1. Introduction

Endothelin (ET)-1 is a potent vasoconstrictor. It was first isolated from cultured porcine endothelial cells by Yanagisawa et al. in 1988 (1). Until now, 3 isotypes of endothelins, known as ET-1, ET-2, and ET-3 encoded by separate genes, have been found (2). Evidently, ET-1 and its precursors (prepro ET-1, pro ET-1, and big ET-1) are the predominant isoforms in human endothelial cells (3). ET-1 excerts its functions via two subtypes of endothelin receptors; the ETA and the ETB receptors. ETA receptors are expressed mainly in medial smooth muscle cells (SMCs) to produce contraction, whereas ETB receptors on vascular endothelial cells induce relaxation of medial SMCs via prostacyclin and nitric oxide (NO) (4). It is now recognized that ETB receptors are also present on the SMCs and can mediate vasoconstriction (5). ET-1 plays an important role in the regulation of vascular tone and blood pressure (6,7). In addition, there is evidence that ET-1 may contribute to a number of cardiovascular diseases, including hypertension, heart failure, renal failure, pulmonary hypertension, vasospasm after subarachnoid hemorrhage and atherogenesis (6-9).

Evidence is mounting that ET-1 plays an important role in atherogenesis and hypercholesterolemia. This notion is supported by studies showing that preproET-1 mRNA expression is increased in human atherosclerotic lesions (10). ET-1 also shows chemotaxis toward monocytes by inducing monocyte chemotactic protein-1 (MCP-1) (11) and increasing production of cellular adhesion molecules (12). Plasma ET-1 concentrations are elevated in cholesterol-fed rats (13), rabbits (14), and patients with symptomatic atherosclerosis (15). Previous immunohistochemical studies with human materials show that ET-1 immunoreactivity is present not only in vascular endothelial cells, but in intimal foamy macrophages, together with intimal and medial SMCs (16-18). In vivo, the administration of a selective ETA receptor antagonist reduced atherosclerotic

lesions in hyperlipidemic hamsters (19) and in apolipoprotein E (apoE)-deficient mice (20). Together, these findings suggest that ET-1 has proatherogenic properties.

Recently, we demonstrated that there is an increased immunoreactivity of ET-1 and ETB receptors in human and mouse atherosclerotic lesions (21,22). Infiltrated macrophages and T lymphocytes exclusively express the ETB receptor. This notion is consistent with the findings that ETB receptors are upregulated in human atherosclerotic coronary arteries (23) and that ETB receptor is present on mouse and rat peritoneal macrophages (24-25), on human peripheral blood monocytes (26), and on macrophages within human atherosclerotic plaque (27). ET-1 induces human monocytes to produce inflammatory chemokines (11) and oxygen free radicals (28). Thus, the ETB receptor located on macrophages may be involved in ET-1-mediated inflammatory processes leading to the development of atherosclerosis.

We also found that accumulation of foamy macrophages and T lymphocytes may modulate the switching of ET receptor subtypes from ETA to ETB in vascular SMCs (21). In vitro study showed that the switching of ET receptor subtypes occurs in accordance with phenotypic changes in vascular SMCs: ETA receptor is expressed predominantly in SMCs of contractile phenotype, whereas ETB receptor is expressed preferentially in SMCs of synthetic phenotype29. Mitogenic activity mediated through the ETB receptor is built up in vascular SMCs of the synthetic phenotype (29). Therefore, the stimulation of ET-1, potentially released from foamy macrophages and T lymphocytes, may play an active role in SMC proliferation and migration during atherogenesis, and the process may be mediated through the ETB receptor in SMCs.

Conversely, the release of NO from vascular endothelial cells mediated by ETB receptor is vasculoprotective because inhibition of the L- arginine/NO

pathway accelerates lesion progression in hypercholesterolemic rabbits (30,31) and low density lipoprotein receptor-deficient mice (32). Furthermore, the endogenous effect of vascular ETB stimulation in vivo favors vasodilatation (4,5). Indeed, systemic and selective blockade of the ETB receptor induces hypertension and increases peripheral vascular resistance (33,34).

Although ETA receptor has been shown to be involved in atherosclerosis in animals (19,20), the pathophysiological roles of the ETB receptor in atherogenesis have not been fully elucidated. In this study, we examined the effect of non-selective ETA/ETB receptor antagonist SB209670 (35-37) on atherosclerotic lesions in apoE-deficient mice, a suitable animal model of atherosclerosis (38-41). We found that chronic administration of SB209670 reduces diet-induced hypercholesterolemia and atherosclerosis in apoE-deficient mice.

2. Materials and methods

2.1 Experimental animals and study design

All experimental procedures were approved by the Animal Center Commitee of Tsukuba University. Homozygous apoE-deficient mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and animals were derived from brother-sister matings. Before the study, animals were kept on standard mouse chow (0.075% cholesterol, 4% fat, Oriental yeast, Chiba, Japan). At the age of 10 weeks, 94 male mice were randomly divided into four groups: 1. Mice fed a Western-type diet (21% fat, 0.15% cholesterol; Harlan Teklad no.88137) with placebo (0.9% saline)(n=24), 2. Mice fed the Western type diet with SB209670 treatment (n=24), 3. Mice fed the chow diet with placebo (n=23), 4. Mice fed the chow diet with SB209670 treatment (n=23). Dosing was continued until the end of the 12-week observation period. Mice were given SB209670 (10mg/kg/day) (Smithkline Beecham Pharmaceuticals, PA) or placebo by subcutaneously