RESEARCH PAPER

Influence of rosuvastatin on the NAD(P)H oxidase activity in the retina and electroretinographic response of spontaneously hypertensive rats

P Sicard[1], N Acar[2], S Grégoire[2], B Lauzier[1], AM Bron[2,3], C Creuzot-Garcher[2,3], L Bretillon[2], C Vergely[1] and L Rochette[1]

[1]Laboratory of Experimental Cardiovascular Pathophysiology and Pharmacology, Faculties of Medicine and Pharmacy, Dijon, France; [2]Eye and Nutrition Research Group, UMR FLAVIC, INRA, Dijon, France and [3]Department of Ophthalmology, University Hospital, Dijon, France

Background and purpose: Retinal complications may be encountered during the development of hypertension as a response to oxidative stress. Statins may reduce the risk of developing hypertension and ocular diseases. We evaluate the effects of rosuvastatin (ROSU) on retinal functionality and oxidative stress levels in normotensive and spontaneously hypertensive rats (SHR).

Experimental approach: Wistar Kyoto (WKY) and SHR were treated for 3 weeks with rosuvastatin ($10 \, mg \, kg^{-1} \, day^{-1}$). Electroretinograms (ERG) were recorded before and after rosuvastatin treatment. Reactive oxygen species (ROS) were determined in the retina with dihydroethidium staining and NAD(P)H oxidase activity was evaluated.

Key results: Retinal ganglion cell ROS and retinal NAD(P)H oxidase activity were higher in SHR than in WKY rats, respectively (17.1 ± 1.1 vs 10.2 ± 1.2 AU, $P < 0.01$; 38095 ± 8900 vs $14081 \pm 5820 \, RLU \, mg^{-1}$; $P < 0.05$). The ERG b-wave amplitude in SHR was significantly lower than that in WKY rats. Rosuvastatin reduced SBP in SHR but did not change plasma lipid levels. Rosuvastatin treatment in SHR significantly decreased ROS levels (11.2 ± 1.3, $P < 0.01$), NAD(P)H activity in retinal ganglion cells (9889 ± 4290; $P < 0.05$), and increased retinal plasmalogen content in SHR, but did not modify the ERG response.

Conclusions and implications: Rosuvastatin, beyond lowering cholesterol levels, was able to lower ROS in the retina induced by hypertension, but without improving retinal function in SHR. These findings point to a complex relationship between ROS in the pathogenesis of retinal disease and hypertension.
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Introduction

Oxidative stress underlies the development and the persistence of several cardiovascular and ocular diseases such as hypertension and retinopathies (Sagar *et al.*, 1992; Du *et al.*, 2003; Moreno *et al.*, 2004; Touyz, 2004). Superoxide anion, produced by mitochondria and oxidases, is of particular interest because it can react with nitric oxide (NO) to produce peroxynitrite, a toxic oxidative species known to

have deleterious effects (Halliwell *et al.*, 1999), including lipid peroxidation and cytotoxicity (Chemtob *et al.*, 1995). Moreover, in the major models of hypertension, including spontaneously hypertensive rats (SHR), reduced NO availability has been implicated as a major cause of endothelial dysfunction in hypertension (Schiffrin *et al.*, 2000) and an increase in arterial blood pressure is associated with production of free radicals in many organs (Touyz, 2004).

The development of eye diseases, associated with increased oxidative stress, is relatively common in patients with elevated blood pressure (Ciulla *et al.*, 2003). For instance, signs of hypertensive retinopathy, from arteriolar narrowing to isolated microaneurysms and haemorrhages are strongly

related to blood pressure, but inconsistently associated with plasma cholesterol levels. Indeed, the retina is constantly exposed to several sources of oxygen species (UV radiation, high mitochondrial activity) and contains a high level of polyunsaturated fatty acids, making it more susceptible to lipid peroxidation (SanGiovanni and Chew, 2005). It has been suggested that, in the eye, oxygen-derived free radicals play a role in diseases such as cataracts, uveitis, age-related macular degeneration (AMD) (Austin and Mamdani, 2005) or glaucoma (Moreno *et al.*, 2004). Retinal cells are equipped with multiple reactive oxygen species (ROS) scavengers, including enzymes such as endogenous peroxidases (Kortuem *et al.*, 2000). Statins are inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMGCoA) reductase, and are widely used for their potent properties to reduce cholesterol levels in humans. However, their beneficial effects go beyond this pharmacological definition, as it has been shown that, among many other effects, they are able to exert anti-inflammatory and antioxidant properties (Davignon, 2004). For instance, in experimental models, rosuvastatin at $10 \, \mathrm{mg \, kg^{-1} \, day^{-1}}$ is able to reduce the development of hypertension (Susic *et al.*, 2003) by increasing NO bioavailability and decreasing oxidative stress (Bayorh *et al.*, 2005). Moreover, recent papers suggest that statins may reduce the risk of developing ocular diseases such as AMD (McGwin *et al.*, 2005) and primary open-angle glaucoma (McGwin *et al.*, 2004). Therefore, statins should be of use in the treatment of hypertension-associated retinal dysfunction. The aim of this study was to assess the role of rosuvastatin, a new hydrophilic HMGCoA reductase inhibitor, on retinal function, lipid composition and oxidative stress level in normotensive and hypertensive rats. In this paper, we are able to show for the first time that retinal functionality in SHR is impaired, relative to that in WKY rats. We also provide data showing increased retinal oxidative stress in SHR can be lowered by treatment with rosuvastatin.

Methods

Animals and treatment

All experiments were performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and with the French legislation and animal quarters (agreement number A21 231010EA). The animals were housed under controlled temperature ($21 \pm 1^\circ\mathrm{C}$) and light conditions (12-h light/12-h dark cycle). The light intensity measured at various locations of the animal quarters was less than 20 lux. Eleven-week-old male adult SHR (200–220 g, $n = 10$) and WKY rats (200–230 g, $n = 10$) purchased from Charles River Laboratories (L'Arbresle, France) were used. They received either vehicle (water) or rosuvastatin ($10 \, \mathrm{mg \, kg^{-1} \, day^{-1}}$, AstraZeneca, Rueil-Malmaison, France) by gavage for 3 weeks. The rosuvastatin dose was chosen according to previous studies in rats (Susic *et al.*, 2003; Mooradian *et al.*, 2005; Otto *et al.*, 2005; Sicard *et al.*, 2005).

Standard laboratory rat chow (Purina) and tap water were available *ad libitum*. All animals were allowed to acclimatize for at least 7 days before experimental manipulations.

Systolic blood pressure and heart rate measurement

Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious rats every week using a non-invasive tail-cuff method (Bioseb, Chaville, France). The rats were handled repeatedly and allowed to adapt to the restraint chamber for 3 days before the actual measurements started. A mean of six measurements was considered as one individual SBP.

Electroretinography

The electroretinogram (ERG) was recorded *in vivo* before rosuvastatin treatment (11 weeks of age) and at the end of the gavage period (14 weeks of age). The following ERG measurement procedures were adapted from those described by Doly *et al.* (1993). Before ERG recordings, the rats were dark-adapted for at least 3 h. All further procedures were carried out under dim red light ($\lambda > 650 \, \mathrm{nm}$) and at a constant temperature of $25^\circ\mathrm{C}$. The animals were anaesthetized with an intramuscular injection of ketamine ($120 \, \mathrm{mg \, kg^{-1}}$ body weight) and xylazine ($6 \, \mathrm{mg \, kg^{-1}}$ body weight) in a saline solution. The pupils were dilated with 0.5% tropicamide (Ciba Vision Ophthalmics, Blagnac, France). An irrigating solution (BSS, Alcon Laboratories, Rueil Malmaison, France) was used to prevent corneal desiccation. After 10 min, the corneal electrode was put in place. The ERG was recorded via the corneal electrode (thin Ag/AgCl wire with a 3-mm ring end) and a reference Ag/AgCl electrode placed on the rat's tongue. The retina was stimulated by a photostimulator (model PS33 PLUS, Grass Telefactor, Astro-Med Inc., West Warwick, RI, USA) delivering light flashes (white light, $6500 \, \mathrm{mcds \, m^{-2}}$) to the eye through fibre optics and a white sphere that mimics a Ganzfeld dome. One flash was delivered every minute and the average of ten individual ERGs was considered as one measurement. The ERG response was amplified using a low-pass filter setting of 1 Hz and a high-pass filter of 1000 Hz. After amplification, the signal was digitized and processed. The amplitudes were determined for each recording and were measured from the baseline (a-wave) or from the peak of the a-wave (b-wave).

Tissue collection and processing

At the end of the 3-week rosuvastatin treatment, the animals were anaesthetized with sodium thiopental ($60 \, \mathrm{mg \, kg^{-1}}$, intraperitoneal) and heparin was intravenously injected ($500 \, \mathrm{IU \, kg^{-1}}$). Blood samples were collected from the aorta and immediately centrifuged at $4^\circ\mathrm{C}$. The plasma was removed, aliquoted, instantaneously frozen in liquid nitrogen and stored at $-80^\circ\mathrm{C}$ until assessed. The animals were killed by decapitation. One eye from each animal was enucleated and the retina was removed, immediately frozen in liquid nitrogen and stored at $-80^\circ\mathrm{C}$ for lipid analyses and chemiluminescence experiments. The other eye was embedded on OCT (Dako, Trappes, France) for $10 \, \mu\mathrm{m}$-thick cryosections.

In situ *detection of superoxide anion*

The oxidative fluorescent probe dihydroethidium (DHE, Molecular Probe, Cergy Pontoise, France) was used to localize

superoxide anion (Oudot et al., 2006). The freshly frozen eye sections were incubated in a light-protected humidified chamber at room temperature with DHE ($5 \mu M$) for 5 min, and then counterstained with a nuclear tracer: 4′,6-diamidino-2-phenylindole (DAPI, $30 \mu g \, ml^{-1}$, Sigma, Lyon, France). The slides were fixed for 10 min in acetone and were immediately analysed with a computer-based digitizing image system (Microvision, Evry, France) using a fluorescent microscope (Eclipse 600, Nikon, Champigny-Sur-Marne, France) connected to a video camera (Tri CCD, Sony, Paris, France). Fluorescence was detected with 510–560 nm excitation and 590 nm emission filters. Automatic computer-based analysis was performed with the same threshold for all sections ($\times 500$ magnification). Three cell layers were delimited (ganglion cells, bipolar cells and photoreceptor cells). Nucleus quantification was performed in each layer. Results are expressed as DHE/DAPI ratio. To verify the specific detection of superoxide anion with DHE, cryosections from fresh-frozen retinas of WKY and SHR were incubated with superoxide dismutase (SOD, $300 \, U \, ml^{-1}$, Dako) or apocyanin (APO, $100 \mu M$) for 10 min before DHE incubation.

NAD(P)H oxidase activity measured by lucigenin-enhanced chemiluminescence

The capacity of the retina to produce superoxide anion in a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent way was assessed using an LB 9507 luminometer (Berthold Systems, Aliquippa, PA, USA) by measuring superoxide-enhanced lucigenin ($0.5 \mu M$, Sigma) chemiluminescence in the presence or absence of NAD(P)H ($30 \mu M$, Sigma) (Sicard et al., 2006). The results are expressed in relative light units per gram of dry tissue. To verify the specific effect of NAD(P)H oxidase and superoxide anion, WKY and SHR retinas were incubated with apocynin ($100 \mu M$, Sigma) or superoxide dismutase ($300 \, U \, ml^{-1}$, Dako) before NADPH incubation.

Retinal lipid analyses

Total lipids from the retinas were extracted according to the Folch procedure (Folch et al., 1957) using 2:1 chloroform-methanol (v:v) and 0.73% NaCl in water. One aliquot of total lipids was used to determine the proportions of lipid classes (phospholipids, cholesterol, cholesteryl esters, diacylglycerols, triacylglycerols) using a combination of thin layer chromatography on silica gel-coated quartz rods and a flame ionization detector (Iatroscan System, Iatron, Tokyo, Japan) according to the technique first published by Ackman (1981). The remaining part of total lipids was used to determine retinal fatty acid composition. Total lipids were transesterified with boron trifluoride in methanol (7% w/v) according to Morrison and Smith (Grech et al., 1996). The fatty acid methyl esters (from acyl radicals) and dimethylacetals (DMA, from alkenyl radicals as an index of plasmalogen levels) were analysed on a Hewlett-Packard (Palo Alto, CA, USA) 5890 series II gas chromatograph equipped with a split/splitless injector, a flame ionization detector, and a BPX 70-silica capillary column ($120 \, m \times 0.5 \, mm$ internal

diameter film thickness $0.25 \mu m$; SGE, Melbourne, Australia). The injector and the detector were maintained at 250 and $280^\circ C$, respectively. Hydrogen was used as the carrier gas (inlet pressure 300 kPa). The oven temperature was fixed at $60^\circ C$ for 1 min, then increased from 60 to $200^\circ C$ at a rate of $20^\circ C \, min^{-1}$ and left at this temperature until the end of the analysis. DMA were identified by comparison with commercial or synthetic standards and data were computed using DIAMIR software (JMBS Ins., Portage, MI, USA).

Determination of plasma cholesterol levels

Total and high-density lipoprotein (HDL) cholesterol as well as triglyceride plasma concentrations were measured in a Cobas Fara analyser (Roche, Basel, Switzerland). Low-density lipoprotein (LDL) cholesterol was calculated with Friedwall methods.

Statistical analysis

All data are expressed as means $\pm$ s.e.m. Statistical analyses were performed with a two-factor analysis of variance (ANOVA) test (SigmaStat); the two factors were rat strain (SHR vs WKY) and rosuvastatin treatment. ANOVA was followed by inter-group pair-wise comparisons with Tukey HSD multiple comparisons.

Results

Physiological and hemodynamic parameters

Body weights were the same for WKY and SHR rats (Table 1) whether they received rosuvastatin treatment or not. HDL, LDL and total cholesterol levels in plasma were higher in WKY than in SHR ($P < 0.05$). No difference was observed between WKY and SHR for triglyceride plasma levels. Rosuvastatin treatment did not modify plasma cholesterol and triglyceride levels in WKY or SHR. SBPs were dramatically higher in SHR than in WKY (175 mm Hg ± 2 vs 131 ± 1 mm Hg, $P < 0.001$). In SHR, 3 weeks of rosuvastatin treatment decreased SBP by 18 mm Hg ($P < 0.01$). HR was similar for SHR and WKY animals, and was not modified by rosuvastatin treatment.

Retinal function

Before any treatment (11 weeks of age), the ERG b-wave amplitude was significantly lower in SHR than in WKY

Table 1 Body weight and plasma lipid levels of WKY and SHR after 3 weeks of treatment with rosuvastatin

Rats treatments	WKY		SHR	
	Control	ROSU	Control	ROSU
Rat weight (g)	301 ± 6	290 ± 5	298 ± 3	296 ± 6
Total cholesterol (mg l^{-1})	710 ± 30	690 ± 20	$520 \pm 20*$	480 ± 10
HDL (mg l^{-1})	360 ± 10	350 ± 10	$280 \pm 10*$	280 ± 10
LDL (mg l^{-1})	260 ± 20	230 ± 10	$150 \pm 20*$	130 ± 10
Triglycerides (mg l^{-1})	450 ± 60	530 ± 70	480 ± 60	580 ± 50

Abbreviations: WKY, Wistar Kyoto; SHR, spontaneously hypertensive rats; HDL, high-density lipoprotein; LDL, low-density lipoprotein, ROSU, rosuvastatin. Results are presented as means $\pm$ s.e.m.
*$P < 0.05$ SHR control vs WKY control; $n = 10$ for each group.

(Figure 1). This was maintained at 14 weeks, even though a significant increase in the ERG b-wave amplitude was observed in SHR rats between 11 and 14 weeks of age ($P < 0.05$). Treatment with rosuvastatin did not modify the ERG b-wave amplitudes in SHR or WKY groups. No changes were observed in the oscillatory potentials (OPs) in the recorded ERGs. No variations were observed in the ERG a-wave amplitude whatever the animal strain, the age and the gavage administered (data not shown).

Retinal oxidative stress

Superoxide anion production was analysed in the three retinal cell layers: photoreceptors (Figure 2a), bipolar cells (Figure 2b) and in ganglion cells (Figure 2c). No differences were observed in photoreceptors and in bipolar cells in SHR, WKY with or without rosuvastatin treatment. However, markers of superoxide anion production were 40% higher in SHR ganglion cells than in those from WKY ($P < 0.01$). Rosuvastatin treatment for 3 weeks decreased superoxide anion production in SHR ganglion cells ($P < 0.01$). No differences were observed in the other retinal layers of SHR and WKY rats. Retinal NAD(P)H oxidase activity (Figure 3) in SHR was more than two-fold higher in WKY rats. Rosuvastatin treatment for 3 weeks significantly decreased retinal NAD(P)H oxidase activity in SHR, without affecting that of WKY rats.

Retinal lipid composition

Proportions of retinal lipid classes in rosuvastatin-treated and non-treated WKY and SHR rats are presented in Table 2.

In retinas, phospholipids were the major lipid class with approximately 87% of total lipids. The remaining lipids were exclusively cholesterol in the free and esterified forms; no diacylglycerols or free fatty acids were detected. No differences were observed between treated and non-treated WKY and SHR for total saturated, total monounsaturated and total polyunsaturated fatty acid levels (data not shown). Among the polyunsaturated fatty acids, the levels of docosahexaenoic acid (DHA; 22:6 $n-3$) were found to be unaffected by the animal strain and by the rosuvastatin treatment (data not shown).

The retinal plasmalogen composition (DMA 16:0/C16:0 and DMA 18:0/C18:0) of treated and non-treated WKY and SHR is presented in Table 3. No differences were observed between non-treated WKY and SHR animals for DMA 16:0/ C16:0 and DMA 18:0/C18:0 ratios. However, rosuvastatin treatment increased DMA C16:0/C16:0 by 10% and DMA C18:0/C18:0 by 5% in SHR as compared to SHR control ($P < 0.05$), due to increased DMA C16:0 and DMA C18:0 concentrations ($P < 0.05$).

Discussion

Our study demonstrated that (1) the retinal functionality was lower in SHR than in WKY controls, (2) SHR displayed an increase in superoxide anion production in retinal ganglion cells, an increase in NAD(P)H oxidase activity and (3) that rosuvastatin was able to decrease these markers of retinal oxidative stress in SHR but has no effect on the retinal functionality.
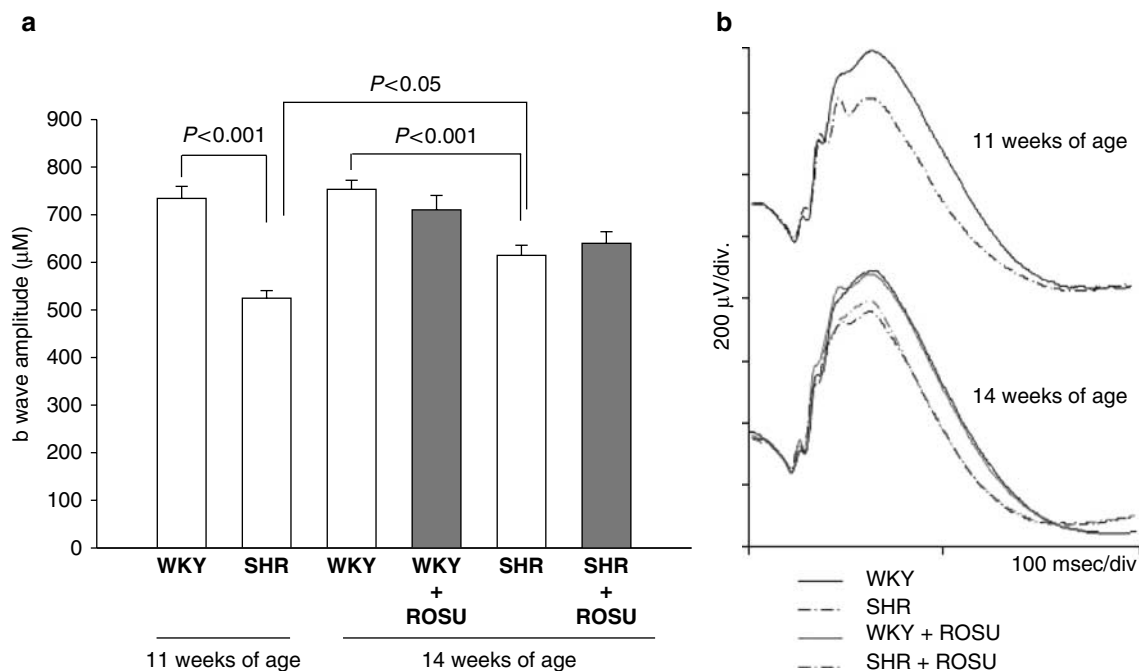

Figure 1 (a) ERG b-wave amplitudes of WKY and SHR at 11 and 14 weeks of age treated with vehicle (water) or ROSU (10 mg kg^{-1} day^{-1} for 3 weeks). Results are expressed as means ± s.e.m. ($n = 10$ for each group). (b) Representative ERG traces of WKY and SHR at 11 and 14 weeks of age treated with vehicle (water) or rosuvastatin. ERG, electroretinogram; ROSU, rosuvastatin; WKY, Wistar Kyoto; SHR, spontaneously hypertensive rats.
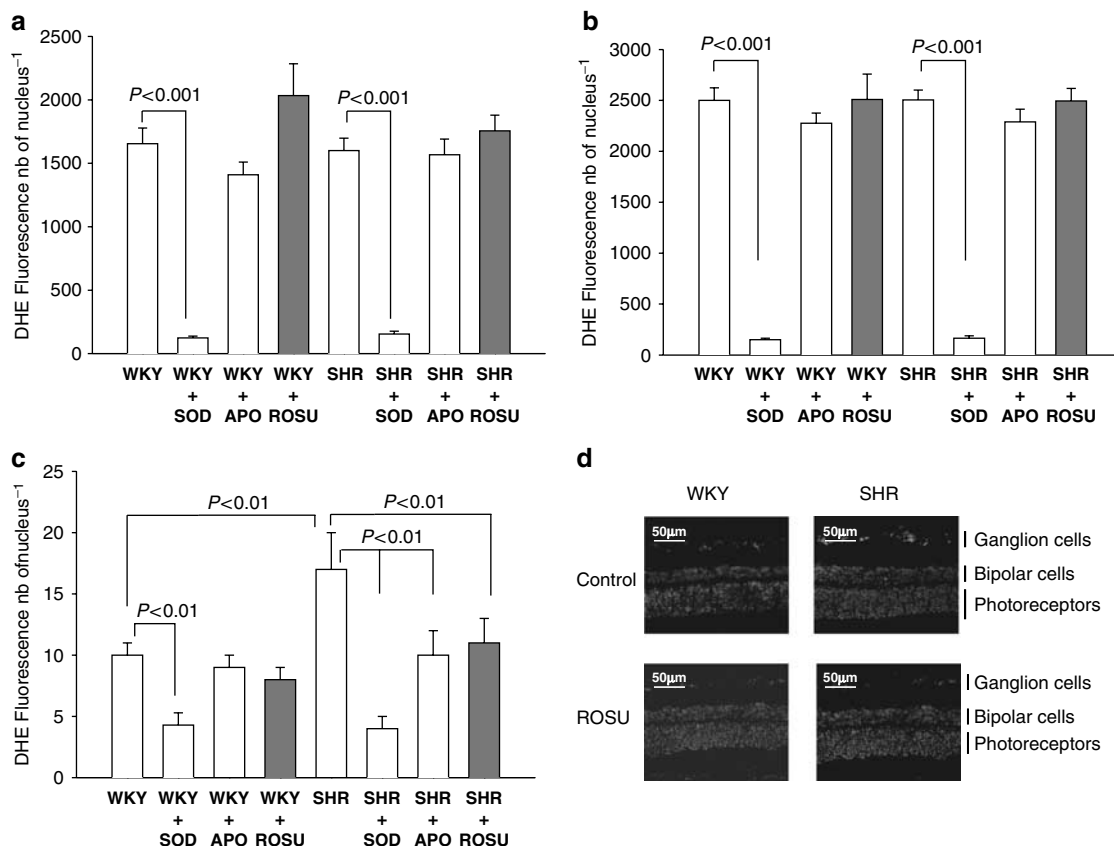
Figure 2 Effect of ROSU (10 mg kg^{-1} day^{-1} for 3 weeks) on DHE fluorescence intensity (**a**) in photoreceptor cells (**b**) bipolar cells and (**c**) ganglion cells (**d**). Localization of this fluorescence on frozen eye sections. The slices were incubated *in vitro* with or without SOD or APO. Results are expressed as means ± s.e.m. (*n* = 10 for each group). ROSU, rosuvastatin; DHE, dihydroethidium; SOD, superoxide dismutase; APO, apocyanin.
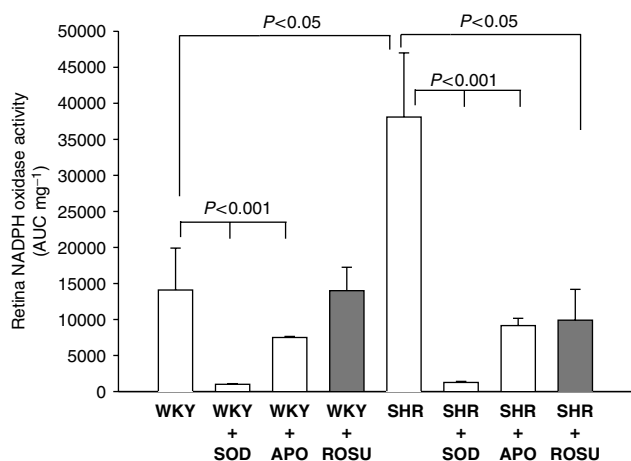

Figure 3 Effect of ROSU (10 mg kg^{-1} day^{-1} for 3 weeks) on retinal NAD(P)H oxidase activity evaluated with lucigenin in WKY and SHR. The retinas were incubated *in vitro* with or without SOD or APO. Results are expressed as means ± s.e.m. (*n* = 10 for each group). ROSU, rosuvastatin; WKY, Wistar Kyoto; SHR, spontaneously hypertensive rats; SOD, superoxide dismutase; APO, apocyanin.

Table 2 Retinal phospholipid, free cholesterol and esterified cholesterol levels of SHR and WKY treated or not with rosuvastatin for 3 weeks (% of total lipids)

Rats treatments	WKY		SHR	
	Control	ROSU	Control	ROSU
Phospholipids	88.7 ± 0.8	86.2 ± 1.2	86.9 ± 0.6	87.2 ± 1.3
Free cholesterol	7.9 ± 0.7	9.4 ± 0.6	8.7 ± 0.6	8.1 ± 0.6
Esterified cholesterol	3.3 ± 0.4	4.5 ± 0.7	4.3 ± 0.9	4.8 ± 1.0
Phospholipids/total cholesterol	7.6 ± 0.6	6.3 ± 0.5	6.9 ± 0.4	6.4 ± 0.7

Abbreviations: SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto; ROSU, rosuvastatin.
Results are mean ± s.e.m (*n* = 6 for each group).

SHR is a genetically engineered/selected model of hypertension and appears to have some visual dysfunctions (Rogers *et al.*, 1993) caused by retinal capillary remodelling (Bhutto and Amemiya, 1997; Dosso *et al.*, 1999) and probably related to increased blood pressure. Bellini *et al.* (1995) demonstrated that the electrical activity of the retina is altered early in the course of hypertension in humans. Population based-studies show that hypertensive retinopathy signs are strongly associated with blood pressure, but inconsistently associated with cholesterol and other risk factors of atherosclerosis (Yu *et al.*, 1998; Wong and McIntosh, 2005). Hypertension leads to dysfunction of many

Table 3 Retinal composition in plasmalogen composition in SHR and WKY treated or not with rosuvastatin for 3 weeks (% of total fatty acids)

Rats Treatments	WKY		SHR	
	Control	ROSU	Control	ROSU
Plasmalogens				
DMA 160	0.66 ± 0.03	0.65 ± 0.01	0.71 ± 0.02	0.79 ± 0.01*
C160	16.12 ± 0.65	15.48 ± 0.33	15.72 ± 0.07	15.87 ± 0.17
DMA 160/C160	0.041 ± 0.002	0.042 ± 0.001	0.045 ± 0.001	0.050 ± 0.001*
DMA 180	2.43 ± 0.04	2.48 ± 0.02	2.36 ± 0.02	2.47 ± 0.04*
C180	21.91 ± 0.20	22.02 ± 0.06	21.51 ± 0.13	21.57 ± 0.08
DMA 180/C180	0.111 ± 0.002	0.113 ± 0.001	0.110 ± 0.001	0.114 ± 0.001

Abbreviations: SHR, spontaneously hypertensive rats; WKY, Wistar Kyoto; DAM, dimethyacetals; ROSU, rosuvastatin.
Results are mean ± s.e.m.
*SHR control vs SHR ROSU, $P < 0.05$ ($n = 6$ for each group).

organs including the eye, which is affected by retinal artery occlusion (Klein *et al.*, 1997).

Electroretinography is an accurate method to record retinal electrical activity and gives more relevant data on retinal cells dysfunction than other techniques such as light discrimination and visual acuity measurement, used to characterize the visual function of SHR (Rogers *et al.*, 1993). Rogers *et al.* (1993) suggested that the reason for retinal dysfunction in SHR was a reduced number of photoreceptors. Loss of photoreceptors is linked to a reduction in ERG a-wave amplitude (Machida *et al.*, 2000). As we did not observe any differences in the ERG a-wave amplitude between SHR and WKY, our results do not support this hypothesis. However, we clearly observed a retinal functional defect in SHR that may be linked to dysfunction of the inner retinal cell layers, mainly amacrine and bipolar cells, since the b-wave is generated by the response of these cells (Bui and Fortune, 2004). Nevertheless the mechanisms for this finding remain partly unresolved. Studies suggested that the amplitude of the b-wave can be in some situations an indicator of retinal oxygenation (Block and Schwarz, 1998; Luhmann *et al.*, 2005). It is now well known that OPs are one of the most sensitive parameters to be affected in retinal ischemia (Wachtmeister, 1998; Tzekov and Arden, 1999). However, in our studies, we were not able to show any modification in OPs of SHR when compared to WKY.

Two experimental studies have suggested that retinal damage induced by ischemia/reperfusion in SHR was closely related to oxidative stress and was reduced by antioxidant treatment such as superoxide dismutase (Szabo *et al.*, 1992; Hirose *et al.*, 2004). While it is well known that SHR exhibit increased levels of oxidative stress in several organs including the kidney, vessels (Rodriguez-Iturbe *et al.*, 2003; Touyz, 2004) and the brain (Ikeda *et al.*, 2003), no studies have evaluated the basal oxidative stress level in SHR retinas. Our study showed, for the first time, that superoxide anion production was higher in SHR ganglion cell layers than in WKY rats and that this was associated with an increase in NAD(P)H oxidase activity. NAD(P)H oxidase activity is known to be upregulated in SHR organs (vessels, heart, kidneys) (Griendling *et al.*, 2000; Rodriguez-Iturbe *et al.*, 2003), and could play an important role in the development of hypertension. Only a few results are available on the role of NAD(P)H oxidase in the retina. Al-Shabrawey *et al.* (2005)

demonstrated that NAD(P)H oxidase activity was required for any hypoxia-stimulated increase in vascular endothelial growth factor expression and retinal neovascularization. The oxidative damage resulted in cell death, in part by apoptosis, causing morphologic changes in the retina and a decline in retina function (Cingolani *et al.*, 2006).

Lipid composition in the retina, in particularly the level of DHA, and the balance between $n-6$ and $n-3$ fatty acids are very important for visual functioning (SanGiovanni and Chew, 2005). Chronic dietary DHA deficiency alters the recovery of rod photoreceptor response in rodents and in nonhuman primates (Jeffrey *et al.*, 2002; Niu *et al.*, 2004). The ERG a-wave and b-wave could also be defective in rats consuming a balanced diet containing small amounts of $n-3$ polyunsaturated fatty acids with isomerized double bonds (Acar *et al.*, 2002), thus reinforcing the crucial role of DHA in retinal functionality. In our models, there was no difference between SHR and WKY retinas with regard to lipid content including DHA, plasmalogen and the $n-6/n-3$ ratio.

In our experimental conditions, the animals were given $10 \, mg \, kg^{-1} \, day^{-1}$ of rosuvastatin and this treatment did not lower plasma LDL, HDL, total cholesterol or triglyceride levels. These results confirm other studies showing that chronic rosuvastatin treatment (3 months) with the same concentration did not modify plasma cholesterol levels (Susic *et al.*, 2003). Furthermore, Davignon (2004) showed that the anti-inflammatory, antiaggregant and antioxidant effects of statin therapy were independent of the plasma cholesterol lowering effects.

In contrast, we observed an antihypertensive effect of rosuvastatin in the SHR model from the first week of treatment as has already been described by our group and others (Susic *et al.*, 2003; Sicard *et al.*, 2005). Statin therapy decreases inducible NO synthase and increases endothelial NO synthase expression in the vascular wall, which contributes to an enhancement of NO availability and can therefore restore endothelial function in hypertensive rats (Bayorh *et al.*, 2005). A small, but possibly clinically relevant, reduction in blood pressure associated with statin therapy has been reported in a number of studies; this effect has been observed in hypertensive patients (Glorioso *et al.*, 1999; Borghi *et al.*, 2000; Ferrier *et al.*, 2002).

To our knowledge, there are no studies available on the effect of rosuvastatin treatment in retinal function and

oxidative stress levels. In SHR, rosuvastatin was able to decrease the oxidative stress, demonstrated by the reduction in NAD(P)H oxidase activity in the ganglion cells. This effect was not associated with a decrease in retinal cholesterol levels. These results are in accordance with those of Erdos *et al.* (2006), who demonstrated that rosuvastatin inhibited specific NAD(P)H oxidase activity. However, rosuvastatin did increase phospholipid plasmalogens by 10% for DMA C16:0/C16:0 and by 5% for DMA C18:0/C18:0. Previous studies have suggested that plasmalogens may be protective by serving as antioxidants during lipoprotein oxidation (Zoeller *et al.*, 1988; Engelmann, 2004). Lovastatin increased the plasmalogen portion in erythrocyte membrane phospholipids (Brosche *et al.*, 1996). The increase in plasmalogen concentration in the retina of SHR treated with rosuvastatin could then be taken as a reinforcement of antioxidant defences.

Ko *et al.* (2005) suggested that an elevation of antioxidant concentrations might diminish the increase in ROS, but could not prevent subsequent damage. A number of studies concerning the role of oxidative stress in ocular degenerative pathways (Du *et al.*, 2003; Moreno *et al.*, 2004) concluded that the use of antioxidants could be a good therapeutic tool to prevent retinal degeneration. In addition, the relationship between eye diseases such as glaucoma, AMD and the use of cholesterol-lowering drugs has been examined in several clinical studies, but the results have been contradictory (Sen *et al.*, 2002; McGwin *et al.*, 2004, 2005; Wilson *et al.*, 2004). However, some of the results suggest that primary prevention with statins might reduce the risk of developing AMD or retinopathy (Sen *et al.*, 2002; McGwin *et al.*, 2005).

In conclusion, our study demonstrated, for the first time, that the increase in oxidative stress in retinal ganglion cells, if the SHR, was associated with increased NAD(P)H oxidase activity. In this genetically hypertensive model, rosuvastatin therapy could decrease production of retinal superoxide anion through the inhibition of NAD(P)H oxidase activity, independently of a reduction in plasma and tissue cholesterol levels, but might not be sufficient to restore retinal function. These findings point to a complex relationship between oxidative stress in the pathogenesis of retinal disease and hypertension. Additional investigations are warranted to investigate whether statins or other antioxidant agents may provide additional protection against retinal hypertensive diseases, particularly the role of isoprenoid metabolism, in the regulation of fundamental cellular processes in the retina.

Acknowledgements

Conflict of Interest

The authors state no conflict of interest.

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