerosed glomeruli were found, probably because the study did not last long enough. The present study also shows that high plasma cholesterol levels produce not only renal capillary damage but also vascular functional changes since, in segmental arteries from hypercholesterolaemic rabbits, acetylcholine but not sodium nitroprusside relaxation was reduced. This supports the concept that a reduction in endothelium-dependent relaxation is a common alteration in hypercholesterolaemia. A reduced nitric oxide (NO) availability has been proposed as one of the mechanisms underlying the endothelial dysfunction associated with high cholesterol levels, a consequence of both a decreased synthesis and increased degradation [14]. In addition, the present data suggest that an increase in TXA2/PGH2 availability, which can counteract the effect of relaxing factors, might participate in the reduction of the acetylcholine relaxation observed in the renal arteries from hypercholesterolaemic rabbits. In fact, it has been shown that oxidized LDLs are able to increase the release of thromboxane A2 [15]. In agreement with this, Husain et al. [16] have observed that the i.v. administration of aspirin improved acetylcholine-mediated vasodilation in patients with atherosclerosis. Similarly, it has been reported that the infusion of a TXA2/PGH2 receptor antagonist increased the reduced renal vasodilation response to acetylcholine in cholesterol-fed rats [17].

The current study also shows that atorvastatin treatment prevents the development of glomerulosclerosis in hypercholesterolaemic rabbits, even in the presence of high plasma cholesterol levels, since atorvastatin treatment reduced but did not normalize them. In addition, atorvastatin reduced renal lipid content. These data are in agreement with experimental and clinical studies showing that statins reduce the severity of glomerular injury and preserve renal function in renal disease [6–8]. The mechanisms underlying this beneficial effect could involve inhibition of monocyte infiltration, LDL oxidation, extracellular matrix accumulation and mesangial cell proliferation [7,8,12]. In addition, atorvastatin administration was able to prevent the acetylcholine-induced relaxation reduction induced by the intake of a cholesterol-rich diet, a result which was reported previously [7]. This beneficial effect on endothelial function could account for an increase in NO availability, since statins can up-regulate endothelial NO synthase expression and also prevent its down-regulation induced by oxidized LDL [18,19]. In addition, as the present data indicate,
a reduction of TXA₂/PGH₂ availability can also be involved in the amelioration of endothelial dysfunction. This suggestion is based on the fact that prior incubation with ifetroban does not improve further the relaxation to acetylcholine in rabbits treated with atorvastatin. Indeed, it has been shown that the urinary metabolite of TXA₂ was reduced after 3 months of treatment with statins in patients with hypercholesterolaemia [15].

In summary, all these data suggest that high plasma cholesterol levels in rabbits were associated not only with a reduction in acetylcholine-induced vasorelaxation, but also with diffuse glomerulosclerosis. Treatment with atorvastatin prevented glomerular damage and endothelial dysfunction. These beneficial effects exerted by atorvastatin were observed in animals in which cholesterol plasma levels were high enough to expect deleterious effects. Therefore, these data suggest that atorvastatin can play a protective role against both structural and vascular renal damage associated with hypercholesterolaemia.

Acknowledgements. We thank Mrs Blanca Martínez and Mr Antonio Carmona for their technical assistance. This work was supported by grants from Comisión Interministerial de Ciencia y Tecnología (SAF98-0077) and from Fondo de Investigaciones Sanitarias (FIS 98/0003-02). E.C. received the support of a grant from Parke Davis S.L. (Spain).

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