Original Research

Atorvastatin protects against deleterious cardiovascular consequences induced by chronic intermittent hypoxia

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Abstract

Chronic intermittent hypoxia (IH), a major component of obstructive sleep apnea (OSA), contributes to the high risk of cardiovascular morbidity. We have previously demonstrated that IH-induced oxidative stress is involved in the hypertension and in the hypersensitivity to myocardial infarction. However, the mechanisms underlying these cardiovascular alterations are still unclear, as well as the role of potential protective treatment. Atorvastatin has pleiotropic actions, including increasing nitric oxide (NO) bioavailability and reducing inflammation and oxidative damage. The aim of this study was to evaluate the beneficial effect of a two time course of this treatment against the deleterious cardiovascular consequences of IH. Rats were divided into two groups subjected to chronic IH or normoxic (N) exposure. IH consisted of repetitive one-minute cycles (with only 30 s of a 5% inspired O_2 fraction) and was applied for eight hours during daytime, for 14 (simultaneous protocol) or 28 d (delayed protocol). Atorvastatin (10 mg/kg/ d) or its vehicle was administered during the 14 d simultaneous protocol or the last 14 d of the delayed protocol. For both protocols, systolic arterial pressure was significantly increased by 14 d IH exposure. Atorvastatin prevented this deleterious effect in the simultaneous protocol. Carotid artery compliance and endothelial function were significantly altered after 28 d but not after 14 d of IH exposure. Delayed atorvastatin administration preserved these vascular parameters. IH also increased hypersensitivity to myocardial infarction after 14 d exposure, and atorvastatin abolished this deleterious effect. IH also enhanced cardiac NADPH expression and decreased aortic superoxide dismutase activity after 14 d exposure. Atorvastatin significantly restored these activities. In conclusion, whereas IH rapidly increased blood pressure, myocardial infarction hypersensitivity and oxidative stress, compliance, endothelial function and the structural wall of the carotid artery were only altered after a longer IH exposure. Atorvastatin prevented all these deleterious cardiovascular effects, leading to a potentially novel pharmacological therapeutic strategy for OSA syndrome.

Keywords: atorvastatine, intermittent hypoxia, cardiovascular injuries, rat, OAS syndrome

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Introduction

Obstructive sleep apnea (OSA) syndrome, characterized by episodic cessation of airflow during sleep, is a highly prevalent disease, affecting 5–20% of adults.[1] OSA is strongly associated with increased cardiovascular morbidity and mortality. Patients with OSA syndrome have higher rates of systemic hypertension, coronary heart disease and stroke.[2,3] The altered endothelial and vascular function, inflammation and oxidative stress induced by OSA could be involved in mediation of deleterious cardiovascular consequences.[4] The gold standard treatment, continuous positive airway pressure reverses some of the chronic consequences of OSA;[5] but has limited long-term acceptance in some individuals.[6] It is thus very important today to develop innovative treatment against these deleterious consequences. Recurrent intermittent hypoxia (IH), a major component of sleep apnea, is known to be a key factor in determining deleterious cardiovascular consequences. Further to this, we have demonstrated that in a rodent model of IH exposure chronic IH induces both cardiac[7] and vascular injuries.[8,9] Recent animal studies showed that

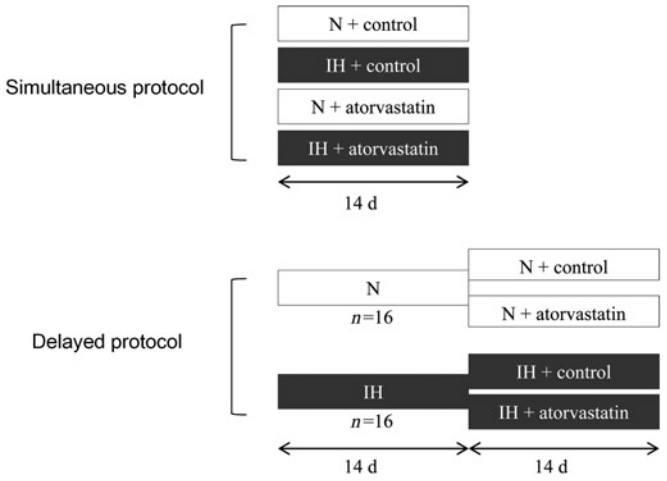

Figure 1 Schematic representation of both of the protocols used. $N = 8$ rats in each group. Rats were subjected to intermittent hypoxia (IH groups) or normoxia (N groups) and were treated with 10 mg/kg/d of atorvastatin or its vehicle (NaCl 0.9%, control or C groups)

oxidative stress is involved in IH-induced hypertension and myocardial infarction hypersensitivity.[10,11] However, the effect of IH on arteriolar reactivity and vascular wall distensibility is still unclear. It has been observed that the balance between the production of superoxide anions ($O_2^{\cdot -}$) and nitric oxide (NO) in the vessel wall plays an important role in the pathogenesis of vascular remodeling. We hypothesized that $O_2^{\cdot -}$ generated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase during IH could limit NO bioavailability, thereby impairing endothelium-dependent vasodilation.

Additionally, potential drugs targeting oxidative stress may have a protective effect against the deleterious consequences of IH. Statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, are known to reduce the number of cardiovascular events in patients with coronary heart disease and hypercholesterolemia. Their use is mostly well tolerated and safe: the commonly reported hepatic adverse effect is both asymptomatic and reversible. Pleiotropic protective effects-induced by statins include improvement of endothelial function, antithrombotic actions, reduction of vascular inflammatory process and an antioxidant effect.[12] Atorvastatin is also able to prevent endothelial dysfunction and hypertension experimentally, via the reduced production of reactive oxygen species (ROS).[13] This antioxidant effect proceeds from NADPH oxidase inhibition[14] or an increase in antioxidant defenses, such as enhanced superoxide dismutase (SOD) activity.[15] We have previously observed in the rat that atorvastatin is able to prevent IH-induced infarction aggravation as well as cardiac NADPH oxidase increase.[16,17]

Taken together, these observations allow one to assume that atorvastatin may also have beneficial actions on the deleterious vascular consequences of IH.

The aim of this study therefore was to investigate whether simultaneous or delayed atorvastatin treatment could prevent IH-induced cardiovascular injury, by protecting the myocardium and vessels from ROS.

Methods

Animals

This investigation conformed to the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and with French law and local ethical committee guidelines for animal research. The protocol received approval from the Direction des Services Vétérinaires de l'Isère, France. Male Wistar rats (275–299 g) were provided by Janvier (Le Genest-St-Isle, France), housed in controlled conditions and provided with standard rat chow.

IH protocol

We have developed an animal model of IH to study the influence of this condition on cardiovascular parameters. Rats were placed in plexiglas chambers connected into a timed solenoid valve system distributing nitrogen and air. IH chambers were flushed with air–nitrogen for 30 s to achieve hypoxia (5% O_2) followed by 30 s of compressed air to restore normoxia (N, 21% O_2). Control rat chambers were flushed with similar cycle of air–air only. This one-minute cycle was repeated for eight hours during 14 or 28 d according to experimental design. Body weight was registered daily.

Experimental design

Two experimental protocols for IH/N exposure and atorvastatin treatment were studied (see Figure 1):

- *Simultaneous protocol*: with four subgroups ($n = 8$ in each)

Rats were exposed to 14 d of IH or N and received simultaneously during the same period 10 mg/kg/d of either atorvastatin or its vehicle (NaCl 0.9%, control groups) by intraperitoneal injection just before exposure, in agreement with the literature.[18,19]

- *Delayed protocol:* with another four subgroups ($n = 8$ in each)

First, rats were exposed to 14 d of IH or N without atorvastatin (for induction of cardiovascular consequences), and then were exposed to 14 additional days of IH or N and concomitantly treated with either atorvastatin or its vehicle.

Indeed, it has been previously shown that 14 d of IH exposure induces cardiac injuries, hypertension and increased oxidative stress[10,11] and that these effects could disappear after 28 d of IH exposure.[10,11] The same duration (14 d) of protective treatments has been shown to be efficient against these IH-induced deleterious effects.[10,11] According to these previous results, these both IH exposure time courses have been chosen here with different beginnings of atorvastatin treatment in order to explore its protective effect after pathological dysfunction instauration.

All the experiments have been performed the following day of the last IH exposure.

Hematocrit

The hematocrit was measured at the end of both protocols by collecting blood in a specific glass tube (Brand, Finland) and centrifuging for 10 min at 1000 rpm.

Arterial blood pressure

Depending on the protocol, two methods were used.

Simultaneous protocol: As previously described,[7] arterial blood pressure was measured by a carotid catheter after 14 d of exposure and treatment. Rats were anesthetized by pentobarbital (60 mg/kg), the right carotid artery was cannulated with polyethylene tubing (PE-50) connected to a pressure transducer (Statham), and diastolic and systolic arterial blood pressure (SAP) was recorded on a data-acquisition system (PowerLab, ADInstruments, Oxford, UK).

Delayed protocol: The non-invasive method of plethysmography was used before and after atorvastatin treatment. Rats were anesthetized by 1% isoflurane. SAP was recorded using a piezo-electric sensor placed around the rat's tail, which was connected to the same data-acquisition systems and then analyzed with Chart 5 software (PowerLab). Carotid catheterization was also used at the end of this protocol to assess a difference in sensibility of these two methods.

Ischemia–reperfusion on the isolated heart

The experimental protocol used here has been described in details in our previous work.[10,11] Hearts were rapidly excised, immersed in 4°C Krebs–Henseleit (KH) solution and perfused using the Langendorff technique at a constant pressure. After 20 min of stabilization, a no-flow global ischemia was induced by stopping the perfusion for 30 min. The heart was then reperfused for 120 min. Coronary flow (CF) was measured periodically throughout the ischemia–reperfusion procedure, by collecting the effluent. Heart rate and left ventricular developed pressure (LVDP=difference between left ventricular systolic pressure and left ventricular end-diastolic pressure [LVEDP]) were both continuously recorded. At the end of the ischemiare-perfusion protocol, infarct size was determined using a colorimetric technique coupled to a computerized planimetric analysis and was expressed as a percentage of the both ventricles.

Vascular mechanical properties and reactivity

A segment of the left carotid artery was quickly excised and placed in a physiological buffer of the following composition: 135 mmol/L NaCl, 5 mmol/L KCl, 1.6 mmol/L $CaCl_2$, 1.17 mmol/L $MgSO_4$, 0.44 mmol/L KH_2PO_4, 2.6 mmol/L $NaHCO_3$, 0.34 mmol/L Na_2HPO_4, 5.5 mmol/L D-glucose, 0.025 mmol/L EDTA, 10 mmol/L HEPES (pH 7.4). The vessel was cleaned of adhering connective tissue and fat, and was then cannulated and mounted onto a pressure arteriograph (Living Systems Instrumentation Inc., Burlington, VT, USA,). The experiments were performed at 37°C in an organ bath filled with physiological buffer. Following a 15-min equilibration period, the vessel was transilluminated under an inverted microscope connected to a camera and a computerized system, allowing the continuous recording of the vessel outer diameter (OD). OD recording was performed while increasing the intravascular (transmural) pressure from 0 to 175 mmHg by steps of 25 mmHg (5 min per step). Compliance at 150 mmHg was defined as the diameter change in absolute volume at this pressure.

After the mechanistic study, the reactivity study was performed at 75 mmHg and the carotid artery was contracted using the smooth muscular cell-dependent vasoconstrictor phenylephrine (PE, 1 μmol/L) until maximum vasoconstriction was reached. The endothelial function was assessed by testing the relaxant effect of acetylcholine (Ach, 1 μmol/L) on carotid arteries precontracted with PE. Endothelium-independent function was assessed by testing the relaxant effect of sodium nitroprusside (SNP, 1 μmol/L), a nitric oxide (NO) donor, on carotid arteries precontracted with PE.

Intima-media thickness

OCT-embedded aorta and carotid sections (10 μm, $n = 8$ in each group) were stained with hematoxylin and eosin to assess global tissue morphology and elastic fibers. Intima-media thickness (up to 30 measurements on three non-contiguous vessel sections per animal) was measured with a light microscope (Eclipse 80i, Nikon France SAS, Champigny-sur-Marne, France).

Western blot analysis of NADPH oxidase expression

Myocardial and aortic samples ($n = 4$ in each group) for simultaneous and delayed protocols were collected, and protein concentrations were assessed using a Pierce BCA Protein assay kit (Thermo Scientific). Proteins were separated on a 12% SDS-acrylamide gel and transferred to nitrocellulose membranes, which were incubated with anti-p47-phox antibody (1/500; Santa Cruz sc-14015). Bound antibody was visualized by use of horseradish peroxidase-conjugated goat anti-rabbit antibody (1/5000; Santa Cruz sc-2004). Equal loading was confirmed by β-actin immunoblotting, on the same membranes. Relative densitometry was then calculated (Image J software).

Superoxide dismutase activity

SOD activity was measured using the SOD reagent Kit (#900-157, Stressgen) in the homogenate from the same groups. The superoxide anions are experimentally generated from the conversion of xanthine to uric acid by xanthine oxidase, and they convert WST-1 into WST-1 formazan (colored product that absorbs light at 450 nm). Absorbance is read at 450 nm every minute for 10 min. SOD activity from the sample is then determined from percent inhibition of the rate of this WST-1 formazan formation.

Statistical analysis of data

All data are presented as means ± standard error of the mean (SEM). SAP, OD, carotid contraction and relaxation, SOD activity, NADPH expression, intima-media thickness

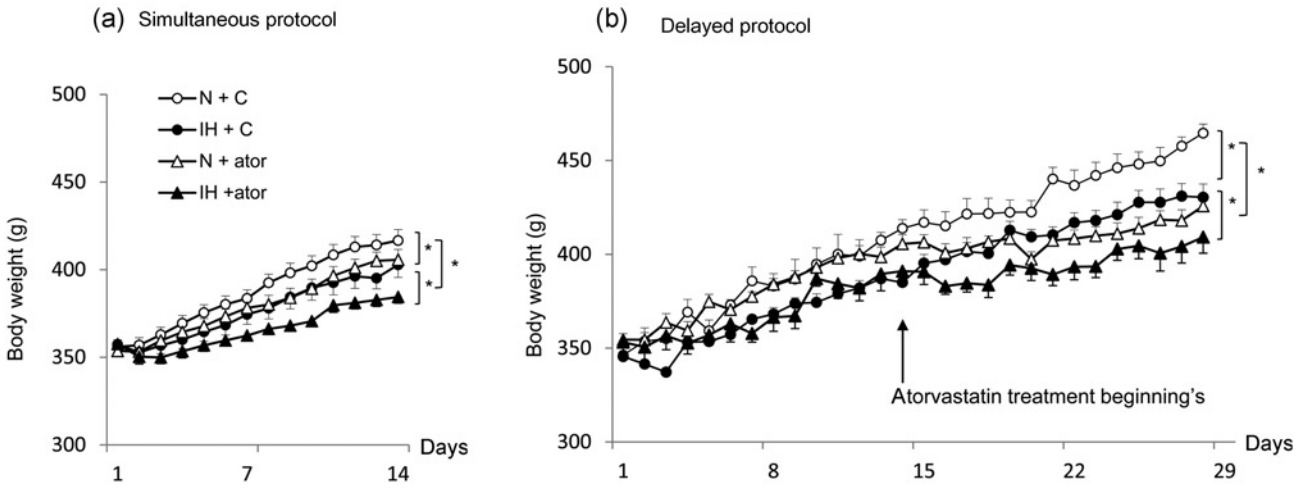

Figure 2 Body weight evolution during intermittent hypoxia (IH) or normoxia (N) exposure and atorvastatin treatment, which was significantly lower in IH than in N groups, after both 14 (a) and 28 d (b). Atorvastatin-treated rats also showed decreased body weight compared with control non-treated animals. $N = 8$ per group and data are mean ± SEM $^{*}P < 0.05$, two-way repeated measures analysis of variance

Table 1 Hematocrit from the eight experimental groups at the end of the simultaneous (14 d) and delayed (28 d) protocols

Hematocrit (%)	N + C	IH + C	N + ator	IH + ator
Simultaneous protocol	43.0 ± 1.6	46.6 ± 1.8*	41.4 ± 1.5	47.8 ± 0.4*
Delayed protocol	44.7 ± 1.6	56.0 ± 1.6*	42.5 ± 0.9	49.4 ± 1.5*†

$N = 8$ and data are mean ± SEM
*$P < 0.05$ versus corresponding N group
†$P < 0.05$ versus corresponding IH group, ANOVA

(IMT) and I/D data were compared by using two-way analysis of variance (ANOVA), with exposure and treatment corresponding to each factor. A two-way repeated-measures ANOVA was used for data with a time variability. *Post hoc* multiple comparisons were performed by using Tukey's tests. Statistical significance was set at $P < 0.05$.

Results

Body weight and hematocrit

Body weight was significantly lower in hypoxic than in normoxic groups, whatever the protocol used. Atorvastatin administration globally reduced the body weight of animals (Figure 2), as previously observed.[20] This effect should be due to its hypocholesterolemic effect but this remains to be clarified.

The increased hematocrit observed in IH groups compared with control groups constitutes an index of the hypoxia performed. Atorvastatin administration significantly lowered the IH-induced increase in hematocrit without abolishing it, only after 28 d (Table 1).

Blood pressure

As previously shown,[11] SAP was significantly enhanced in the IH compared with the N groups after 14 d in both protocols (Figure 3a, carotid catheterization and Figure 3b, plethysmography). At the end of the delayed protocol (at 28 d), IH tended to increase SAP measured by either carotid catheterization (Figure 3c) or plethysmography (same profile of data which are not shown) but this effect was not statistically significant.

In the simultaneous protocol, atorvastatin treatment abolished the IH-induced SAP increase, since there was no difference between N and IH groups (Figure 3a). In the delayed protocol, atorvastatin appeared to have no protective effect, since SAP at 28 d tended to be higher in the IH group compared with in the N group (Figure 3c). Moreover, diastolic, mean arterial pressure in the simultaneous protocol (at 14 d) and in the delayed protocol (at 28 d) measured by carotid catheterization have the same profile but was not presented here.

Myocardial infarction

Figure 4 shows that infarct size was significantly higher in the IH compared with the N group (IH + C: 53.2 ± 2.0% versus N + C: 41.3 ± 2.7%, $P = 0.025$) after 14 d exposure. Atorvastatin treatment significantly prevented the aggravation of infarction induced by IH exposure since the infarct size was not different between the IH + ator (41.6 ± 2.0%) and the N + ator (40.3 ± 2.4%) groups. The ventricular area measured in the four experimental groups was not different between groups (data not shown) indicating that IH did not affect this parameter. After 30-min global ischemia, CF and LVDP decreased markedly at reperfusion, and LVEDP increased significantly during ischemia in all hearts. All parameters measured were not significantly different between the four groups throughout the ischemia–reperfusion protocol. No difference in functional ventricular recovery was seen between the four groups during reperfusion (these data are presented as online supplementary data, in Table 2).

Vascular mechanical properties and reactivity

At the end of the simultaneous protocol, isolated carotid artery OD was similar between normoxic and IH groups

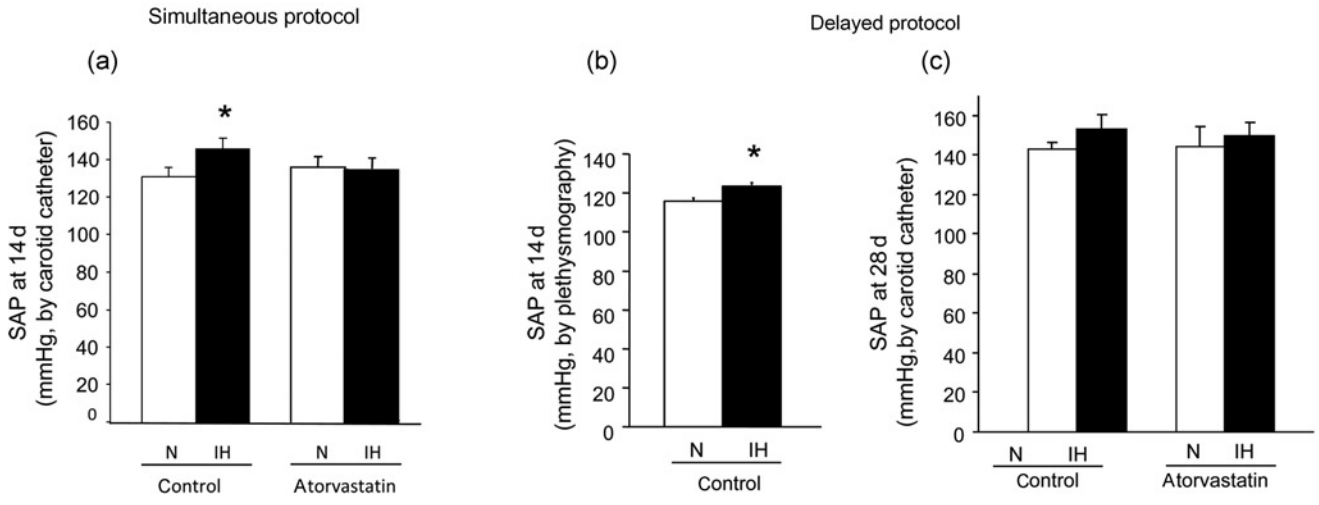

Figure 3 Systolic arterial pressure (SAP), measured invasively by catheterization or by plethysmography, after exposure to intermittent hypoxia (IH) or normoxia (N), in the simultaneous protocol (a, after 14 d with atorvastatin treatment) and in delayed protocol (b, at 14 d before atorvastatin treatment and c at 28 d after atorvastatin treatment). $N = 8$ per group and data are mean $\pm$ SEM $^{*}P < 0.05$ versus corresponding N group, two-way analysis of variance (ANOVA) for simultaneous protocol and two-way repeated measures ANOVA for delayed protocol

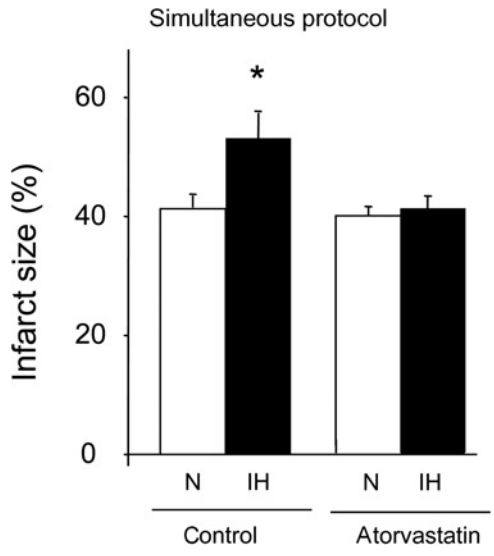

Figure 4 Infarct size expressed as a percentage of the ventricles in isolated hearts subjected to 30-min global ischemia followed by 120-min reperfusion, from rats exposed for 14 d to either intermittent hypoxia (IH) or normoxia (N) and treated or not with atorvastatin as per the simultaneous protocol. Data are mean $\pm$ SEM, $n = 8$ per group. $^{*}P < 0.05$ versus N group, two-way analysis of variance

throughout incremental increases in intraluminal pressure (data not shown). Compliance at 150 mmHg was also similar between normoxic and IH groups and was not modified by atorvastatin treatment (Figure 5a).

At the end of the delayed protocol, isolated carotid artery OD was not different between IH and N groups (data not shown), but compliance at 150 mmHg was statistically reduced in IH compared with N groups, supporting carotid rigidity induced by longer IH-exposure. Atorvastatin treatment prevented this mechanical alteration, since compliance was not different between the IH and the N-treated groups (Figure 5b).

The endothelial function of the carotid artery, assessed by Ach relaxation, was similar in the four groups at 14 d (simultaneous protocol, Figure 6a). However, endothelial-dependent vasodilation at 28 d (delayed protocol, Figure 6c) was significantly altered in the IH ($9.9 \pm 2.0\%$) than in the N ($41.1 \pm 1.4\%$) group. Atorvastatin delayed administration significantly prevented this IH-induced endothelial dysfunction. Moreover, endothelial-dependent vasodilation at 28 d (delayed protocol, Figure 6c) was globally lower than that at 14 d (simultaneous protocol, Figure 6a). That could be explained by the global increase in SAP observed (N groups, delayed protocol, Figure 3c) or by different experimental conditions. Finally, no difference in vasodilation to SNP was seen between the four groups in both protocols, showing no modification of the endothelium-independent function (Figure 6b and d).

Structural vascular wall

Hematoxylin and eosin staining on carotid artery samples at the end of the delayed protocol showed a significant IMT-increase in the IH (39.5 ± 1.9 μm) compared with the N (33.7 ± 2.6 μm) groups. Delayed atorvastatin administration abolished this deleterious effect (Figure 7).

Cardiovascular oxidative stress

At the end of the simultaneous protocol, myocardial NADPH oxidase (p47-phox subunit) expression was significantly increased by IH exposure, and atorvastatin treatment prevented this IH-induced pro-oxidative effect. No effect on aortic NADPH oxidase expression was seen after IH exposure (data not shown). IH exposure also significantly decreased aortic SOD activity, without modifying it in the myocardium, for the simultaneous protocol. Atorvastatin treatment preserved this antioxidative enzyme (Figure 8).

At the end of the delayed protocol, neither NADPH oxidase expression nor SOD activity measured in the

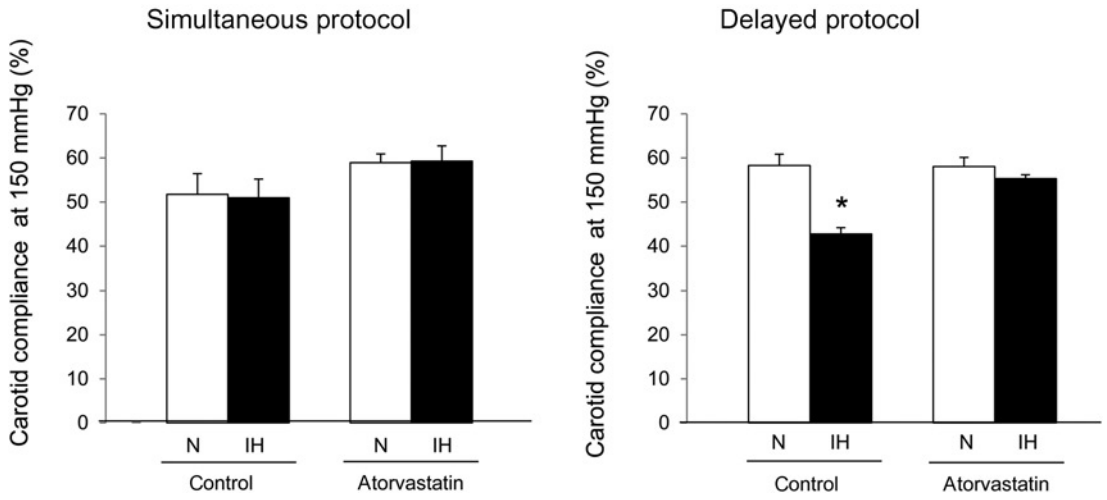

Figure 5 Effect of intermittent hypoxia (IH) or normoxia (N) exposure and atorvastatin treatment on the mechanical properties of the carotid artery at the end of the both protocols. Vascular compliance at 150 mmHg is expressed in the percentage of change in outer diameter between 0 and 150 mmHg intraluminal pressure. $N = 4$ per group and data are mean ± SEM $^{*}P < 0.05$ versus corresponding N group, two-way analysis of variance

myocardium and the aorta was modified by IH exposure or atorvastatin treatment (data not shown).

Discussion

The purpose of this study was to characterize the vascular injuries induced by IH exposure for 14 and 28 d. In addition, we sought to determine whether simultaneous or delayed atorvastatin administration could prevent these IH-induced alterations, by protecting the vessel from ROS.

Hemodynamic and cardiovascular alterations induced by IH

This study confirms our previous observations in rats exposed to IH for 14 d, which are that IH induces an early increase in arterial blood pressure.[11] It seems that this effect was maintained after a longer exposure, since SAP tended to be higher in the IH compared with the N group at 28 d, even if it was not statistically significant. For hemodynamic measurement, catheterization technique was more sensible than tail plethysmography, and gave more information as diastolic and mean arterial blood pressures. However, this invasive technique had limitations since it can be used only at the end of protocols. Finally, global lower SAP values from tail plethysmography can be explained by the influence of isoflurane. It is already known from previous studies that isoflurane decreases heart rate and mean arterial pressure in rats.[21]

We also confirm here that exposure to IH for 14 d is able to induce hypersensitivity to myocardial infarction.[11] We also noticed here that no difference in functional ventricular recovery was seen between the different groups during reperfusion, whereas atorvastatin treatment significantly prevented the IH-induced aggravation of infarction. This dissociation between functional parameters and infarction aggravation by IH or prevention by statin treatment are in accordance with previous results, showing that functional ventricular recovery is not modified either by IH[7,22] or by protective agents[11] independently of their respective effect on infarction. Moreover, infarct size development and functional parameters are not systematically dependent and infarct size measurement is considered as more reliable measure of cardiac ischemia–reperfusion sensitivity.[23] Finally, IH could have a different impact on the two ventricles (which was not investigated here) leading to an interventricular difference in sensitivity to ischemic injury (with a possible larger extent of the right ventricle injury) explaining potentially the discrepancy between IH-induced worsening of infarction and no difference in the left ventricular function recovery seen.

On the other hand, we observed that vascular mechanical and functional damage appears to increase with the length of exposure, and were seen at 28 d IH exposure but not at 14 d. Indeed, in the carotid artery, mechanical properties (seen by compliance at 150 mmHg pressure) and endothelial function were significantly reduced after 28 d in the IH group. It has been already shown that hypoxia induces a time-course response on endothelium-dependent vasodilation, depending of the vascular bed. In this study, IH attenuates endothelium-dependent vasodilation in skeletal muscle resistance arteries for 14 d and longer exposures (4 and 8 weeks) do not exacerbate it.[24] We also showed no difference in response to SNP in both protocols, confirming that the alteration in vasodilation observed was linked to endothelial cells and NO bioavailability. The impaired endothelial-dependent vasodilation seen after 28 d of IH exposure could thus potentially result from the early oxidative stress induced and the hypertension development. It seemed that IH also led to vascular remodeling, since IMT from the carotid artery was significantly higher after 28 d IH exposure. Accordingly, we have previously observed in mouse aorta an IH-induced increase in IMT and elastic fibers disorganization.[9] These vascular alterations could represent potential adaptations to IH-induced increased blood pressure.

However, a variety of experimental models of chronic IH has been developed. Indeed, the duration, frequency and

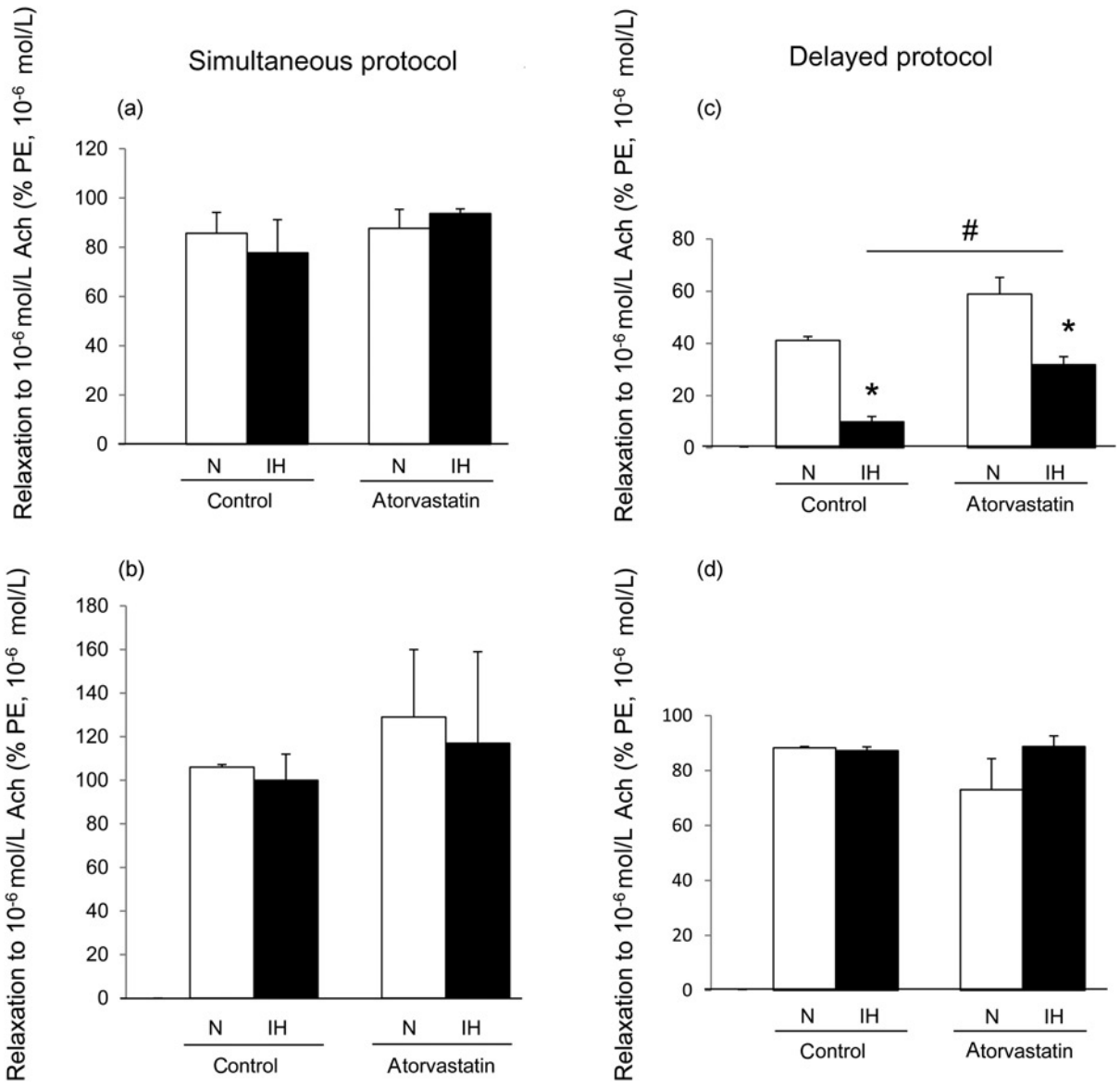

Figure 6 Relaxation in response to acetylcholine (Ach) and sodium nitroprusside (SNP) in carotid arteries precontracted with phenylephrine (PE), at the end of the both protocols. Vasodilation responses were measured under a no flow condition at 75 mmHg intraluminal pressure. Data are mean ± SEM, $n = 4$ per group. $^{*}P < 0.05$ versus corresponding N group and $^{\#}P < 0.05$, two-way analysis of variance

severity of hypoxic episodes used are critical factors determining whether chronic exposure to IH has beneficial or harmful effects[25]. Thus, chronic cyclic IH can have beneficial effects when few daily cycles of moderate intensity are used[26]. In contrast, more severe protocols with chronic repetitive short cycles designed to reproduce the cyclic IH pattern seen in OSA patients (as used here) result generally in deleterious cardiovascular effects.

Effect of IH on oxidative stress

We showed that NADPH-oxidase expression in the heart was enhanced after 14 d of IH exposure. SOD activity was also significantly attenuated in the thoracic aorta, suggesting that oxidative stress is involved in the early vascular consequences of IH. The role of this stress in the cardiovascular damage induced by a longer IH exposure remains to be clarified. Others studies using other IH-models demonstrate that IH has variable effects, depending on the length of exposure and the organ examined.[27] They found that IH does not affect oxidative stress markers in the circulation or cardiovascular system after one week, but that IH increases lipid peroxidation, NADPH oxidase expression and phosphorylation in the liver. All of these effects become statistically significant after four weeks. Another study shows in mouse that two weeks of IH exposure induces cardiac oxidative stress while following four weeks of exposure it is normalized to normoxic hearts.[10,11] In the IH model used here, it seems that oxidative stress is concomitant with IH induced alteration in blood pressure, suggesting that hypoxia inducible factor (HIF-1α) activation[28,29] is potentially implicated.

Indeed, we previously demonstrated that our IH-model is able to increase myocardial HIF-1α activity.[22] Thus, the mechanisms underlying cardiovascular damage induced by IH exposure remain to be determined, in particular the role of oxidative stress. It is possible that effects of chronic IH on oxidative stress could be only transient and could be relieved by some adaptive mechanisms.

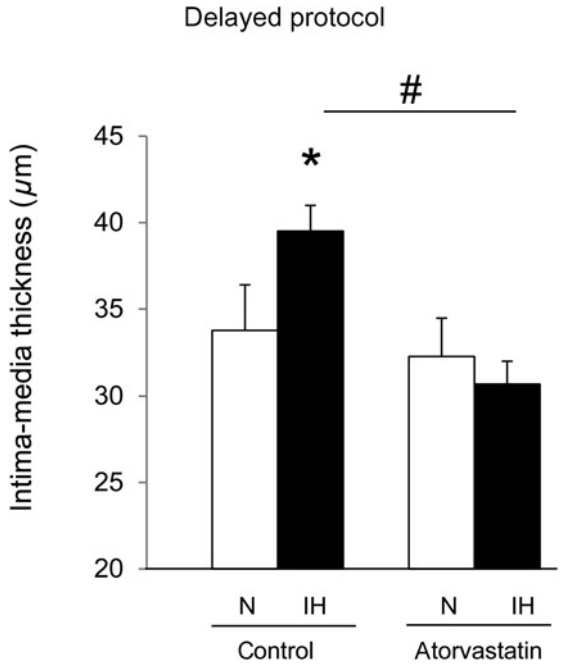

Figure 7 Intima-media thickness measured by hematoxylin and eosin staining, at the end of the delayed protocol in rat carotid arteries (10 × 40 magnification and digitally magnified insets). Data are mean ± SEM, $n = 8$ per group. $^{*}P < 0.05$ versus corresponding N group and $^{\#}P < 0.05$, two-way analysis of variance

Atorvastatin effect against IH-induced cardiovascular damages

Here we showed a spectacular protective effect of atorvastatin against the deleterious cardiovascular consequences of IH exposure. Indeed, this statin, administered throughout the 14-d IH exposure, was able to prevent IH-induced SAP and infarction increases, as well as cardiac NADPH oxidase increase and aortic SOD decrease in rats.

After a first 14 d exposure to IH, delayed atorvastatin administration (throughout the 14 following days) prevented late IH-induced vascular injuries. This treatment was even able to preserve carotid artery compliance and endothelial function, and prevented remodeling at 28 d IH exposure, even if it was started 14 d after the start of IH. This beneficial effect could be due to its early observed antioxidant property. Indeed, it has been recently shown in hypertensive rats that statins improve plasmatic and vascular oxidative stress and preserve endothelial NO synthase and Cu/Zn-SOD.[30]

Finally, the protective effect of atorvastatin could depend on the vascular bed studied[31] and could be completed by studying reactivity of resistance vessel such as mesenteric or skeletal muscle resistance arteries.

However, in view of pleiotropic effects of statins, mechanisms underlying the beneficial effects of atorvastatin observed here remain to be clearly identified and the role of oxidative stress should be clarified (by using an antioxidant instead of atorvastatin for example). It would also be interesting to investigate the effects of this drug in a chronic-like condition (i.e. longer than 28 d of IH exposure model) where many other changes could take place. These results may be more potentially relevant for clinical practice.

Conclusion and perspectives

These results show an early IH-induced hypertension, myocardial hypersensitivity to infarction and oxidative stress,

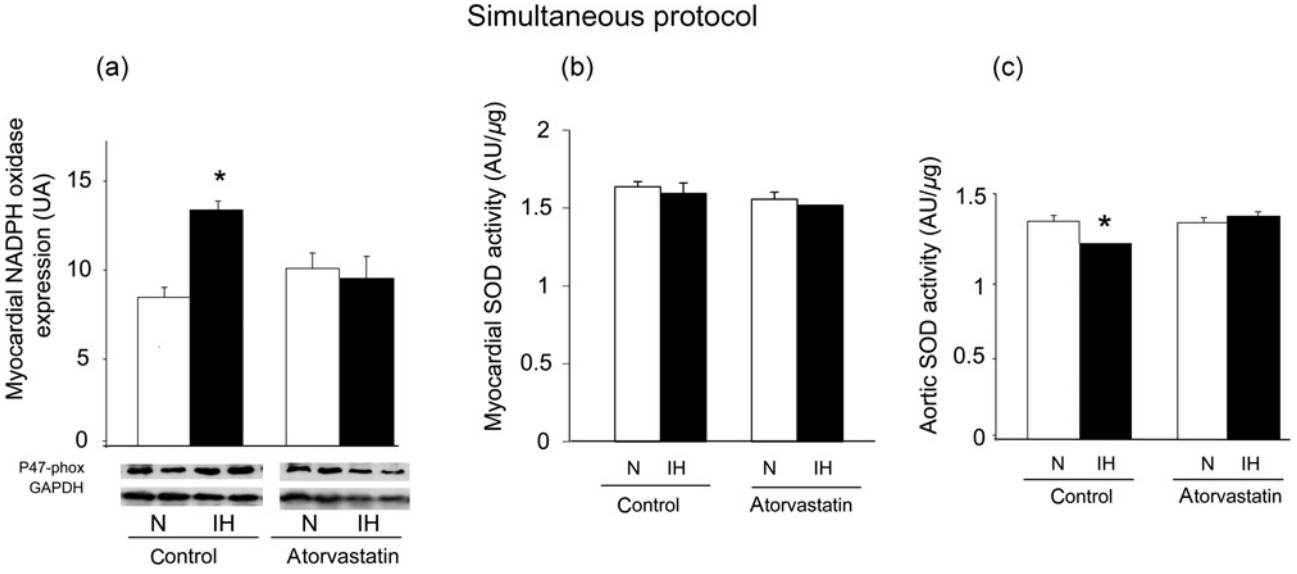

Figure 8 Western blot analysis of cardiac cytosolic ventricular p47-phox content (a) and superoxide dismutase (SOD) activity in the heart (b) and thoracic aorta (c) of rats exposed for 14 d to either normoxia (N) or intermittent hypoxia (IH) and treated or not with atorvastatin throughout exposure (simultaneous protocol). Data are mean ± SEM, $n = 4$ per group. $^{*}P < 0.05$ versus N group, two-way analysis of variance

with late evidence of impairment in carotid artery structure and function. Atorvastatin administration was efficient against early IH-induced cardiovascular injuries as well as against late mechanical and endothelial dysfunction. Thus, this treatment was able to confer cardiovascular protection against IH exposure even in a pathological context, which is more clinically relevant. Moreover, the beneficial effects of atorvastatin observed in the present study suggest that oxidative stress plays a pivotal role in the deleterious vascular consequences of IH. It has been shown clinically that oxidative stress is correlated to the severity of OSA,[32,33] and so it could potentially contribute to the development of hypertension and endothelial dysfunction in OSA patients.[34] Additional clinical studies are now required to explore more precisely the mechanisms by which oxidative stress is involved in these deleterious vascular effects. To conclude, the protective effect of atorvastatin observed here could lead to the development of clinical perspectives and a potentially novel pharmacological therapeutic strategy for OSA syndrome.

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