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PRESERVATION OF ERECTILE FUNCTION BY STATINS IN A RAT MODEL OF ERECTILE DYSFUNCTION INDUCED BY HYPERCHOLESTEROLEMIA

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ABSTRACT

Background and objective

To assess the effects of hypercholesterolemia (HC) on the quality of erections and to evaluate the effects of stain therapy in a rat model of erectile dysfunction (ED) induced by HC.

Material and methods

Sprague–Dawley rats were randomly divided into three groups (n=12 in each): control, HC, and HC with simvastatin treatment (HC+SS). The control was fed a normal chow diet, and the HC and HC+SS were fed a high-fat and high-cholesterol diet for 12 weeks. The HC+SS received simvastatin once daily via oral gavage for 12 weeks. Subsequently, the intraperitoneal glucose tolerance test (IPGTT), intracavernous pressure and mean arterial pressure, lipid profiles, expression of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS), oxidative stress (8-hydroxy-2-deoxyguanosine, 8-OHdG level), serum testosterone levels, and the ratio of collagen fibers (CF) and smooth muscle (SM) were evaluated in the serum and corpora tissue.

Results

IPGTT was not different among all groups. The HC showed markedly lower erectile parameters than the control. In contrast, the HC+SS showed preserved erectile function, improved lipid profiles, increased eNOS and nNOS, decreased oxidative stress, and minimized change in SM/CF ratio.

Conclusions
Our results suggest that oxidative stress damage by HC may cause ED and that statin therapy may have beneficial effects on preserving erectile function by improving lipid profiles and minimizing damage caused by oxidative stress.

Key Words: *erectile dysfunction; hypercholesterolemia; statin*

INTRODUCTION

Based on experimental results indicating that increased total cholesterol (TC) and decreased high-density lipoprotein cholesterol (HDL-C) are closely related with the risk of erectile dysfunction (ED),[1] many people believe that hypercholesterolemia (HC) is one of the causes of ED. Although the way in which ED could be caused by HC has not been clearly elucidated, many studies have shown that endothelial cells and erectile tissue damage observed during the hypercholesterolemic state are due to oxidative stress injury caused by HC.[2–4]

Pharmacological therapy, particularly with statins, is widely used and has been evaluated as an effective drug for treating HC. Will the correction of HC through statin therapy help preserve erectile function? Theoretically, the answer seems evident. Beneficial effects of statins, which improve erectile function in patient with ED, were reported by recent meta-analysis and systemic reviews of randomized trials.[5–8] However, Trivedi et al. [9] reported that simvastatin-treated group showed no difference in erectile function compared to placebo group. Furthermore, some studies have reported completely different outcomes, suggesting that using statins may cause ED.[10–12] To comprehend the effects of statin on erectile function, it is necessary to examine not only the mechanism of ED induced by HC but also the role of statins in HC and what functional changes are caused by this action. Therefore, in this study, we examined the effects of HC on the penile erection and investigated the effects of stain therapy on erectile function as functional and laboratory aspects in a rat model of ED induced by HC.

METHODS

Preparation of Simvastatin

Pharmaceutical-grade simvastatin tablets (20-mg tablets; Zocor Tab, MSD Korea, Merk & Co. Inc, Kenilworth, NJ, USA) were finely ground with a mortar and pestle and extensively mixed with fresh 1% methyl cellulose daily.

Animal Groups and Treatment Protocol

Thirty-six, male Sprague–Dawley rats (6-week old) were treated according to a protocol approved by the Institutional Animal Care and Use Committee at the School of Medicine, The Catholic University of Korea (approval Number: CUMC-2015-0123-02), and handled according to the National Institutes of Health guidelines. Rats were randomly divided into three groups (n=12 each): control, the normal control group; HC, HC induced by a high-fat and high-cholesterol diet; and HC+SS, HC rats administered simvastatin. All rats in the HC and HC+SS were fed a high-fat and high-cholesterol diet produced by Feedlab (Gyionggi-do, Korea). Rats in the control were fed a normal chow diet for 12 weeks. The formulation of high-fat and high-cholesterol diet is presented in Table 1. Rats in the HC+SS received simvastatin (10 mg/kg) for 12 weeks. Simvastatin was mixed with 1% methyl cellulose and administered orally once per day using an 8-Fr red Rob-Nel catheter. The intraperitoneal glucose tolerance test (IPGTT) was performed in all rats at 11 weeks. At 12 weeks, all rats were weighed, and intracavernosal pressure (ICP) and mean arterial pressure (MAP) were measured. Subsequently, blood was sampled from the internal carotid artery and corporal tissue was

TABLE 1 Composition of the High-fat and High-cholesterol Diet

Formulation	High-fat and high-cholesterol diet (HFD 45% cal + 2% Cholesterol)
	(g%)
Protein	24
Carbohydrate	41
Fat	24
Ingredient	g
Casein (from milk)	233.1
Corn starch	84.8
Sucrose	201.4
Dextrose	116.5
Cellulose	58.3
Soybean oil	29.1
Lard	206.9
Mineral mixture	52.4
Vitamin mixture	11.7
TBHQ	0.0
L-Cystine	3.5
Choline bitartrate	2.3
Total	1,000.0
Cholesterol	20
Cholic acid	5

HFD = high-fat diet; TBHQ = tertiary-butylhydroquinone.

collected. After the experiment, rats were euthanized using a 30% volume displacement rate of CO_2 in an induction chamber. The results from rats with delayed growth, disease transmission, and deaths that occurred during invasive procedure were excluded from the baseline data.

Intraperitoneal Glucose Tolerance Test

During week 11 of the experiment, an IPGTT was performed for all rats after fasting for 16 h. After intraperitoneal injection of 50% glucose (2 g/kg body weight), blood was sampled from the tail vein before and 15, 30, 60, and 120 min. Blood glucose concentrations were measured using a glucose meter (Accu-Chek; Roche Diagnostics, Indianapolis, IN).

Serum Levels of TC, Low-density Lipoprotein/Very Low-density Lipoprotein Cholesterol, High-density Lipoprotein Cholesterol, and Triglycerides

The serum TC, low-density lipoprotein cholesterol (LDL-C)/very low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels were measured by spectrophotometer using commercial assay kits. The detailed experimental procedure is the same as in our previous study.[13]

ICP and MAP Measurement

ICP and MAP were continuously monitored and measured by placing needles connected to the pressure transducer and recorder in the corpus cavernosum of the penile proximal portion and the right carotid artery. After placing pressure checking needles, cavernosal nerve stimulation was performed by giving electrical stimulation to the pelvic ganglion lateral to the prostate using a bipolar electrical stimulator. After ICP and MAP measurement, the corpus cavernosum was collected and split into two parts. The first part was cryopreserved, while the other part was fixed in formalin. The detailed experimental procedure is the same as in our previous study.[13]

Masson's Trichrome Staining

Masson's trichrome staining was performed in paraffin-embedded corporal tissue sections. The tissue processing and dyeing processes are the same as those in our previous study.[13] The stained muscle tissue was expressed in red color, and its distribution was estimated using Adobe Photoshop CS 8.0.

Endothelial Nitric Oxide Synthase Expression Test: Western Blot Analysis

In the corpora tissue, endothelial nitric oxide synthase (eNOS) expression was examined by Western Blot analysis. The tissue processing and the detailed experimental procedure are the same as in our previous study.[13]

Neural Nitric Oxide Synthase Expression Test: Immunohistochemistry

In the corpora tissue, neuronal nitric oxide synthase (nNOS) expression was examined by measuring the mean intensity from a digital image through a confocal microscope after immunohistochemistry. The detailed experimental procedure is the same as in our previous study.[13]

Oxidative Stress in Corpora Tissue (8-Hydroxy-2-deoxyguanosine)

In corpora tissues, oxidative stress was measured by determining the levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidatively modified stretch of DNA. The level of 8-OHdG was measured using commercial assay kits. The detailed experimental procedure is the same as in our previous study.[13]

Serum Testosterone

Serum testosterone measurement was performed using an enzyme-linked immunospecific assay (ELISA) testosterone detection kit (BioVendor-Laboratory Medicine Inc., Brno, Czech Republic).

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Data were evaluated using analysis of variance (ANOVA), with group comparisons performed using the Bonferroni post hoc test. $P<0.05$ was considered statistically significant.

RESULTS

Baseline Data

During this experiment, two anesthesia-related deaths (one in the control and one in the HC) and two technical errors (failure to catheterize the internal carotid artery; one in the HC and one in the HC+SS) occurred. Therefore, the test results of four rats were excluded from the baseline data.

Difference in Body Weight Gain and Serum Lipid Profiles

Body weight gain and serum levels of TC, LDL/VLDL-C, HDL-C, and TG of the three groups are presented in Table 2. HC showed a significantly higher body weight gain compared to the control. Administration of simvastatin significantly reduced body weight gain. HC showed increased serum TC, LDL/VLDL-C, and TG levels and decreased HDL-C levels compared to the control, whereas the lipid profiles of the HC+SS significantly improved compared with those of the HC.

Intraperitoneal Glucose Tolerance Test

Changes in blood glucose levels in each group are shown in Figure 1. There were no statistically significant differences in blood glucose levels among the three experimental groups ($P=0.3873$). High cholesterol diet and statin treatment did not show any relevance to the rise of blood glucose level.

In vivo Assessment of Erectile Function

Changes in ICP and MAP during resting and cavernosal nerve stimulation in the control, HC, and HC+SS are shown in Figure 2. The mean peak ICP±SD and ICP/MAP ratio ±SD for the control, HC, and HC+SS are shown in Table 3. The HC showed decreased peak ICP and ICP/MAP ratios compared with the control, and the ratios significantly improved in the HC+SS.

Masson's Trichrome Staining

The mean smooth muscle (SM)/collagen fiber (CF) ratio ±SD for the control, HC, and HC+SS is shown in Table 2 (Figure 3d). Compared with the control, the HC exhibited decreased SM and increased CF (Figure 3a, b). However, the HC+SS showed increased SM and decreased CF compared with the HC (Figure 3b, c).

Expression of eNOS in Corpora Tissue

The protein expression of eNOS and P-eNOS in the corpora tissue was evaluated using Western blot analysis. The mean densities ±SD of P-eNOS/

TABLE 2 Body Weight Gain, Lipid Profiles, SMC/CF Ratio in Corpora Tissue, Serum Testosterone Level, Expression of eNOS and nNOS in Corpora Tissue, and Oxidative Stress Marker

	Control	HC	HC+SS	p-value
Body weight gain (g)	281±40.31	352.39±46.68	300.9±33.03	0.003[a] 0.002[b] 0.006[c]
Lipid profiles				
Total cholesterol (μM)	92.38±5.18	170.76±23.71	125.92±3.45	<0.001[a] <0.001[b] <0.001[c]
LDL/VLDL-cholesterol (μM)	48.99±5.29	136.30±23.99	85.13±4.32	<0.001[a] <0.001[b] <0.001[c]
HDL-cholesterol (μM)	43.38±3.01	34.46±3.29	40.78±1.75	<0.001[a] <0.001[b] <0.001[c]
Triglyceride (mM)	1.13±0.15	2.73±0.19	1.62±0.27	<0.001[a] <0.001[b] <0.001[c]
Masson's Trichrome staining in corpora tissue				
SMC/CF ratio	0.14±0.05	0.05±0.02	0.10±0.06	0.013[a] <0.001[b] <0.001[c]
Serum testosterone level				
Serum testosterone (pmol/L)	3.30±0.99	1.27±0.95	1.43±0.59	<0.001[a] <0.001[b] 0.317[c]
Expression of eNOS in corpora tissue				
P-eNOS/eNOS	0.53±0.07	0.25±0.04	0.46±0.06	<0.001[a] <0.001[b] <0.001[c]
Expression of nNOS in corpora tissue				
Expression of nNOS	279.86±86.57	67.05±21.15	161.46±47.73	<0.001[a] <0.001[b] <0.001[c]
Oxidative stress marker				
8-OHdG (ng/mL)	3.01±0.97	10.24±1.39	7.55±0.97	<0.001[a] <0.001[b] <0.001[c]

HC = high-fat and high-cholesterol diet; SS = simvastatin; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein; HDL = high-density lipoprotein; SMC = smooth muscle cell content; CF = collagen content; eNOS = endothelial nitric oxide synthase; P-eNOS = phosphorylated endothelial nitric oxide synthase; nNOS = neural nitric oxide synthase; 8-OHdG = 8-Hydroxy-2-deoxyguanosine.
Data are expressed as mean ± standard deviation.
[a]*One-way ANOVA test, overall comparison.*
[b]*Comparison between the control and HC groups.*
[c]*Comparison between the HC and HC+SS groups.*

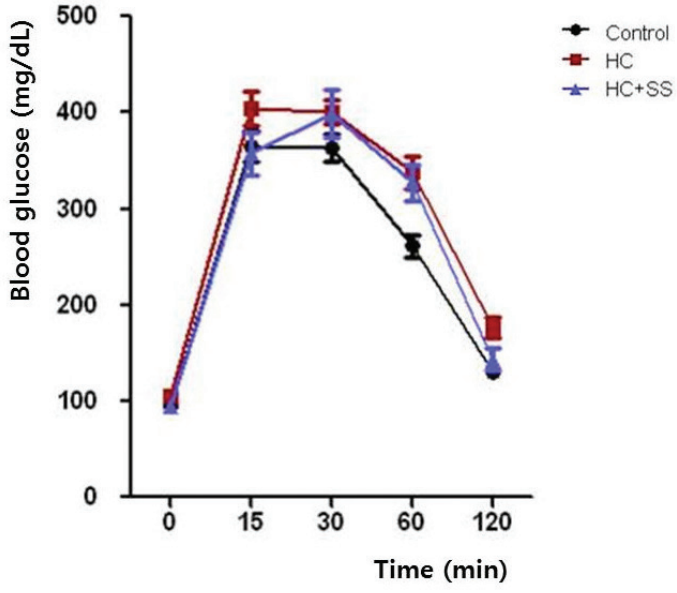

FIG. 1 Intraperitoneal glucose tolerance test (IPGTT). The blood glucose levels, measured at before and 15, 30, 60, and 120 min after intraperitoneal injection of 50% glucose. There were no statistically significant differences among the experimental groups ($p = 0.3873$).

eNOS for the control, HC, and HC+SS are shown in Table 2. There was no difference in total eNOS protein expression among the three groups. However, HC showed a significantly lower expression of P-eNOS protein compared with the control, and this decrease significantly improved in the HC+SS (Figure 4a, b).

Expression of nNOS in Corpora Tissue

The expression of nNOS in the dorsal penile nerve was analyzed using immunohistochemical staining (Figure 5a–c). The mean intensities ±SD of the nNOS-positive areas for the control, HC, and HC+SS are shown in Table 2. The expression of nNOS significantly decreased in the HC compared with the control; however, it was significantly greater in the HC+SS than in the HC (Figure 5d).

Measurement of Oxidative Stress in Corpora Tissues

Levels of oxidative stress in corpora tissues were assessed quantitatively by measuring 8-OHdG using ELISA. The mean levels ±SD of 8-OHdG for the control, HC, and HC+SS are shown in Table 2. HC showed a significantly greater oxidative stress than that in the control group. However, oxidative stress was significantly lower in the HC+SS than that in the HC.

Serum Testosterone

The mean levels ±SD of serum testosterone for the control, HC, and HC+SS are shown in Table 2. Serum testosterone level was lower in the HC and HC+SS than that in the control. Statistically significant difference was not found between the HC and HC+SS.

DISCUSSION

The findings to be noted in our study were as follows: (1) during IPGTT, no abnormal blood glucose level elevations were observed in the HC and HC+SS compared to the control; (2) statin treatment preserved erectile function by ameliorating lipid profiles and reducing oxidative stress caused by HC; (3) NOS and nNOS protein expressions were increased in the HC+SS, which may contribute to the preservation of erectile

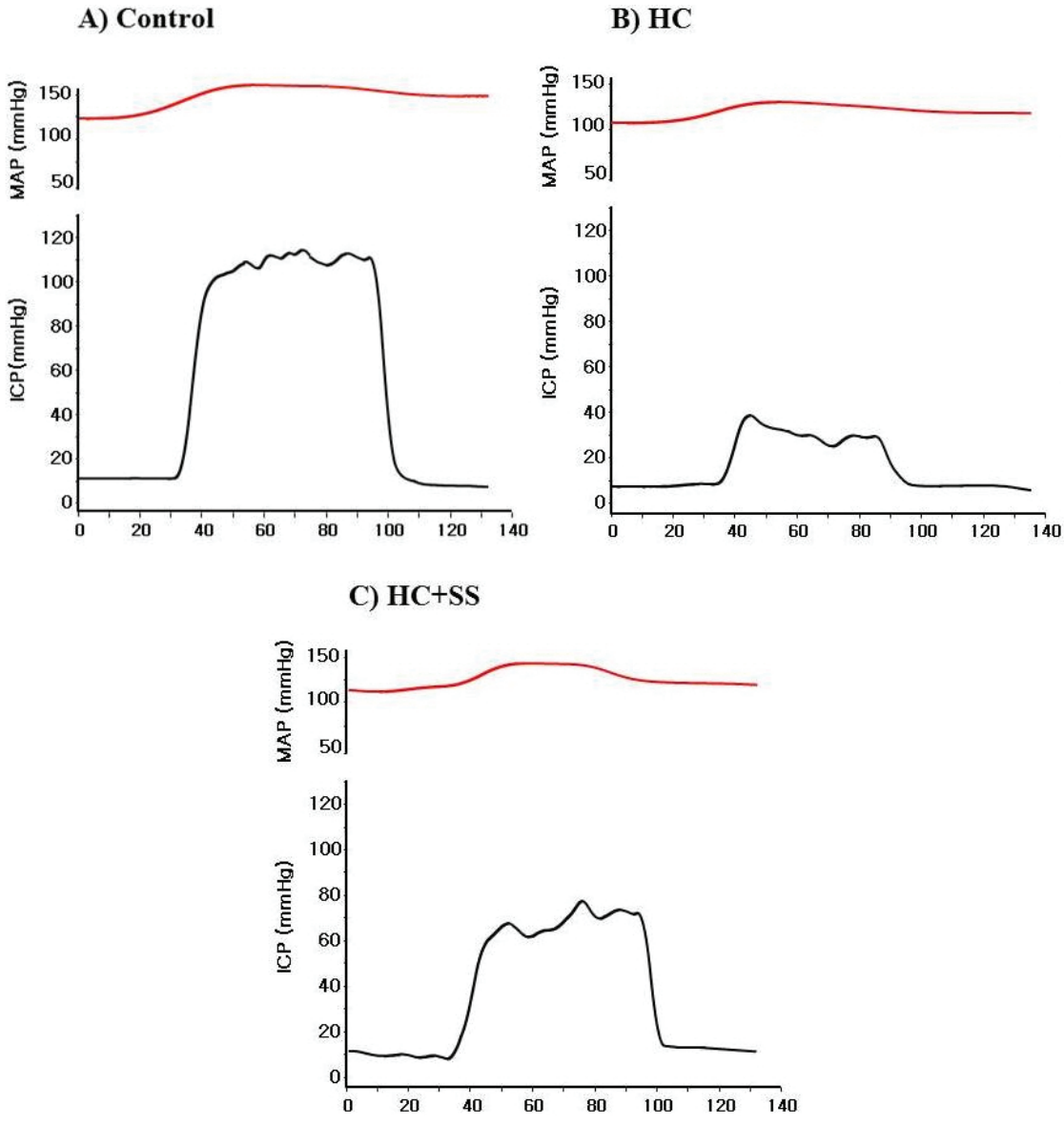

FIG. 2 In vivo Assessment of Erectile Function (ICP and MAP) . The changes in ICP and MAP during resting and cavernosal nerve stimulation in the control, HC, and HC+SS groups (a,b,c).

function in association with increased NO bioactivity; and (4) the decreased level of 8-OHdG and the increased expressions of eNOS, nNOS, and SM/CF ratio in the HC+SS may be explained by antioxidant effects of statins.

It is believed that endothelial dysfunction and erectile tissue dysfunction are closely related to the main mechanism of ED.[14,15] Many studies have been performed to clarify the link between HC and ED.[8,16] The results of these studies indicated that dysfunction of endothelium-dependent and endothelium-independent relaxations of the corpus cavernosum,[2,17–19] decreased cavernosal content of endothelial cells, decreased SM function, and increased collagen content[17] were observed in hypercholesterolmic men and

TABLE 3 Intracavernous Pressure in Response to Electrical Stimulation of the Cavernous Nerve in Rats from Each Experimental Group

	Control	HC	HC+SS	p-value
Peak ICP	106.87±10.03	28.25±8.04	76.37±23.20	<0.001[a] <0.001[b] <0.001[c]
ICP/MAP ratio	0.86±0.15	0.26±0.05	0.69±0.21	<0.001[a] <0.001[b] <0.001[c]

HC = hypercholesterolemia; ICP = intracavernosal pressure; MAP = mean arterial pressure; SS = simvastatin.
Data are expressed as mean ± standard deviation.
[a]One-way ANOVA test, overall comparison.
[b]Comparison between the control and HC groups.
[c]Comparison between the HC and HC+SS groups.

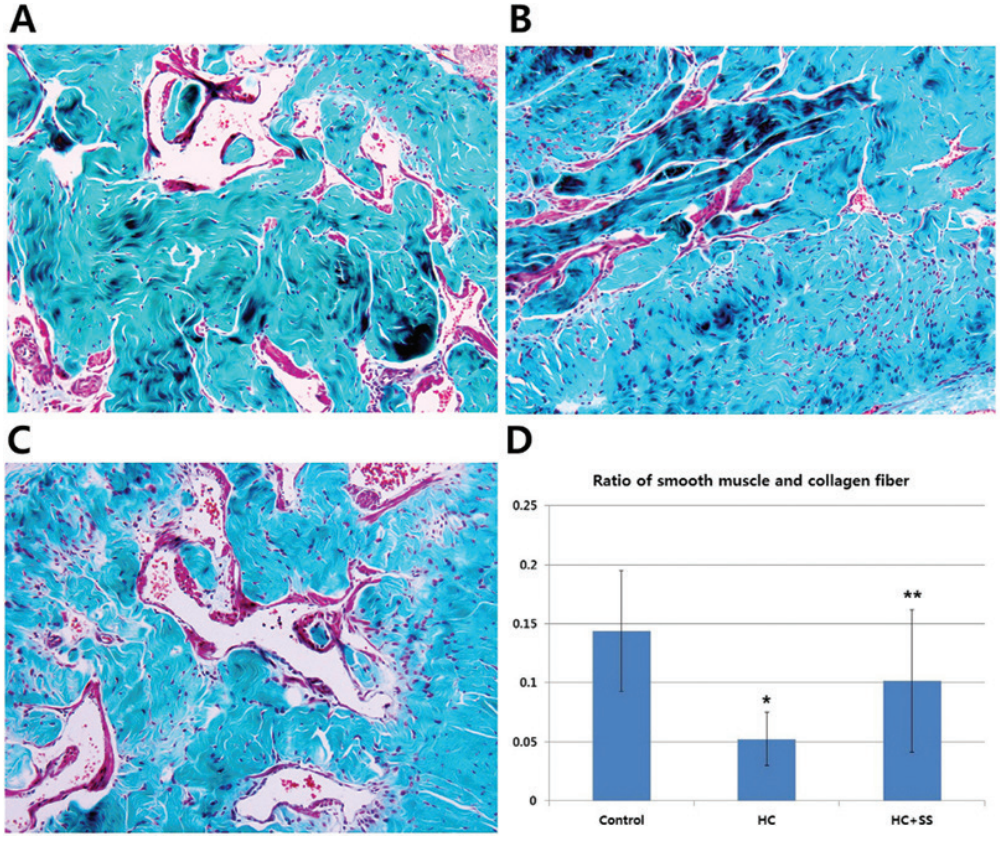

FIG. 3 Masson's trichrome staining of the corpora tissue. Smooth muscle and collagen fibers are shown in red and blue, respectively (magnification: x200) (a,b,c). Ratio of smooth muscle and collagen fibers in the corpora tissue (d).
Data are expressed as mean ± standard deviation (SD).
** Significant difference between the control and hypercholesterolemia (HC) groups.*
*** Significant difference between the HC and HC+SS groups.*

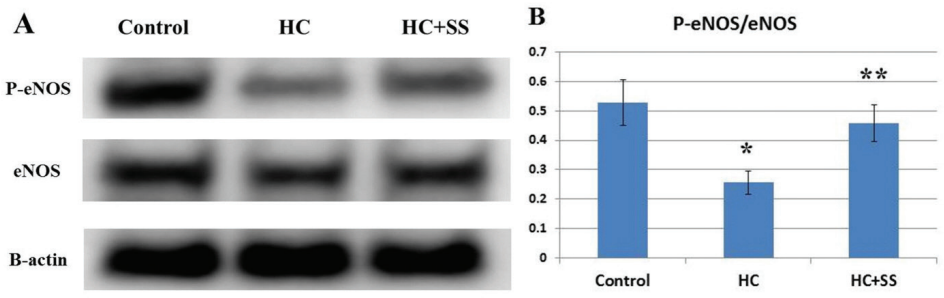

FIG. 4 Phosphorylated-endothelial nitric oxide synthase (P-eNOS) protein expression in corpora tissue. Western blot analysis of P-eNOS and eNOS in corpora tissue (a). Densitometric analysis of P-eNOS relative to eNOS (b).
Data are expressed as mean ± standard deviation (SD).
** Significant difference between the control and hypercholesterolemia (HC) groups.*
*** Significant difference between the HC and HC+SS groups.*

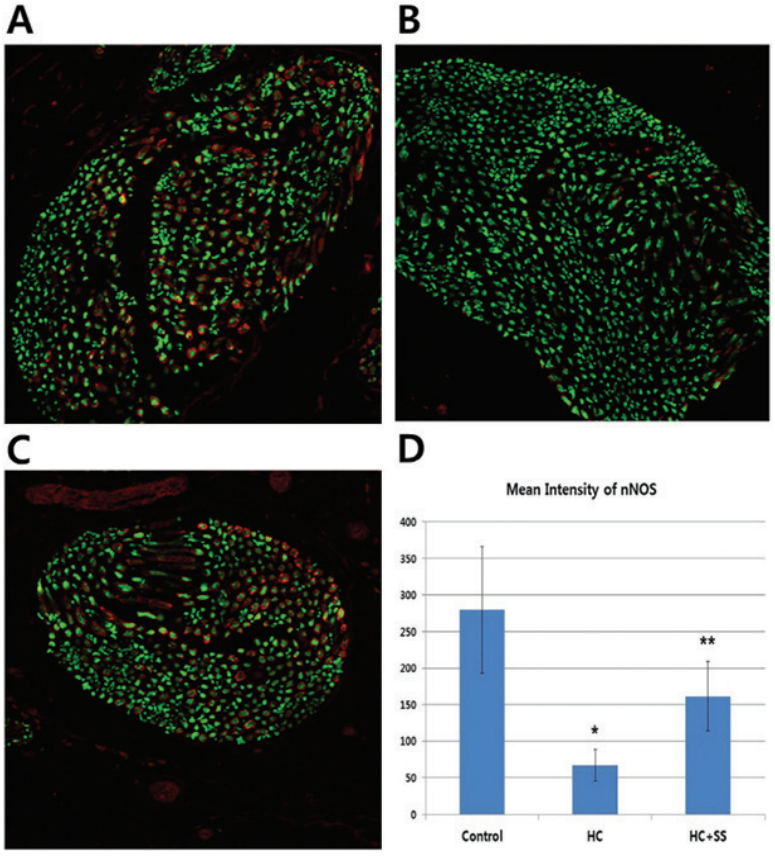

FIG. 5 Immunostaining of neuronal nitric oxide synthase (nNOS) in the dorsal penile nerve. Immunostaining for nNOS (red) and β-III tubulin (green) in the dorsal penile nerve. (magnification x400) (a,b,c). Mean intensity of nNOS expression for the dorsal penile nerve cross section (d).
Data are expressed as mean ± standard deviation (SD).
** Significant difference between the control and hypercholesterolemia (HC) groups.*
*** Significant difference between the HC and HC+SS groups.*

animals. These findings are thought to be caused by oxidative stress damage induced by HC in vascular endothelial cells and erectile tissue.[2–4]

The occurrence of oxidative stress due to HC is thought to be caused by the breakdown of the balance between the formation of superoxide anions and detoxification of superoxide anions by antioxidant defense systems. In HC, the protein expression of nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase and eNOS uncoupling were increased,[20] which led to increased formation of vascular superoxide anions, which in turn causes imbalance. Superoxide anions can not only directly inactivate NO,[21] which is the most important neurotransmitter that mediates the relaxation of the SM layer during erectile process, but also react with NO to form reactive nitrogen species. Peroxynitrite, one of the reactive nitrogen species, is especially highly toxic and can induce tissue injury, eNOS uncoupling, and oxidative damage to DNA, proteins, and lipids.[22]

Therefore, the imbalance created by the increased formation of superoxide anions in HC induces oxidative stress, and this may reduce eNOS activity and NO production,[23–27] impair the expression of eNOS and nNOS,[23–25,28] and dysregulate the cyclic guanosine monophosphate (cGMP) signal transduction pathways.[4] These various actions are thought to be the mechanisms of ED in HC.

In our study, decreased eNOS and nNOS expressions, increased 8-OHdG levels, and decreased SM/CF ratios in the corpus cavernosum were observed in the HC compared to the controls. We thought that these results suggest the occurrence of oxidative stress due to HC, which may cause vascular endothelial and corporal tissue damage, resulting in a decrease in the peak ICP and ICP/MAP ratio compared to the control.

Among the various drugs used for HC treatment, statins (HMG-CoA reductase inhibitor) are the most routinely used drug for first-line therapies because they are well-tolerated cholesterol lowering agents. Statins inhibit the enzyme HMG-CoA reductase, which converts HMG-CoA into mevalonate during de novo synthesis of cholesterol and results in the reduction of hepatic synthesis of cholesterol, with a compensatory increase in the number of receptors with a high affinity for LDL-C on the hepatocyte membrane.

Interestingly, many studies reported that statins exhibit antioxidant activities in a variety of ways, including inhibition of the uptake and generation of oxidized-LDL,[29,30] attenuation of vascular and endothelial superoxide anion formation by inhibition of NADH oxidases,[31–33] and preservation of the relative levels of vitamin E and C, and endogenous antioxidants[34–36] independent of the cholesterol-lowering mechanism.[37] The antioxidant activities of statins are known to have clinically beneficial functions, called pleiotropic actions, in vascular tissue, kidney, bone, and glucose metabolism.[38] These antioxidant properties of statins may lead to an increase in NO bioavailability, promotion of re-endothelialization, and inhibition of inflammatory responses, especially in vascular tissue, resulting in the restoration or improvement of endothelial function. Therefore, the endothelium-dependent effects of statins are speculated to contribute to most of the advantageous effects of statin therapy for ED. In our study, the HC+SS showed increased eNOS and nNOS expressions and decreased 8-OHdG levels in the corporal tissue. These findings demonstrated that the antioxidant effects of statins diminished oxidative stress damage by HC in the vascular endothelium. Consequently, the production of NOS, preservation of the SM/CF ratio in the corpus cavernosum, and preservation of erectile function were observed through increased peak ICP and ICP/MAP ratios.

Several studies have reported that statins have negative effects on ED.[10,12] Corona et al.[39]

reported that statin treatment may cause overt primary hypogonadism and may be regarded as a risk factor for ED. Hyyppa et al.[40] and Schooling et al.[41] reported that statin therapy reduced serum testosterone levels in hypercholesterolemic men and women. These reports seemed to be based on the ability of statins to impede presqualenic steroid synthesis within the testis by inhibiting mevalonate formation and eventually hinder testosterone production. However, Sniderman et al.[42] suggested that reduced serum testosterone levels may have less clinical significance regarding the occurrence of ED due to statin therapy because the average changes are small, the range of normal values for testosterone is wide, and the relationships between testosterone concentration, sex drive, and function are unclear. Moreover, a few studies have reported that diet-induced HC lowers serum and testicular testosterone levels by an unknown mechanism and suggested that HC is an independent risk factor for testicular dysfunction.[43–45] In our study, the HC and HC+SS showed decreased testosterone levels compared with the control. However, the HC and HC+SS showed differences in erectile function. Therefore, we presumed that the preservation of erectile function by statin therapy may be due to the antioxidant effect of statins, which is either independent of the reduced testosterone production by statins or can be explained by the possibility that statins did not decrease testosterone levels enough to cause erectile ED. Therefore, future studies should be conducted to elucidate the mechanism responsible for lower testosterone levels in HC and whether statin therapy can reduce testosterone levels enough to cause ED.

CONCLUSION

The results of this study suggest that oxidative stress damage by HC may cause ED and that statin therapy may have beneficial effects on preserving erectile function by ameliorating lipid profiles and minimizing oxidative stress damage. We suggest that clinicians should consider a lipid profile test during the evaluation of patients with ED and statins as a treatment option for ED patients with HC.

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