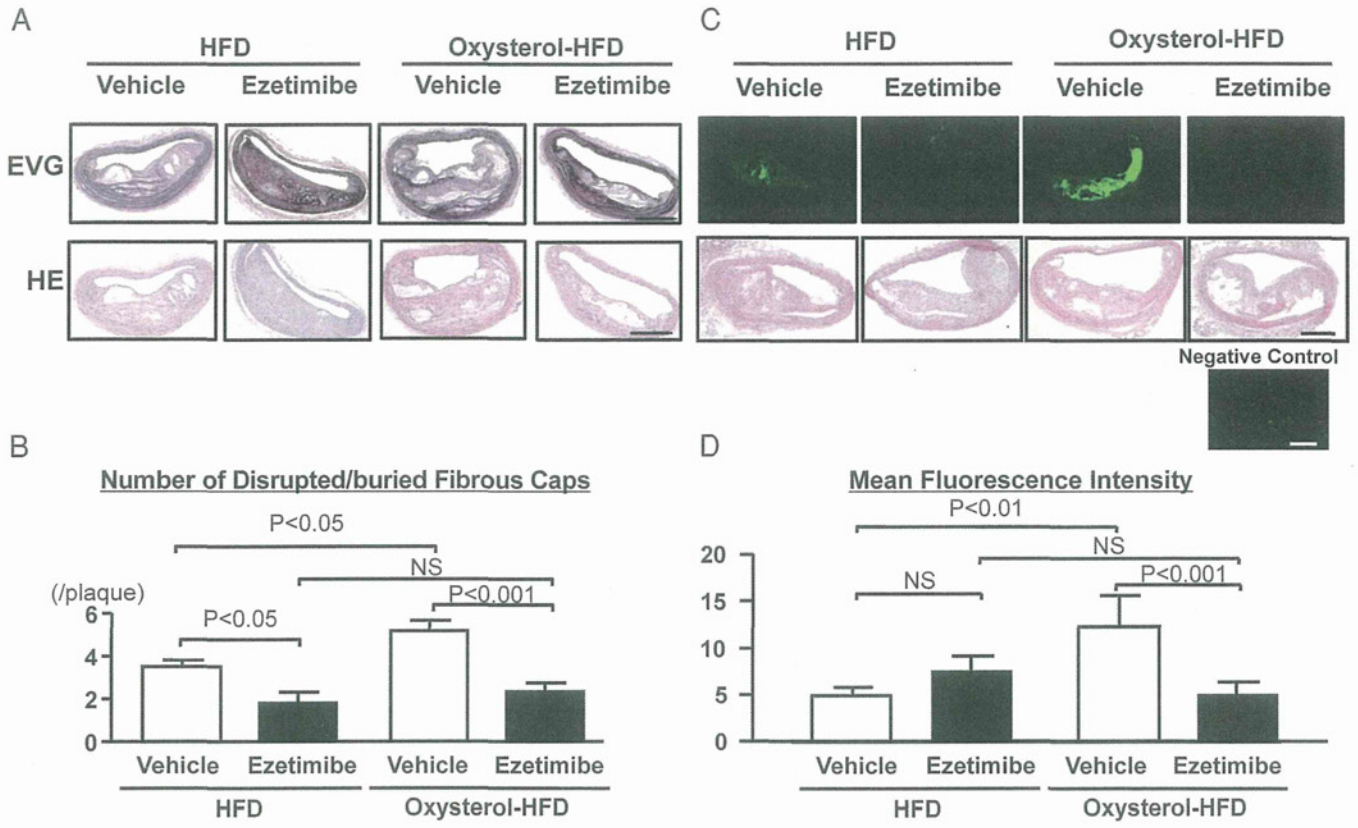

Fig. 1. Atherosclerotic plaque rupture in the brachiocephalic arteries of ApoE$^{-/-}$ mice. A, Photomicrographs of brachiocephalic artery plaques stained with Elastica van Gieson (EVG) or hematoxylin and eosin (HE). Bar indicates 100 μm. B, Quantitative comparison of the number of disrupted/buried fibrous caps. Data are indicated as mean ± SEM (n = 10 to 13). C, In situ zymography of activated gelatinase in brachiocephalic artery plaques in ApoE$^{-/-}$ mice. C, Upper panels: fluorescence microscopy photomicrographs (green fluorescence indicates gelatinase activity). Negative control zymograms of the oxysterol-HFD-fed group were incubated in the presence of 1 mM EDTA. D, Lower panels: photomicrographs of serial sections stained with HE. Bar indicates 100 μm. D, Quantitative analysis of gelatinase activity. Data are reported as the mean ± SEM (n = 6 to 9). Data were compared using 2-way ANOVA followed by Bonferroni's multiple comparison tests.

oxysterol-HFD-fed groups at week 4 (before Ang II infusion). At week 5 (1 week after Ang II infusion), plasma lipid peroxide levels were greater in the oxysterol-HFD-fed group than in the HFD-fed group. This difference became insignificant at week 8 (4 weeks after Ang II infusion). Treatment with ezetimibe significantly reduced lipid peroxide levels in both the HFD- and oxysterol-HFD-fed groups.

Oxysterol Levels in Plasma and Liver

Total oxysterol concentrations tended to increase in the livers of mice in the oxysterol-HFD-fed group compared to those in the HFD-fed group (**Table 4**). When the ratio of total oxysterols to cholesterol was examined, the ratio was higher in the oxysterol-HFD-fed group than in the HFD-fed group. Treatment with ezetimibe inhibited such an increase in the total oxysterol/cholesterol ratio. No significant changes were observed in plasma oxysterol levels among the 4 groups (**Table 5**).

Atherosclerotic Plaque Destabilization and Rupture in the Brachiocephalic Artery

Histopathological and immunohistochemical analyses of plaques in the brachiocephalic artery were performed to examine atherosclerotic plaque destabilization markers (lipid core area, fibrous cap thinning, and Mac-3-positive area) (**Table 6**). The presence of dietary oxysterols did not alter fibrous cap thickness but increased the lipid core area, mac-3-positive area, and plaque area. These changes in plaque destabilization markers were associated with increased MCP-1-positive areas. The number of disrupted/buried fibrous caps, a histopathological feature of plaque rupture, was then examined (**Fig. 1A** and **1B**). Feeding mice with oxysterol-HFD increased the incidence of plaque

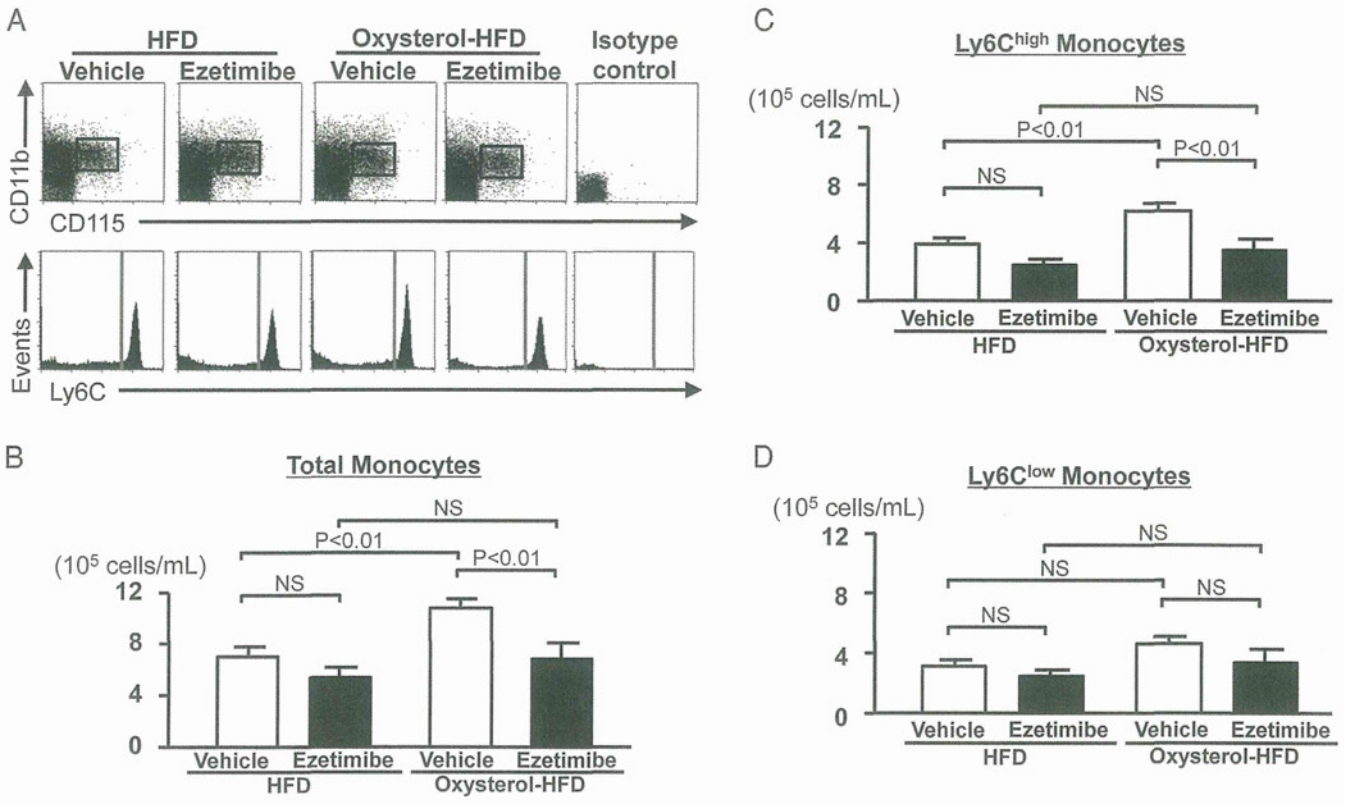

Fig. 2. Flow cytometric analysis of blood monocytes in ApoE$^{-/-}$ mice. A, Representative flow cytometry dot plots and histograms from ApoE$^{-/-}$ mice. The number of blood monocytes was determined by co-expression of CD115 and CD11b. Blood CD115^{+}CD11b^{+} monocytes were divided into Ly6C^{hi} and Ly6C^{lo} subsets. B, Total blood CD115^{+}CD11b^{+} monocytes. C, Total blood Ly6C^{hi}CD115^{+}CD11b^{+} monocytes. D, Total blood Ly6C^{lo}CD115^{+}CD11b^{+} monocytes. Data are reported as the mean ± SEM (n = 5 to 9). Data were compared using 2-way ANOVA followed by Bonferroni's multiple comparison tests.

rupture compared with the HFD-vehicle-fed group.

Treatment with ezetimibe prevented the oxysterol-HFD-induced increase in plaque destabilization markers (i.e., lipid core area, monocyte area, plaque size) and rupture. Treatment with ezetimibe also increased fibrous cap thickness in both the HFD- and oxysterol-HFD-fed groups.

In Situ Zymography

Analysis of gelatinase activity, reflecting the combined activity of MMP-2 and MMP-9, by *in situ* zymography showed enhanced gelatinase activity in areas of monocyte/macrophage infiltration. Treatment with ezetimibe inhibited increased gelatinase activity in the oxysterol-HFD-fed group (**Fig. 1C** and **1D**).

Flow Cytometric Analysis of Blood Monocyte Subsets

The monocyte Ly6C^{hi} subset is reported to play a critical role in atherosclerotic plaque progression in mice[30, 31]. Oxysterol-HFD feeding increased the number of circulating monocytes at week 8 (**Fig. 2**). This monocytosis was associated with an increase in Ly6C^{hi} subsets, but not with increases in Ly6C^{lo} subsets. Treatment with ezetimibe prevented monocytosis of the Ly6C^{hi} subsets induced by oxysterol-HFD.

Immunohistochemical Analysis of 7-Ketocholesterol and ApoB48

Immunohistochemical analysis showed the presence of 7-ketocholesterol (a representative oxysterol derived from diet) in brachiocephalic artery plaques (**Fig. 3**). Quantitative analysis revealed an increase in 7-ketocholesterol-positive areas in the oxysterol-HFD-fed group and treatment with ezetimibe inhibited this increase. Serial sections of brachiocephalic artery plaques were immunostained with antibodies for either 7-ketocholesterol or ApoB48 (a lipoprotein synthesized in the small intestine, incorporated into chylomicrons, and essential for intestinal lipid absorption) (**Fig. 3C**). Interestingly, 7-ketocholesterol-positive areas colocalized with ApoB48-positive areas.

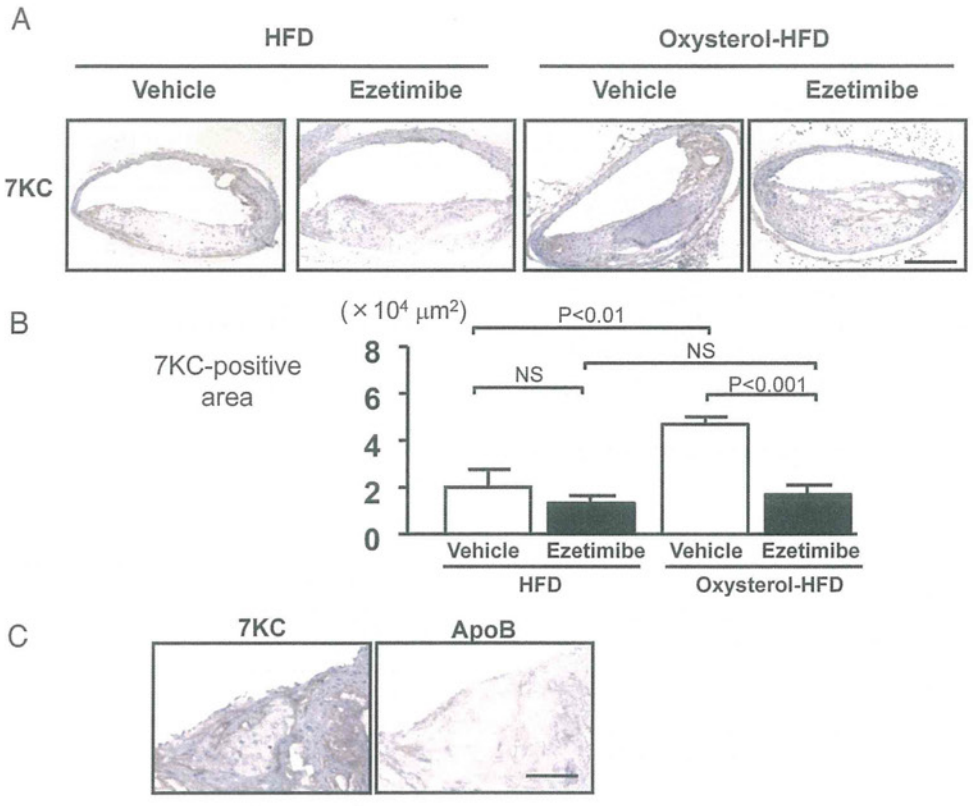

Fig. 3. Immunohistochemical analyses of 7-ketocholesterol (7KC) in the brachiocephalic arteries of ApoE$^{-/-}$ mice. A, Photomicrographs of brachiocephalic artery plaques stained with 7KC. Scale bar indicates 100 μm. B, Quantitative analysis of the 7KC-positive area. Data are reported as the mean ± SEM (n=4 to 6). Data were compared using 2-way ANOVA followed by Bonferroni's multiple comparison tests. C, Co-localization study: representative photographs of serial sections immunohistochemically stained with 7KC and ApoB. Scale bar indicates 50 μm.

Atherosclerotic Plaque Destabilization and Rupture in ApoE$^{-/-}$CCR2$^{-/-}$ Mice

To determine the role of the MCP-1-CCR2 pathway in atherosclerotic plaque rupture, ApoE$^{-/-}$CCR2$^{-/-}$ mice were used[22]. In ApoE$^{-/-}$CCR2$^{-/-}$ mice, oxysterol-HFD feeding did not induce plaque destabilization and rupture in brachiocephalic artery plaques (**Fig. 4A**, **4B** and **Table 7**). In addition, double-knockout mice did not display an increase in monocyte infiltration, MCP-1 expression, and systemic monocytosis of Ly6C^{hi} monocyte subsets at week 8 (**Fig. 4C** and **4D**). There was no difference in serum cholesterol levels between HFD and oxysterol-HFD groups (**Table 7**).

Discussion

Hyperlipidemic mice with advanced atherosclerotic brachiocephalic artery plaques appear to be a suitable animal model for elucidating the molecular and cellular mechanisms behind human plaque rupture. This model is used because, unlike other models of aortic atherosclerosis, brachiocephalic artery plaques represent several key histological features of ruptured human plaques, including an increase in plaque destabilization markers (monocyte infiltration/activation, lipid accumulation, fibrous cap thinning due to decreased smooth muscle content) and evidence of disrupted/buried fibrous caps[16-19]; however, this model has been criticized because it does not display other histological features of human plaque rupture including occlusive thrombosis[16-19], which is thought to be a reflection of differences in the coagulation and thrombolytic systems between the 2 species. In addition, the incidence of rupture in this original hyperlipidemic mouse model is too infrequent (10-20% without HFD feeding[18] and 40-60% per plaque after HFD feeding[17]) to allow for mechanistic examina-

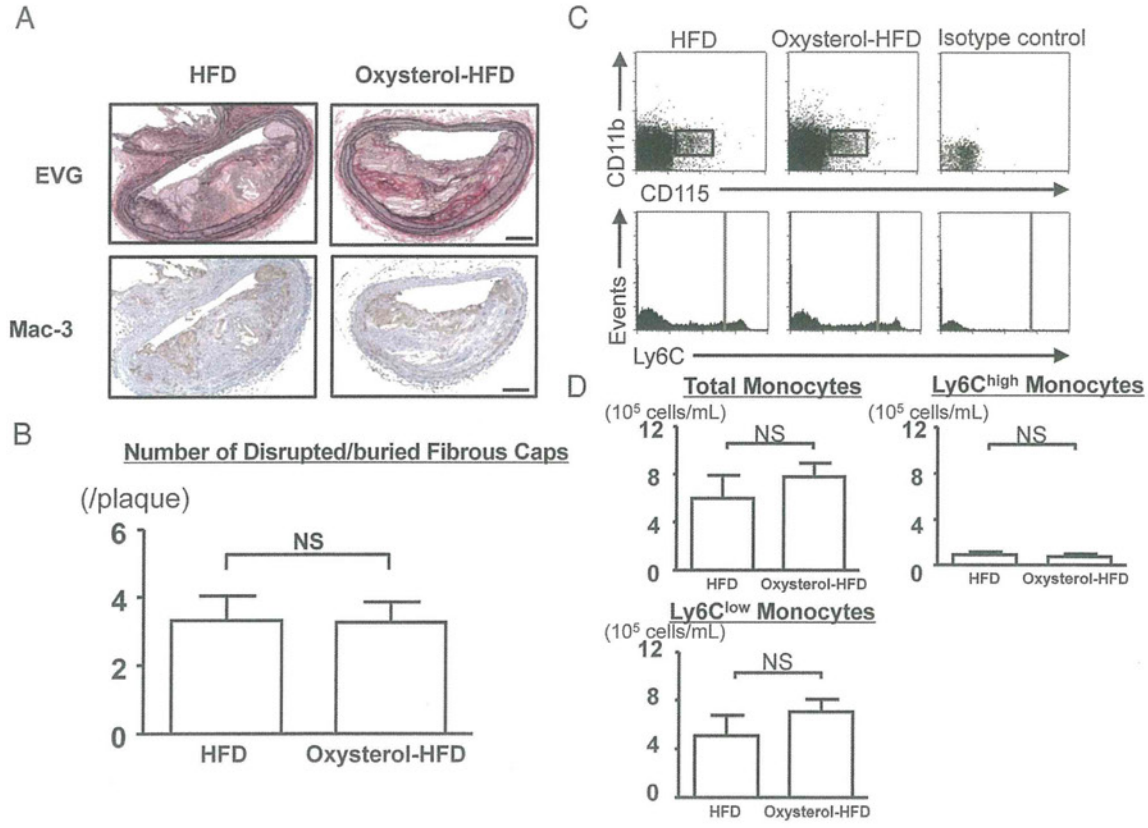

Fig. 4. Atherosclerotic plaque rupture in the brachiocephalic arteries of ApoE$^{-/-}$CCR2$^{-/-}$ mice. A, Photomicrographs of brachiocephalic artery plaques stained with EVG and immunostained with mac-3. B, Quantitative comparison of the number of disrupted/buried fibrous caps and the mac-3-positive area. Scale bar indicates 100 μm. n=6 to 7. C, Flow cytometric analysis of blood monocytes in ApoE$^{-/-}$ CCR2$^{-/-}$ mice. A, Representative flow cytometry dot plots and histograms of blood. D, The number of blood monocytes was determined by co-expression of CD115 and CD11b. Blood CD115^{+}CD11b^{+} monocytes were divided into Ly6C^{hi} and Ly6C^{lo} subsets. Data are reported as the mean ± SEM (n=6 to 9). Data are compared using the unpaired-t test.

tions and analysis of the effects of new modalities that inhibit plaque destabilization and rupture. In a previous study that examined the therapeutic effects of anti-atherosclerotic drugs[17], an excess of 60 animals was needed per group to ensure that the studies were adequately powered. To overcome this problem, we performed chronic infusion of angiotensin II in addition to HFD feeding in ApoE$^{-/-}$ mice because this has been used to enhance the features of atherogenesis, such as monocyte-mediated inflammation, lipid accumulation, and MMP activation[22, 24]. We found that chronic angiotensin II infusion could increase the incidence of plaque rupture from less than 1 rupture per plaque (authors' unpublished observation) to 3.4 per plaque (**Fig. 1A** and **1B**); therefore, in the present study, we used this modified model to investigate whether dietary oxysterols accelerate plaque rupture and whether ezetimibe has therapeutic effects on atherosclerosis.

We report here for the first time that oxysterol-HFD feeding does not affect plasma lipid levels but enhances plaque destabilization and rupture. Mechanistic analyses of the plaques showed that oxysterol-HFD increased the infiltration of monocytes/macrophages, MCP-1 expression, and lipid core size in the plaques. Monocytosis associated with an increase in the Ly6c^{hi} monocyte subset in circulating blood was also noted in the oxysterol-HFD-fed group. Importantly, monocyte activation and other pathogenetic features seen in plaques in ApoE$^{-/-}$ mice fed oxysterol-HFD were blunted in ApoE$^{-/-}$CCR2$^{-/-}$ mice fed oxysterol-HFD. Accumulating evidence in humans and animals indicates that activated monocytes/macrophages play a key role in causing plaque destabilization and rupture by secreting MMP-9/gelatinase-B and MMP-2/gelatinase-A, which break down the

Table 7. Characteristics of brachiocephalic artery plaques, lipid profile and TBARS in ApoE$^{-/-}$ CCR2$^{-/-}$ mice at week 8

	HFD group	Oxysterol-HFD group
Plaque area ($\times 10^4$ μm^2)	28.1 ± 3.7 (n=6)	26.1 ± 6.3 (n=8)
Fibrous cap thickness (μm)	2.1 ± 0.2 (n=6)	1.9 ± 0.4 (n=8)
Lipid core area ($\times 10^4$ μm^2)	6.9 ± 2.0 (n=6)	5.6 ± 1.2 (n=8)
Mac-3-positive area ($\times 10^4$ μm^2)	4.5 ± 0.8 (n=6)	3.8 ± 0.7 (n=8)
MCP-1-positive area ($\times 10^4$ μm^2)	6.7 ± 1.4 (n=6)	4.3 ± 0.6 (n=8)
Serum cholesterol levels (mg/dL)	1,326 ± 80 (n=5)	1,207 ± 78 (n=8)
Plasma lipid peroxide (μM)	6.2 ± 0.4 (n=5)	6.3 ± 0.3 (n=8)

Data are expressed as the mean ± SEM.

extracellular matrix (a determinant of the integrity of fibrous caps)[3, 32]. Gough *et al.*[18] reported that macrophage-targeted expression of a constitutively active MMP-9 mutant resulted in increased incidence of plaque rupture in advanced brachiocephalic artery plaques of ApoE$^{-/-}$ mice. Clinical studies have shown a correlation between MMP-9 and MMP-2 and cardiovascular events in patients with acute coronary syndrome[33, 34]; therefore, we examined gelatinase activity using *in situ* zymography and found enhanced gelatinase activity in areas of inflammatory cell infiltration in the oxysterol-HFD-fed group. In *in vitro* experiments, addition of oxysterol (7-KC) to murine macrophages induced MCP-1 production but did not alter MMP secretion. The data from this study and an aforementioned study[18] are consistent with the idea that enhanced monocyte-mediated inflammation and the resulting increase in macrophage MMP activity can induce plaque destabilization and rupture.

Oxysterol measurements using gas-liquid chromatography mass spectrometry showed that dietary oxysterol intake resulted in an increase in liver oxysterol concentrations, but did not lead to an increase in plasma oxysterol concentrations. In contrast, immunohistochemical analysis showed an increase in oxysterols (7-KC) in monocyte-positive areas of the plaques, which was greater in the oxysterol-HFD-fed group than in the HFD-fed group. Interestingly, 7-KC appeared to co-localize with ApoB48 (a lipoprotein involved in lipid absorption in the small intestine). Because dietary oxysterols did not affect oxidized LDL/cholesterol ratios in the plasma, it is unlikely that oxysterols incorporated into LDL play a major role in the pathogenesis of plaque destabilization and rupture; therefore, these data suggest a major role for monocyte/macrophage accumulation of diet-derived oxysterols in causing plaque destabilization and rupture.

Another new finding of the present study was that treatment with ezetimibe prevented plaque destabilization and rupture induced by dietary oxysterols. It is likely that these observed therapeutic effects resulted largely from the lipid-lowering effects of ezetimibe, which markedly decreases LDL cholesterol, chylomicron, and VLDL cholesterol levels and increases HDL cholesterol levels, as reported in patients with dyslipidemia. Ezetimibe also inhibited oxysterol accumulation in the liver and plaques, monocyte infiltration/activation, and MMP activity. The latter therapeutic effects might not necessarily be totally explained by the lipid-lowering effects of ezetimibe treatment; rather, they may result partly from a unique effect of ezetimibe, such as inhibition of dietary oxysterol absorption in the small intestine and reduced accumulation of oxysterols in atherosclerotic plaques, which can prevent plaque destabilization and rupture. These data suggest that ezetimibe could be a new therapeutic modality for subjects who overindulge in oxysterol-rich Western food and in patients at high risk for cardiovascular disease owing to high plasma oxysterol levels. Because Western diets have become prevalent in non-Western countries in this era of global civilization, the quantity of oxysterols consumed in the diet is of clinically importance for the paradigm shift in the pathogenesis of atherosclerotic vascular diseases. These data indicate a primary role for monocyte infiltration/activation via the MCP-1-CCR2 pathway and the resultant increase in combined gelatinase activity (MMP-2 + MMP-9) in plaque destabilization and rupture induced by dietary oxysterols in this model. These data suggest a mechanism that connects dietary oxysterols with the pathogenesis of plaque destabilization and rupture. Our results also suggest that inhibiting the absorption of dietary oxysterols with ezetimibe may be a useful treatment for high-risk patients with increased dietary oxysterol intake or whose plasma oxysterol concentrations are elevated.

Acknowledgments

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Conflicts of Interest

No.

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Original Article

Angiotensin II receptor blockers improve endothelial dysfunction associated with sympathetic hyperactivity in metabolic syndrome

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Objectives: Renin–angiotensin system inhibitors are preferred for the treatment of hypertension with metabolic syndrome (MetS). Underlying endothelial dysfunction and sympathetic nervous system (SNS) activation are critically involved in the pathogenesis of hypertension in MetS. We investigated whether treatment with angiotensin II type 1 receptor blockers (ARBs) improves endothelial and autonomic function in patients with MetS.

Methods and results: We conducted a prospective, randomized, open-label, blinded endpoint trial. Sixty patients with MetS were randomized into three treatment groups: telmisartan, candesartan, or diet therapy (control; $n = 20$ each), and treated for 6 months. To evaluate the endothelial function of forearm resistance arteries, blood flow and vascular resistance were measured using a strain-gauge plethysmograph during intra-arterial infusion of acetylcholine (ACh) or sodium nitroprusside (SNP). At 6 months, both telmisartan and candesartan comparably decreased blood pressure. Furthermore, ARB treatment ameliorated impaired forearm vasodilation in response to ACh. Telmisartan had a greater effect than candesartan on ACh-induced forearm vasodilation. In contrast, forearm vasodilation in response to SNP was comparable between the telmisartan and candesartan-treated groups. ARB treatment increased high-molecular-weight (HMW) adiponectin levels and baroreflex sensitivity, but telmisartan had a stronger effect than candesartan. In addition, only telmisartan treatment significantly decreased plasma norepinephrine concentrations, blood pressure variability, and heart rate variability based on spectral analysis.

Conclusion: These findings indicate that ARBs improve impaired endothelial and baroreflex function, and increase HMW adiponectin levels in patients with MetS. Telmisartan exhibited more beneficial effects than candesartan, and only telmisartan reduced sympathetic hyperactivity, despite similar depressor effects.

Keywords: angiotensin II, autonomic function, endothelial function, metabolic syndrome

Abbreviations: ACh, acetylcholine; ARBs, angiotensin II receptor blockers; BRS, baroreflex sensitivity; FBG, fasting blood glucose; HDL-C, high-density-lipoprotein cholesterol; HMW, high molecular weight; HOMA-IR, homeostasis Model Assessment of Insulin Resistance; LF/HF-HRV, low-frequency power/high-frequency power in heart rate variability; LF-SBPV, LF power/total power in SBP variability (normalized unit); MetS, metabolic syndrome; SNP, sodium nitroprusside; SNS, sympathetic nervous system; TNF, tumor necrosis factor

INTRODUCTION

Metabolic syndrome (MetS) is characterized by visceral obesity, impaired fasting glucose, dyslipidemia, and hypertension [1,2]. Several studies suggest that endothelial function is impaired in MetS [3–6]. Endothelial dysfunction is a predictable marker of cardiovascular events and can be measured in the forearm resistance vessels [7,8] and brachial artery [9–11]. Endothelial dysfunction has not yet been established in MetS [12]. Endothelial dysfunction in the resistance arteries occurs in the early stages of hypertension, and cannot be detected by measurements of flow-mediated vasodilation (used to determine endothelial function of the conduit artery) [13,14], which may lead to contradictory findings in patients with MetS. A recent study demonstrated that endothelium-dependent vasodilation in resistance arteries, but not in the brachial conduit artery, is inversely associated with a 5-year risk of a composite cardiovascular endpoint [15].

Insulin resistance and the sympathetic nervous system (SNS) have important roles in the pathogenesis of MetS [16–19]. Urinary excretion of catecholamine metabolites becomes elevated and more pronounced as the number of symptoms of MetS increases [20]. Sympathetic neural discharge is markedly potentiated [17], leading to increased insulin levels and elevated blood pressure [19]. Thus, treatments targeting the activation of the SNS are reasonable for patients with MetS, because SNS activation enhances

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hypertension, insulin resistance [19], and endothelial dysfunction [21]. Furthermore, SNS activation is important for the occurrence and progression of hypertension leading to hypertensive organ damage in MetS [16]. In addition, patients with MetS present with cardiovascular autonomic imbalance, such as reduced baroreflex sensitivity (BRS) [22].

In hypertensive patients with MetS, renin–angiotensin system inhibitors such as angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (ARBs) are preferred [23,24]. It has not yet been determined, however, whether ARBs have beneficial effects on endothelial and autonomic dysfunction in patients with MetS. The aims of the present study were to investigate whether ARB treatment improves endothelial and autonomic function in patients with MetS, and if so, whether these effects are class effects of ARBs. We divided the patients with MetS into three treatment groups: telmisartan, candesartan, and diet therapy (as a control). Telmisartan and candesartan are widely used long-acting ARBs that have powerful blood pressure-lowering effects [24]. Therefore, we examined endothelial function based on forearm endothelium-dependent vasodilation in response to acetylcholine (ACh) or sodium nitroprusside (SNP) infusion assessed by venous-occlusion plethysmography [25–27]. We also examined autonomic function by measuring plasma norepinephrine concentrations, spectral analysis of blood pressure and heart rate variability, and BRS in patients with MetS before and after 6 months of treatment.

METHODS

This prospective, randomized, open-label, blinded endpoint trial was conducted at Kyushu University Hospital between the period of April 2007 and March 2009. The trial was conducted in accordance with the Declaration of Helsinki. The study protocol and the sample sizes of the patient groups were reviewed and approved by the Ethics Committee for Human Research at our institute. Written informed consent was obtained from each individual prior to participation in the study.

Patients

Sixty patients with MetS (MetS group; 34 men, 26 women; mean age 54 ± 8 years) and 10 non-MetS individuals (six men, four women; mean age 51 ± 6 years) were enrolled in the study. Individuals were recruited from among patients admitted into the Heart Center of Kyushu University Hospital from April 2007 to March 2009. MetS was diagnosed in accordance with the current Japanese criteria [28]; the presence of visceral obesity, defined as waist circumference at least 85 cm in men and at least 90 cm in women, was an essential component in conjunction with two or more of the following criteria: serum triglycerides at least 150 mg/dl, high-density-lipoprotein cholesterol (HDL-C) less than 40 mg/dl, SBP at least 130/85 mmHg, and fasting blood glucose (FBG) greater than 110 mg/dl. Any patient with clinical signs of acute infection, autoimmune disorder, severe renal (serum creatinine level >2.0 mg/dl) or hepatic disease, or suspected malignancy was excluded from the study. In addition, individuals with a history of cardiovascular disease, including coronary artery disease, clinical heart failure, cardiomyopathy, valvular heart disease, stroke, and arteriosclerosis obliterans, were also excluded from the study. Insulin resistance in the patients was determined based on plasma high-molecular-weight (HMW) adiponectin levels and the Homeostasis Model Assessment of Insulin Resistance {HOMA-IR; score = [immunoreactive insulin (μU/ml) × FBG (mg/dl)]/405}. Patients without low HMW adiponectin (<4.0 μg/ml) and high HOMA-IR (>2.5) were excluded from the MetS group. Left-ventricular ejection fraction was determined using the modified Simpson method or the single-plane area-length method on echocardiograms. Twenty-four (MetS) and four (non-MetS) patients had a history of smoking; however, they had all quit smoking after admission to the hospital.

Patients in the non-MetS group were admitted to our hospital for atypical chest pain, fatigue, or palpitations. Careful examination was performed to rule out coronary artery disease (by coronary angiography) and other organic heart diseases (by echocardiogram) or arrhythmia (by Holter electrocardiogram or electrocardiogram monitoring during hospitalization and/or electrophysiological study). After the enrollment interview, some patients in the non-MetS group transiently received some medications such as angiotensin-converting enzyme inhibitors, ARBs, or β-blockers from general practitioners for mild high blood pressure and/or palpitations. The patients did not take these medications continuously, however, and we confirmed that they were not diagnosed with MetS upon admission.

We excluded patients with MetS who had already taken the angiotensin-converting enzyme inhibitors or ARBs used in the present study. Some patients in the MetS group were given calcium channel blockers or statins. Because it is ethically unacceptable to discontinue these medications for study purposes, the medications were discontinued only on the day of the study and were restarted immediately after the study ended. Finally, 60 MetS and 10 non-MetS patients were included in the study. The MetS patients were randomly assigned to receive telmisartan with diet therapy, candesartan with diet therapy, or diet therapy only (as a control) in a 1 : 1 : 1 ratio. Treatment allocation was computer-generated by the server at Kyushu University and operated by the Kyushu University Randomization Service Office using a dynamic allocation method that balanced the factors of sex, age, SBP, forearm vascular resistance, and plasma norepinephrine concentration. Once the eligibility of the patient was confirmed, the investigator contacted the Kyushu University Randomization Service Office and was notified of the allocated treatment. The investigator then assigned the treatment to the patient. An independent adjudication committee blinded to the treatment allocation assessed all of the potential outcomes. Allocation was concealed from the investigators until they contacted the Kyushu University Randomization Service Office.

General procedures

We measured endothelial function and plasma norepinephrine concentration with the patients in the supine

position and in a postabsorptive state at a room temperature between 25 and 27°C before and 6 months after starting treatment. All medications were with-held only on the day of the study. With the patients under local anesthesia, the left brachial artery was cannulated with a 20-gauge intravascular cannula for drug infusion; the cannula was connected to a pressure transducer to directly measure arterial pressure. The antecubital vein was cannulated, and blood samples were obtained for serum or plasma chemistry measurements.

Measurements of forearm blood flow and vascular resistance

Forearm blood flow was measured with a strain-gauge plethysmograph, using the venous-occlusion technique as described previously [25–27]. Forearm blood flow (ml/min per 100 ml of forearm volume) was calculated from the rate of the increase in forearm volume, whereas venous return from the forearm was prevented by inflation of a cuff on the upper arm. The pressure in the venous-occlusion or congesting cuff was 40 mmHg. Circulation to the hand was arrested by inflating a cuff around the wrist. The mean of four measurements made at 15-s intervals was used for the subsequent analysis. Forearm vascular resistance was calculated by dividing the mean arterial pressure (DBP and one-third of the pulse pressure in mmHg) by forearm blood flow. Forearm vascular resistance is expressed in units. Forearm blood flow, arterial pressure, and heart rate were measured at rest and during the administration of graded doses of ACh (4, 8, or 16 μg/min) or SNP (0.4, 0.8, or 1.6 μg/min). Each dose of ACh or SNP was infused for 5 min, and forearm blood flow was measured after each infusion.

Assessment of autonomic function

As parameters of autonomic function in the present study, we measured plasma norepinephrine concentrations, spectral analysis for SBP and heart rate variability, and BRS. Blood pressure was monitored using the TaskForce Monitor 3040i (CNSystems, Graz, Austria). The cuff was attached to a finger on the left hand that was supported at the heart level. Electrocardiogram electrodes were attached to the chest. After a minimum of 5 min and once the blood pressure and heart rate readings had stabilized, we obtained three consecutive 5-min recordings of blood pressure and electrocardiogram tracing. Noninvasive brachial blood pressure readings were taken with an appropriately sized cuff. We calculated the low frequency (<0.15 Hz) power/high frequency (0.15–0.4 Hz) power in heart rate variability ratio (LF/HF-HRV) as an indicator of sympatho-vagal balance and the low-frequency power/total power in SBP variability ratio (normalized unit; LF-SBPV) as a parameter of SNS activity [29,30]. Sequence analysis detected sequences of three or more beats during which there was either an increase in SBP and pulse interval (up sequence) or a decrease in SBP and pulse interval (down sequence). BRS was estimated as the mean slope of the up-sequences (Up BRS) and down-sequences (Down BRS), and the mean slope of all sequences was determined as BRS (Sequence BRS) [31,32].

Treatments

All patients in the present study received individual nutritional education every month. The education was performed by the same nutritional instructor, and all patients were asked to follow the calorie-controlled Dietary Approaches to Stop Hypertension diet plan, which emphasizes vegetables, fruits, whole grains, lean meats, and low fat dairy food, and foods rich in magnesium, potassium, calcium, and fiber. The patients were asked to record their daily dietary intake, which was checked by the instructor every month. The patients in the telmisartan and candesartan-treated groups were treated for hypertension with telmisartan (20–40 mg) or candesartan (4–8 mg), respectively, once a day, and in all of them hypertension was successfully treated (<130/85 mmHg) [24].

Primary and secondary endpoint

The primary endpoint of the study was a statistically significant decrease in the forearm vascular resistance response to ACh at the maximum dose (16 μg/min). The secondary endpoint was a statistically significant reduction of the plasma norepinephrine concentration. These prespecified endpoints were compared between the telmisartan-treated and candesartan-treated groups. An Independent Endpoint Classification Committee, without knowledge of the treatment assignment, reviewed all potential cases and determined whether the cases should be classified as having achieved the primary or secondary endpoint.

Statistical analysis

All results are expressed as mean [standard error of the mean (SEM)]. Values before or after treatment were compared between groups using unpaired *t*-tests. Differences between values before and after treatment were tested for statistical significance using a paired-sample *t*-test. Forearm blood flow and vascular resistance responses to graded doses of ACh or SNP were examined by a repeated-measures analysis of variance (ANOVA). Two-way ANOVA was used to compare forearm blood flow and vascular resistance before and after treatment in the telmisartan-treated and candesartan-treated groups. *P* values less than 0.05 were considered statistically significant.

RESULTS

Patient metabolic profiles before and after treatment

Before treatment, the MetS group had significantly different metabolic profiles compared to the non-MetS group (Table 1), and there were no significant differences among the telmisartan-treated, candesartan-treated, and control groups. Reduction of body weight, FBG, fasting blood insulin, serum triglycerides, and HOMA-IR were similar among telmisartan-treated, candesartan-treated, and control groups (Table 2). The reductions in SBP and high-sensitivity C-reactive protein levels were significantly greater in both the telmisartan and candesartan-treated groups than in the control group, but did not differ between the telmisartan-treated and candesartan-treated groups (Table 2). A reduction in serum uric acid was obtained only in the