Experimental study on preventive effects of statin and ARB for metabolic syndrome : using a new animal model, obese stroke-prone spontaneous hypertensive rats

Koichi Masuno, Kumiko Takemori, Takao Inoue, Kazuo Yamamoto
and Hiroyuki Ito

*Department of Pathology, Kinki University, Faculty of Medicine,
Osakasayama, Osaka 589-8511, Japan*

Abstract

SHRSP/IDmcr-*fa*/*fa* (SHRSP/fatty) rats are a new animal model that have the potential to develop severe hypertension, obesity, hyperlipidemia, and hyperglycemia, followed by arteriopathy and glomerulopathy in the kidneys. Thus, SHRSP/fatty rats seem to be the most severe animal model of human metabolic syndrome. Using this unique animal model, we investigated how HMG-CoA reductase inhibitor (statin), a well-known drug for hypercholesterolemia, and angiotensin II receptor blocker (ARB), a widely-used anti-hypertensive drug, affected the pathophysiology related to metabolic syndrome. The statin increased the mRNA expression of adiponectin and leptin, decreased the expression of TNF-alpha gene, and increased the secretion of high molecular weight (HMW) adiponectin, without a lipid-lowering effect.

ARB increased both total adiponectin and HMW adiponectin, independent of blood pressure lowering. Histologically, the incidence of renal lesions, such as angionecrosis and glomerulosclerosis, was decreased in both treated groups. Except for well-known pharmacological effects of these drugs, the additional medicinal benefit of the statin depended on its anti-inflammatory effect, and that of ARB probably depended on its direct effect on adipocytes. It was considered that the increase of HMW adiponectin was enhanced by both pathways, and this may be a common factor of the protective effects of both drugs on pathophysiological damages in SHRSP/fatty rats.

Key words : SHRSP/IDmcr-*fa*/*fa* rats, HMG-CoA reductase inhibitor, angiotensin II receptor blocker, adiponectin

Introduction

It is generally accepted that cardiovascular disease in metabolic syndrome (MS) is associated with four main risk factors, obesity, hyperlipidemia, diabetes, and hypertension.[1] For better understanding of the pathophysiology and establishment of therapeutic strategies against such conditions, we need to reproduce the clinical conditions of MS in an animal model.

As a severe model of MS, SHRSP/IDmcr-*fa*/*fa* (SHRSP/fatty) rats were established by hybridizing stroke-prone spontaneously hyper-

tensive rats of the Izumo strain (SHRSP/Izm) and Zucker fatty (ZF) rats.[2] SHRSP/fatty rats show the combined features of two phyletic lines, such as obesity, hyperlipidemia, diabetes, and severe hypertension.[3] All of these rats suffered from such systemic disorders and died from stroke or renal damage. This means that SHRSP/fatty rats are a unique and useful animal model because their symptoms and causes of death are similar to those humans with MS.

In recent years, HMG-CoA reductase inhibitors (statins) have been generally prescribed for hyperlipidemic patients, especially for hyper-

cholesteremic patients. As is well known, statins have pleiotropic effects, such as inducing the vasohypotonic gene, suppressing the vasoconstrictor gene, and arresting the secretion of inflammatory cytokines. These effects contribute to improvements in hemodynamics as they control the expression of circulation-related genes.[4-7]

On the other hand, angiotensin II receptor blockers (ARB) are generally prescribed for hypertensive patients. In hypertensive animal models, as in humans, ARB have beneficial effects not only for lowering blood pressure but also for the prevention of hypertensive end organ damage.[8-11]

The primary purpose of this experiment was to reveal whether statins or ARB affect the pathophysiology of SHRSP/fatty rats and, if so, are there any additional mechanisms of the beneficial effects of these drugs. The results of this study should reveal the novel pharmacological aspects and modes of action of statins and ARB in SHRSP/fatty rats and give valuable information for the treatment of MS.

Methods

Animals

Male SHRSP/fatty were obtained from the Disease Model Cooperative Research Association (Kyoto, Japan) and were housed in specific pathogen-free conditions at 23°C under a 12-h light/dark cycle. All rats were fed a stroke-promoting diet (Funabasi SP, Funabashi Farms Co., Ltd., Chiba, Japan). Groups of 6 rats were administered 100 mg/kg/day pravastatin (Daiichi Sankyo Co., Ltd., Tokyo, Japan) or 0.1 mg/kg/day olmesartan (Daiichi Sankyo) at 13 weeks of age for 5 weeks, and 8 rats were set as a control. The dosage of ARB was carefully determined in the preliminary study to avoid marked reduction of blood pressure. Also, the dosage of statin was determined according to the results obtained by the manufacture. Body weight and blood pressure were measured once a week by the balance and tail-pulse pick-up methods without anesthesia. All rats were treated according to the Guidelines for Animal Experimentation (Japanese Association for Laboratory Animal Science, 1987). At the end of the experiment, after 12h fasting state, blood samples were drawn under anesthesia with pentobarbital sodium from the posterior vena cava, and then

the animals were euthanized by exsanguination by cutting both the abdominal aorta and vena cava, and their organs/tissues (brain, heart, kidney, liver, lung, pancreas, spleen, testis, thigh muscle, and thoracic aorta) were collected and fixed in 10% neutral-buffered formalin. In addition, retroperitoneum adipose tissue was obtained freshly for adipocyte size determination or kept at −80°C for gene analysis.

Adipocyte isolation and cell size determination

Adipocytes were isolated from retroperitoneum adipose tissue of male SHRSP fatty rats. The number of isolated adipocytes was estimated by counting an aliquot of a predetermined volume of cells on a 6-well plate. Prior to cell size determination, the obtained adipose tissue was processed using protease, filtrated through mesh, and fixed with osmium. This procedure allowed the cells to retain their original spherical shape. Collected fixed cells were poured into a 6-well plate and the cell size of adipocytes was determined using a microscope (LSM 5 PASCAL, Carl Zeiss Co., Ltd., Jena, Germany). Images of adipocytes were captured and the diameter calculated using a Mac SCOPE (Mitani Corporation, Fukui, Japan). Cell diameters were recorded and the mean and standard deviation were automatically calculated in μm after calibration with standard length parameters.

Blood measurements

Blood was drawn from the posterior vena cava under pentobarbital sodium anesthesia and centrifuged. Plasma was obtained and subjected to analysis using an automated clinical chemistry analyzer (Automatic Analyzer 7180; Hitachi, Tokyo, Japan). The measured parameters were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine (Cre), blood urea nitrogen (BUN), total protein (TP), glucose (Glu), free fatty acid (FFA), triglycerides (TG), total cholesterol (T-CHO), and low-density lipids (LDL). In addition, the plasma concentration of insulin, adiponectin and leptin was determined by ELISA (Shibayagi Co., Ltd., Gunma, Japan and B-Bridge International, Inc., CA, USA, respectively). All samples were run in duplicate, and mean values were calculated.

Westernblot study

Fifty-fold-diluted equal amounts of plasma (10 μL) were run on 5-20% gradient polyacrylamide gels (ATTO Corporation, Tokyo, Japan) before being transferred to

polyvinylidene difluoride membranes (ATTO Corporation) and blocked in Tris-buffered saline containing 5% nonfat dry milk for 1 hour at room temperature (RT). The blot was then incubated for 1 hour at RT with adiponectin antibody (Bio Vision, CA, USA). The antigen-antibody complexes were visualized using horse-radish peroxidase-conjugated anti-rabbit anti-body (1 : 3000) and detected by a luminescent image analyzer (Fuji Film, Tokyo, Japan).

Gene analysis

RNA was isolated from homogenized adipose tissue and kidney using QIAzol Lysis Reagent (Qiagen, Maryland, USA) and chloroform, and RNA purification was carried out using the RNeasy Lipid Tissue Mini Kit and RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. All samples were treated with DNase 1 (RNase Free DNase set ; Qiagen).

For first-strand cDNA synthesis, equal amounts (2 μl) of total RNA were reverse-tran-scribed using 100 units of reverse-transcriptase (Takara Bio Inc., Shiga, Japan) and 50 pmol Oligo dT Primer (Takara Bio). Transcript levels for adiponectin, leptin, and TNF-alpha were quantified in adipose tissue and monocyte chemoattractant protein-1 (MCP-1) was quantified in kidney by real-time PCR (7900HT Sequence Detection System ; Applied Biosystems, Foster City, CA, USA). Primers were obtained from Takara Bio. cDNA was amplified under the following conditions : 95°C for 30 seconds, followed by 45 cycles of 5 seconds at 95°C and 30 seconds at 60°C, using SYBR Premix Ex Taq 2 (Takara Bio). The primer and probe concentrations were 100 nmol/L. Formula $2^{-\Delta\Delta Ct}$ ($\Delta\Delta Ct$ is ΔCt WKY-ΔCt control) was used for semiquantification, and the results were normalized to the expression of 18S rRNA (Applied Biosystems). All samples were run in triplicate, and the mean values were calculated.

Pathological examination

The fixed organs/tissues were routinely proces-sed and embedded in paraffin, and the paraffin sections were stained with hematoxylin and eosin and examined microscopically. To com-pare the severity of the renal lesions in each group, the number of arteriolar hyaline degener-ation/necrotic lesions and the accumulation of eosinophilic substances in the glomerular mesan-gium area were assessed. The number of lesions in the right and left kidneys in a single slide was counted, and the mean number and percentage

per kidney was calculated in each animal.

Statistical analysis

Data are presented as the mean±standard error (SE). Statistical differences between mean values were determined using the ANOVA mul-tiple comparisons test. P values less than 0.05 were considered significant. All examined val-ues were tested for statistical differences using the Statistical Analysis Systems (SAS) software ver-sion 8 (SAS Institute, USA).

Results

General observations

SHRSP/fatty rats gained weight for 1-3 weeks after the starting point and then tended to lose weight from 4 to 5 weeks in the control group (Figure 1). In drug-treated groups, the rats gained weight until the endpoint, and the ARB-treated group showed significant increases (p < 0.05) in comparison with the control group at five weeks after the starting point (Figure 1).

On the other hand, blood pressure went up until the endpoint in the control group (Figure 2). In addition, two rats in the control and one rat in statin-treated group could not undergo measurement of blood pressure from 4 to 5 weeks because their condition had worsened. In drug-treated groups, as in the control group, blood
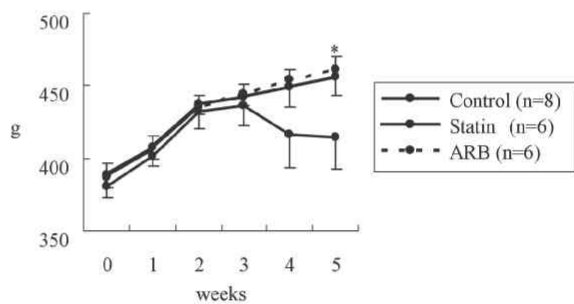
Fig. 1　Body weight of the control, statin-, and ARB-treated groups.
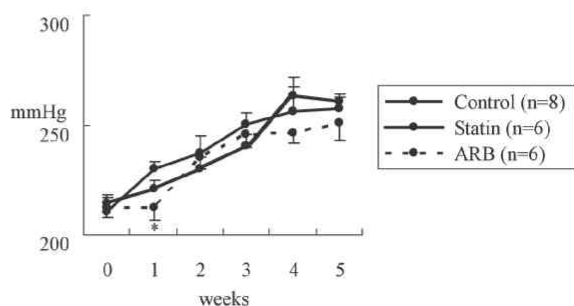
Fig. 2　Blood pressure of the control, statin-, and ARB-treat-ed groups.

pressure also rose until the endpoint, but that in the ARB-treated group tended to be lower than the control group only 1 week after the starting point (Figure 2).

The average cell sizes of adipocytes in statin- and ARB-treated groups were larger than in the control group (Table 1).

Effects on glucose and lipid metabolism

The blood chemistry results are shown in Table 2. Data from WKY/Izm rats are shown as a reference. It was revealed that SHRSP/fatty rats in the control group had similar abnormalities to MS because they showed increases in ALT, Glu, TG, and T-CHO in comparison with WKY/Izm rats. The ARB-treated group showed significantly low levels of T-CHO and LDL and tended to show decreases in FFA and TG. It was suggested that hyperlipidemia was improved in the ARB-treated group. In the statin-treated group, lipid parameters tended to decrease, but these differences were not significant. All three groups showed similar blood glucose values and

were not significantly different. Insulin in the ARB-treated group tended to be higher than in the control group. In contrast, the statin-treated group showed similar values, and no effects of drug treatment were observed.

Western blot analysis of adipokine

The ARB-treated group showed high levels of adiponectin compared with the control group, but the differences were not significant. The statin-treated group showed similar values of adiponectin and leptin (Table 3). A migrating Westernblot image of plasma adiponectin is shown in Figure 3. Adiponectin exists as a polymer in plasma and is categorized according to the polymerization degree as LMW (low molecular weight, trimer), MMW (middle molecular weight, hexamer), or HMW (high molecular weight, multimer).[12,13] HMW adiponectin was hardly detected in the control group, suggesting that SHRSP/fatty rats in the control group had a deficit of HMW adiponectin. On the other hand, HMW adiponectin was clearly observed in

Table 1 Comparison of the average cell size (diameter) of adipocytes.

Items	Unit	Control (n=3)	Statin (n=3)	ARB (n=3)
Adipocyte	μm	98.5±0.2	104.8±0.3**	107.3±1.8**

Mean±SE, ** : P<0.01 (vs. Control)

Table 2 Comparison of biochemical examinations of the blood in the control, statin-, and ARB-treated groups (WKY/Izm data were used as a reference).

Items	Unit	Control (n=6)	Statin (n=5)	ARB (n=5)	WKY/Izm
AST	IU/L	111.8±10.7	120.3±13.2	148.0±29.3	132.4±11.4
ALT	IU/L	125.3±29.4	163.8±38.7	182.6±33.4	35.6±5.2
ALP	IU/L	506.7±23.1	495.5±15.5	505.0±9.3	759.2±13.5
Cre	mg/dL	0.27±0.02	0.19±0.01**	0.17±0.01**	0.27±0.01
BUN	mg/dL	20.3±1.4	18.0±1.5	17.4±0.5	21.0±0.8
TP	g/dL	6.4±0.1	6.2±0.2	6.0±0.1	5.57±0.08
Glu	mg/dL	179.7±9.0	183.5±9.8	189.0±13.1	128.6±4.1
FFA	meq/L	0.93±0.10	0.94±0.13	0.77±0.06	0.85±0.09
TG	mg/dL	526.5±50.5	597.0±94.2	471.4±59.8	29.2±2.1
T-CHO	mg/dL	138.3±6.9	124.8±10.8	106.2±8.5*	87.0±2.3
LDL	mg/dL	11.83±0.79	9.25±2.02	7.00±1.05*	
Insulin	ng/mL	13.6±2.6	14.1±2.7	19.4±3.6	

Mean±SE, * : P<0.05 (vs. Control), ** : P<0.01 (vs. Control)

Table 3 Comparison of obesity related factors in the blood in the control, statin-, and ARB-treated groups.

Items	Unit	Control (n=6)	Statin (n=5)	ARB (n=5)
Adiponectin	μg/mL	6.47±1.23	6.99±1.96	9.35±0.73
Leptin	ng/mL	108.3±10.0	108.4±7.3	92.9±8.6

Mean±SE

statin- and ARB-treated groups, revealing that rats in drug-treated groups had HMW adiponectin in their blood.
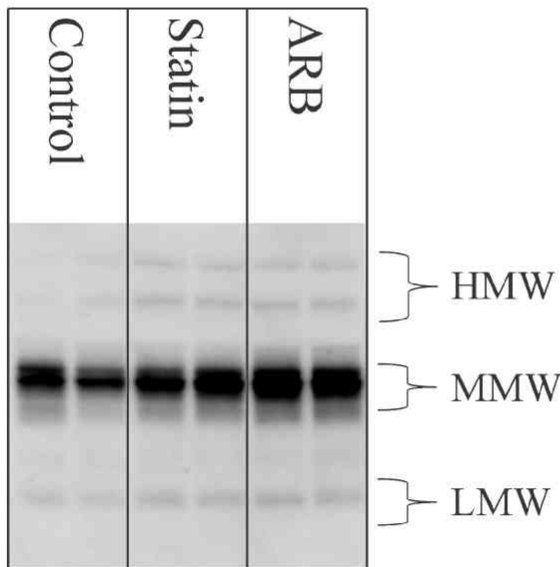
Gene analysis of adipokines and cytokines

The mRNA expression of leptin, adiponectin, and TNF-alpha in adipose tissue was determined by real-time PCR. The statin-treated group showed an increased expression of adiponectin and leptin and a decreased expression of TNF-alpha. The ARB-treated group also showed a tendency towards a decreased expression of TNF-alpha, but there were no changes in adiponectin or leptin expression (Table 4).

The mRNA expression of MCP-1 in kidney was determined by real-time PCR. The ARB-treated group showed an significant decrease and the statin-treated group showed a tendency to decrease compared to the control group (Table 4).
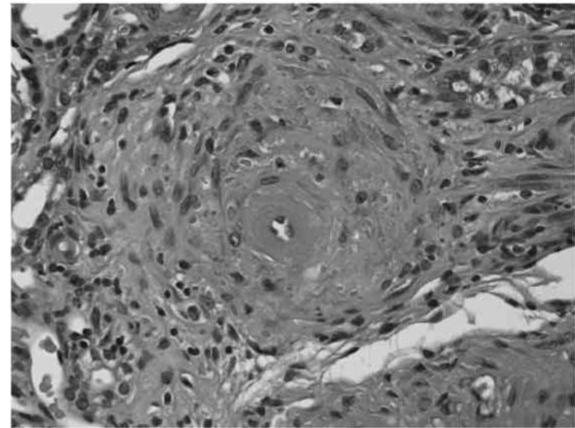
Microscopic findings

Histopathological examination revealed hyaline degeneration or fibrinoid necrosis of arterioles (Figure 4-1) and the accumulation of
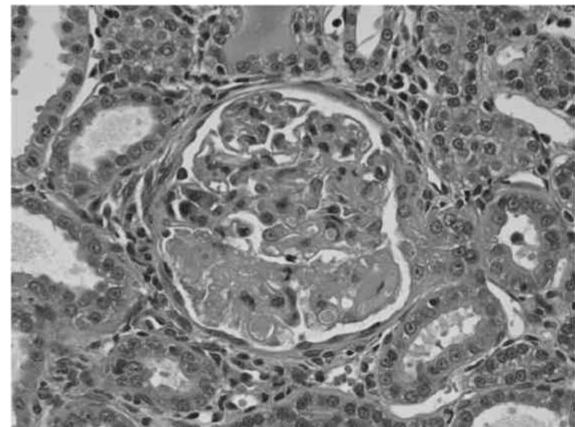
eosinophilic material at the glomerulus (Figure 4-2) with a high incidence in the control group in the kidney (Table 5). However, these lesions were suppressed in both drug-treated groups, and there was no difference between suppression in statin- and ARB-treated groups. Moderate hyperplasia of the islets of the pancreas was observed in all groups, and the degree of this lesion was the same in all groups. No other

Fig. 4-1　Vascular lesion in the kidney (H&E staining) Hyaline degeneration or fibrinoid necrosis of arterioles.

Fig. 3　Western blotting of HMW adiponectin in blood of the control, statin-, and ARB-treated groups.

Fig. 4-2　Glomerular lesion in the kidney (H&E staining) Accumulation of eosinophilic material at the glomerulus.

Table 4　Comparison of adipokine and MCP-1 expression in the control, statin-, and ARB-treated groups.

Items	Control (n=6)	Statin (n=4)	ARB (n=5)
Adiponectin	0.42±0.01	0.50±0.01* (+19%)	0.46±0.02　(+10%)
Leptin	0.84±0.07	1.14±0.07* (+36%)	1.04±0.05　(+24%)
TNF-α	0.90±0.12	0.51±0.09* (−43%)	0.60±0.04　(−33%)
MCP-1	2.08±0.64	0.64±0.23　(−69%)	0.19±0.06* (−91%)

Ratio to 18S rRNA, Mean±SE, ()：each value as a percentage of the control group value, ＋：increase compared with the control group, −：decrease compared with the control group, *：P<0.05 (vs. Control)

Table 5 Comparison of vascular and glomerular lesions in the control, statin-, and ARB-treated groups.

	Control (n=6)	Statin (n=5)	ARB (n=5)
Vascular lesions	11.8±1.2 (19.7±2.5)	3.3±2.1* (2.3±1.5)	3.2±1.5** (6.9±4.4)
Glomerular lesions	6.3±1.0 (1.8±0.5)	2.4±1.9 (0.2±0.1)	2.2±0.6* (0.5±0.3)

Mean±SE, Number of lesions per kidney. ( ): each value as a percentage of the lesions in all arteriole or glomerulus.
* : $P < 0.05$ (vs. Control), ** : $P < 0.01$ (vs. Control)

organs or tissues (brain, liver, spleen, heart, lung, aorta, testis, and thigh muscle) showed any other lesions in any group.

Discussion

The general condition was well preserved both in statin- and ARB-treated groups, although these treatments had no effect on blood pressure. The average cell size of adipocytes in the control group was smaller than in drug-treated groups. It was suspected that loss of body weight was caused by adipocyte atrophy in the control group. This may be due to appetite loss by severe hypertension.

In the present study, investigation of the effects of drugs on glucose/lipid metabolism revealed a decrease in T-CHO and LDL and a tendency towards decreases in FFA and TG in the ARB-treated group. This interesting effect might have resulted from the direct effect of ARB on the AT-2 receptor, because liver and adipose tissue both express the AT-2 receptor.[14,15] In addition, there were no improvements in lipid parameters in the statin-treated group. In general, if HMG-CoA reductase is inhibited by statins treatment, rats have a secondary pathway for synthesizing cholesterol, and so statins have few effects on cholesterol production.[16,17] This might be the reason for the lack of improvement in lipid metabolism in the statin-treated group. Although Glu tended to increase in all groups, it did not reach the reference value for diabetes.[18] Histopathological examination revealed hyperplasia in the islets of the pancreas, and ELISA showed a high blood insulin level in all groups. This suggested that the increase in Glu caused compensatory islet cell hyperplasia and elevated production of insulin. This may cause glomerular lesions, being similar to diabetic glomerulopathy. Investigation into the effect on adipokines revealed a high blood adiponectin level in the ARB-treated group. As mentioned previously, adiponectin is categorized according to the polymerization

degree as LMW, MMW, or HMW, and HMW adiponectin has an markedly suppressive effect on the formulation of lesions.[13,19] Interestingly, HMW adiponectin was detected in both statin- and ARB-treated groups, although it was rarely observed in the control group. Adiponectin, the most abundantly secreted adipokine, has suppressive effects on vascular lesions and diabetes.[20,21] ARB has a stimulatory effect on the secretion of adipokines, especially adiponectin, which acts through the AT-2 receptor in adipocytes.[22] In our study, the total amount of adiponectin secretion and the amount of HMW adiponectin secretion were probably promoted by the same pathway, suggesting that increases in adiponectin suppressed vascular and glomerular lesions of the kidney in ARB-treated groups.

In contrast, the mRNA expression of TNF-alpha was down-regulated in the adipose tissue of the statin-treated group, and the expression of adiponectin was up-regulated. It was reported that statins possess an anti-inflammatory effect that induces the suppression of TNF-alpha and MCP-1 secretion from macrophages in adipose tissue, inhibits abnormal differentiation of adipocytes, and prevents the development of diabetes by stimulating adiponectin secretion.[23,7,24] Therefore, in our study, the secretion of HMW adiponectin was probably caused by the same anti-inflammatory pathway. The decreased mRNA expression of TNF-alpha was one of the most important factors in the prevention of renal vascular and glomerular lesions in the statin-treated group. The mRNA expression of MCP-1 in the kidney was decreased in the statin- and ARB-treated groups. MCP-1 is known as an important mediator of macrophage infiltration and increased expression of MCP-1 was described in an experimental study to cause hypertensive nephrosclerosis in SHRSP.[25] In this study, down-regulation of MCP-1 was caused by the prevention of renal damage in drug-treated groups. In addition, the mRNA expression of leptin was up-regulated in adipose tissue. The

secretion of leptin depended on the size of adipocytes, and the diameter of adipocytes in the statin- and ARB-treated groups was larger than in the control group.[26] This hypertrophy of adipocytes caused mRNA up-regulation in leptin.

The blood leptin level tended to decrease in the ARB-treated group despite an increasing size of adipocytes. SHRSP/fatty rats have a mutation in their leptin receptor, and the concentration of leptin remained quite high. As high concentrations of leptin have negative repercussions for organs, the decrease in leptin might be related to improvements in the clinical condition in the ARB-treated group.[27]

To confirm the beneficial effects of statin and ARB, histopathological examination of the kidney was carried out and revealed that severe arterial lesions, such as angionecrosis and glomerular lesions, including diffuse or segmental deposition of eosinophilic substances, were prevented in the statin- and ARB-treated groups. It was previously reported by this laboratory that the incidence of renal lesions was lower in SHRSP receiving short-term ARB treatment.[10]

As described above, although statins and ARB have different pharmacological effects, both drugs had beneficial effects on SHRSP/fatty rats. Comparing statin and ARB treatments, the common effects were suppression of body weight decreases, improvement of hypertensive arteriopathy and diabetic glomerulopathy, and increased total and HMW adiponectin. In contrast, the different effects were, for statin treatment, mRNA up-regulation of adiponectin and leptin and mRNA down-regulation of TNF-alpha. In addition, in ARB treatment, decreases in T-CHO and LDL, and increases in insulin expression and the total amount of adiponectin were observed.

It was speculated that statin suppressed systemic arteriole and renal damage without a lipid-lowering effect. These advantages were probably caused by the anti-inflammatory effects of statins. ARB improved lipid metabolism, increased adiponectin secretion from adipocytes, and resulted in the suppression of systemic arteriole and renal damage. These effects of ARB might have been caused by their actions on adipocytes. In other words, although the mechanism was similar to the process that suppressed vascular and renal damage, it was probably different from the mechanism leading to enhan-

ced adiponectin secretion.

In conclusion, our study confirmed that SHRSP/fatty rats have the potential to suffer severe hypertension, obesity, hyperlipidemia and hyperglycemia, and to develop arterial degeneration in many organs and tissues, and hypertensive arteriopathy and diabetic glomerulopathy in the kidneys. The severity of these lesions can be reduced by treatment with statins or ARB. In addition, it is considered that adipokines, especially adiponectin, are related to the suppressive effects of both drugs on vascular and renal damage.

References

1. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F (2005) American Heart Association ; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome : an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 112 : 2735-2752

2. Hiraoka-Yamamoto J, Nara Y, Yasui N, Onobayashi Y, Tsuchikura S, Ikeda K (2004) Establishment of a new animal model of metabolic syndrome : SHRSP fatty (fa/fa) rats. Clin Exp Pharmacol Physiol 31 : 107-109

3. Ueno T, Takagi H, Fukuda N, Takahashi A, Yao EH, Mitsumata M, Hiraoka-Yamamoto J, Ikeda K, Matsumoto K, Yamori Y (2008) Cardiovascular remodeling and metabolic abnormalities in SHRSP.Z-Lepr(fa)/IzmDmcr rats as a new model of metabolic syndrome. Hypertens Res 31 : 1021-1031

4. Laufs U, Fata VL, Liao JK (1997) Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase blocks hypoxia-mediated down-regulation of endothelial nitric oxide synthase. J Biol Chem 272 : 31725-31729

5. Seeger H, Mueck AO, Lippert TH (2000) Fluvastatin increases prostacyclin and decreases endothelin production by human umbilical vein endothelial cells. Int J Clin Pharmacol Ther 38 : 270-272

6. Clements JD, Jamali F (2007) Pravastatin reverses the down-regulating effect of inflammation on beta-adrenergic receptors : a disease-drug interaction between inflammation, pravastatin, and propranolol. Vascul Pharmacol 46 : 52-59

7. Inoue T, Node K (2007) Statin therapy for vascular failure. Cardiovasc Drugs Ther. 21 : 281-295

8. Velasquez MT (1996) Angiotensin II receptor blockers. A new class of antihypertensive drugs. Arch Fam Med 5 : 351-356

9. Ito H, Takemori K, Kawai J, Suzuki T (2000) AT1 receptor antagonist prevents brain edema without lowering blood pressure. Acta Neurochir Suppl 76 :

141–145

10. Takemori K, Ishida H, Ito H (2005) Continuous inhibition of the renin-angiotensin system and protection from hypertensive end-organ damage by brief treatment with angiotensin II type 1 receptor blocker in stroke-prone spontaneously hypertensive rats. Life Sci 77 : 2233–2245

11. Hamaguchi R, Takemori K, Inoue T, Masuno K, Ito H (2008) Short-term treatment of stroke-prone spontaneously hypertensive rats with an AT(1) receptor blocker protects against hypertensive end-organ damage by prolonged inhibition of the renin-angiotensin system. Clin Exp Pharmacol Physiol 35 : 1151–1155

12. Crouch E, Persson A, Chang D, Heuser J (1994) Molecular structure of pulmonary surfactant protein D (SP-D). J Biol Chem 269 : 17311–17319

13. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T (2003) Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J Biol Chem 278 : 40352–40363

14. Giacchetti G, Sechi LA, Griffin CA, Don BR, Mantero F, Schambelan M (2000) The tissue renin-angiotensin system in rats with fructose-induced hypertension : overexpression of type 1 angiotensin II receptor in adipose tissue. J Hypertens 18 : 695–702

15. Hirano T, Ran J, Adachi M (2006) Opposing actions of angiotensin II type 1 and 2 receptors on plasma cholesterol levels in rats. J Hypertens 24 : 103–108

16. Björkhem I (1986) Effects of mevinolin in rat liver : evidence for a lack of coupling between synthesis of hydroxymethylglutaryl-CoA reductase and cholesterol 7 alpha-hydroxylase activity. Biochim Biophys Acta 877 : 43–49

17. Yoshino G, Kazumi T, Kasama T, Iwai M, Iwatani I, Matsuba K, Matsushita M, Baba S (1988) Effect of CS-514 (pravastatin) on VLDL-triglyceride kinetics in rats. Atherosclerosis 73 : 191–195

18. Gavin JR 3rd (1998) New classification and diagnostic criteria for diabetes mellitus. Clin Cornerstone 1 : 1–12

19. Hara K, Horikoshi M, Yamauchi T, Yago H, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T (2006) Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 29 : 1357–1362

20. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 116 : 1784–1792

21. Hopkins TA, Ouchi N, Shibata R, Walsh K (2007) Adiponectin actions in the cardiovascular system. Cardiovasc Res 74 : 11–18

22. Lee MH, Song HK, Ko GJ, Kang YS, Han SY, Han KH, Kim HK, Han JY, Cha DR (2008) Angiotensin receptor blockers improve insulin resistance in type 2 diabetic rats by modulating adipose tissue. Kidney Int 74 : 890–900

23. Kiener PA, Davis PM, Murray JL, Youssef S, Rankin BM, Kowala M (2001) Stimulation of inflammatory responses in vitro and in vivo by lipophilic HMG-CoA reductase inhibitors. Int Immunopharmacol 1 : 105–118

24. Mehta S, Farmer JA (2007) Obesity and inflammation : a new look at an old problem. Curr Atheroscler Rep 9 : 134–138

25. Hilgers KF, Hartner A, Porst M, Mai M, Wittmann M, Hugo C, Ganten D, Geiger H, Veelken R, Mann JF (2000) Monocyte chemoattractant protein-1 and macrophage infiltration in hypertensive kidney injury. Kidney Int 58 : 2408–2419

26. Skurk T, Alberti-Huber C, Herder C, Hauner H (2007) Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 92 : 1023–1033

27. Martin SS, Qasim A, Reilly MP (2008) Leptin resistance : a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. J Am Coll Cardiol 52 : 1201–1210