0.9%, P <0.0001). This effect was also observed in SB209670-treated mice (10.6 ± 0.9% vs. 8.0 ± 0.6%, P <0.05). Treatment with SB209670 reduced atherosclerosis by 53% in mice fed the Western-type diet (P <0.001), and by 38% in mice fed the chow diet (P <0.001). Furthemore, the lesion area observed in SB209670-treated mice fed the Western-type diet was similar to that found in control mice fed the chow diet, which probably indicates that the exacerbating effect of the Western-type diet is quantitatively similar to the preventative effect of SB209670 treatment and suggests that the effect is independent of the plasma cholesterol level. And the lesion morphology of the aortic arch was affected by SB209670 treatment: control mice fed a Western-type diet predominantly showed fibrofatty plaques or atheromas, whereas SB209670-treated mice preferencially showed fatty streak lesions or intermediate lesions, and fibrous caps were rarely seen (Fig.4c). However, on the chow diet, SB209670 had no effect on plaque morphology: their lesions were similar to those of SB209670-treated mice on the Western-type diet (data not shown).

4. Discussion

To examine additional effects of ETB receptor antagonism on atherogenesis, we examined the effect of the non-selective endothelin receptor antagonist SB209670 (35-37) on atherosclerotic lesions in apoE-deficient mice, a suitable animal model of atherosclerosis (38-41), because systemic administration of selective ETB receptor antagonists leads to adverse effects such as hypertension and increased peripheral vascular resistance (33,34). The vasoconstrictor effects of ETB antagonism have been shown to result directly from blockade of an endothelial ETB receptor-mediated dilator tone or indirectly from displacement of endogeneously generated ET-1 to vasoconstrictor ETA receptors, or as a result

of reduced clearance of ET-1 by vascular ETB receptors (43-45).

The ETB receptor also play an important role in the regulation of renal tubular sodium excretion (46). ET-1 appears to be natriuretic, an effect that is mediated through activation of proximal tuble sodium cotransport systems (47,48). In this study, renal function of the mice was not examined, however hemodynamic change such as systolic blood pressure was not found. This finding is consistent with the study using SB209670 in human (37).

In this study, we found that treatment with SB209670 reduced atherosclerosis by 53% in mice fed the Western-type diet and by 38% in mice fed the chow diet. Barton et al. (20) reported that in the same animal model on the Western-type diet, treatment with the selective ETA receptor antagonist LU135252 for 30 weeks inhibits atherosclerosis by 31%. Although it is not possible to make a side-to-side comparison between two experiments, the anti-atherosclerotic effect of the non-selective ETA/ETB antagonist SB209670 for 12 weeks in the present study was more prominent than that of the selecive ETA receptor antagonist, thus suggesting that ET-1 acting through the ETB receptor in addition to the ETA receptor plays an important role in atherogenesis.

Treatment with SB209670 led to a reduction in the tissue ET-1 level in the aorta and a concomitant increase in plasma ET-1 in both diet groups. Similar results have been obtained in the studies of selective ETA blockade (20,49). This may be due to displacement of ET-1 from its receptor site followed by overspill into the plasma (20,49). Since infiltrated macrophages and T lymphocytes exclusively express the ETB receptor in atherosclerotic lesions (21,22,27), treatment with SB209670 may inhibit the inflammatory process during atherogenesis and attenuate the extent of atheorsclerosis.

As represented by inhibition of fibroproliferative lesions, the lesion morphology of the aortic atherosclerosis was affected by SB209670 treatment in

mice on the Western-type diet. In vitro studies have shown that in the early passage SMCs (contractile phenotype), the mitogenic activity of ET-1 correlates with ETA receptor binding and density (29,50). However, the mitogenic response is subject to phenotypic modulation because the late passage SMCs (synthetic phenotype) leads to increased ETB expression and the mitogenic activity of ET-1 is inhibited by a non-selective ETA/ETB antagonist, but not by a selective ETA antagonist (29). We previously demonstrated that the switching of ET receptor subtypes from ETA to ETB occurs in human vascular SMCs during atherogenesis (21). This hypothesis is supported by the studies that a density of ETB receptors is increased in the neointima of balloon-injured rabbit arteries (51) and that the administration of the selective ETA receptor antagonist BQ-123 fails to reduce angioplasty-induced neointima formation (51,52), whereas non-selective ETA/ETB antagonists are effective in the rat model (53,54). Furthermore, intimal hyperplasia in an organ culture of human saphenous vein is mainly mediated through the ETB receptor (55). However, some studies stressed that selective ETA receptor antagonism is effective on preventing vascular hyperplasia after injury (49,56,57). Therefore, we propose that blockade of both ETA and ETB receptors in vascular SMCs is essential for an inhibition of the fibroproliferative process during atherogenesis, since selective ETA blockade has no effect on lesion morphology (20).

Selective ETA blockade increases endogenous NO generation and preserves endothelial function, which could be mediated by stimulation of the endothelial ETB receptor (20,58-60). Endothelial function is also preserved by non-selective ETA/ETB blockade associated with increased plasma NOx (59,60). ET-1 modulates NO production through inhibition of NO synthetase (61). Therefore it is conceivable that SB209670-treated mice in this study showed the increased level of plasma NOx. NO inhibits monocyte adhesion (26) and ameliorates

atherosclerosis (30-32), suggesting that the anti-atherosclerotic effect of SB209670 is in part through the effects on NO.

The cholesterol levels in VLDL and IDL fractions were markedly reduced in SB209670-treated mice on the Western-type diet compared with those in control mice. As reported previously, in apoE-deficient mice fed the Western-type diet, the major increase in plasma cholesterol is in the VLDL and IDL lipoprotein fractions (38). Thus, the results suggested that SB209670 relieves diet-induced hypercholesterolemia. The mechanism of its effect remains unclear. However, we speculate that the endothelin receptor antagonist may be involved in the clearance of lipoprotein remnants. Further study is required to determine the interaction between lipid metabolism and the endothelin system.

In conclusion, we found that chronic administration of SB209670 reduced the extent of diet-induced hypercholesterolemia and atherosclerosis in apoE-deficient mice. ET-1 acting through the ETB receptor in addition to the ETA receptor may play an important role in the development of atherosclerosis. The favorable effects of SB209670 in this animal model of atherosclerosis suggest that the non-selective ETA/ETB antagonists may have a therapeutic potential in human atherosclerotic disease.

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