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European Journal of Pharmacology 387 (2000) 313–319

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Increases of vascular endothelin-converting enzyme activity and endothelin-1 level on atherosclerotic lesions in hyperlipidemic rabbits

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Received 19 July 1999; received in revised form 5 November 1999; accepted 12 November 1999

Abstract

The aim of this study was to investigate vascular endothelin-converting enzyme activity and the tissue level of endothelin-1 in the aorta related to atherosclerotic lesions in high cholesterol diet-fed rabbits. Rabbits were fed two atherogenic diets, 0.5% and 1.5% cholesterol, and a normal diet for 16 weeks. Vascular endothelin-converting enzyme activity in the aortic arch and thoracic aorta was significantly increased (2.0–4.4 times) by the atherogenic diet as compared with the normal diet group as well as the levels of lipids and lipid peroxide in plasma were significantly increased. Tissue endothelin-1 levels in both aortas were also elevated (2.3–6.8 times), corresponding well to the increased tissue enzyme activity. In contrast, plasma endothelin-1 levels increased only in the 1.5% cholesterol diet group (2.7 times). These results indicate that the endothelin-converting enzyme activity and the corresponding endothelin-1 level in the vascular walls increase in association with the development of atherosclerotic lesions.

Keywords: Endothelin-converting enzyme; Endothelin; Atherosclerosis; Cholesterol; Lipid peroxide; (Rabbit)

1. Introduction

Biologically vasoactive peptide, endothelin-1, is produced from the post-translational cleavage of the precursor polypeptide, big endothelin-1, at the Trp^{21}–Val^{22} bond by endothelin-converting enzyme (Yanagisawa et al., 1988; Takahashi et al., 1993; Xu et al., 1994). The enzyme is a membrane-bound zinc metalloprotease that has been shown to be expressed in vascular smooth muscle and respiratory epithelial cells, and most abundantly in vascular endothelial cells (Takahashi et al., 1995). Studies of gene-knockout and tissue-distribution of endothelin-converting enzyme suggest that endothelin-converting enzyme-1 is most likely the physiologically relevant protease involved in big endothelin-1 processing (Goto et al., 1996), though two isoenzymes (endothelin-converting enzyme-1 and -2) have been identified (Emoto and Yanagisawa, 1995). In addition, it has been demonstrated that endothelin-converting enzyme-1 was classified into four isoforms, endothelin-converting enzyme-1α (= -1c), -1β (= -1a), -1b and -1d, through alternative splicing of one enzyme gene (Shimada et al., 1995; Valdenaire et al., 1995, 1999; Schweizer et al., 1997).

Generally, atherosclerosis is characterised by fatty streak formation caused by endothelial dysfunction and macrophage foam cell accumulation, and the following fibrosis plaque formation caused by smooth muscle cell proliferation and connective tissue synthesis (Ross, 1993). Several lines of evidence support the implication of involvement of the endothelin system in the pathogenesis of atherosclerosis. It has been demonstrated that endothelin-1 stimulates the proliferation, migration and matrix formation of smooth muscle cells in vitro (Komuro et al., 1988; Hahn et al., 1993; Ohlstein and Douglas, 1993), and endothelin-1 administered exogenously potentiates the neointimal formation after vascular wall injury in vivo (Trachtenberg et al., 1993). In addition, the endothelin-1 release in endothelial cell, monocyte/macrophage and smooth muscle cell has been enhanced by several chemotactic/growth factors or oxidized low density lipoprotein (LDL) (Goto et al., 1996; Kowala, 1997), and this has been thought of as a major risk factor of atherosclerosis.

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0014-2999/00/$ - see front matter
PII: S0014-2999(99)00808-0

Circulating endothelin-1 immunoreactivity has been elevated in patients with diffuse atherosclerotic disease, as well as in animal models of early coronary atherosclerosis and coronary endothelial dysfunction (Lerman et al., 1991; Arendt et al., 1993; Kowala, 1997). Additionally, tissue endothelin-1 level has been increased in the atherosclerotic coronary artery and aorta (Zeiher et al., 1995; Bacon et al., 1996). It has been demonstrated that endothelin-converting enzyme as well as other components of the endothelin system such as prepro endothelin-1, big endothelin-1 and endothelin receptors (both endothelin ET_A and ET_B receptor) are localised in atherosclerotic lesions (Winkles et al., 1993; Bacon et al., 1996; Minamino et al., 1997). Also, early atherosclerotic progression has been decreased by treatment with endothelin ET_A receptor-selective antagonist in hyperlipidemic hamster (Kowala et al., 1995). However, little is known about the quantitative alteration or pathophysiological role of endothelin-converting enzyme in atherosclerotic blood vessels.

The present study was designed to determine whether the endothelin-converting enzyme activity within atherosclerotic vessels is altered in the progression of atherosclerosis by hyperlipidemia in rabbits fed the high cholesterol diet. We demonstrate here that the quantitative increase in vascular endothelin-converting enzyme activity and the corresponding elevation of local tissue endothelin-1 level is associated with the development of atherosclerotic lesions.

2. Materials and methods

2.1. Animal and tissue preparation

Japanese White male rabbits were obtained from Kitayama Labes (Nagano, Japan) and divided into three groups: standard normal (LRC-4, Oriental Yeast, Tokyo, Japan) diet group and two high cholesterol (0.5% and 1.5%) diet groups, all receiving their respective diets for 16 weeks. Each diet was set at 100 g/day, but water was available ad libitum.

After 16 weeks, blood samples were taken for the assay of plasma lipids and lipid peroxide levels. Rabbits were euthanatized with a lethal dose of sodium pentobarbital (Abbot Laboratories, North Chicago, IL). For the assay of enzyme activity and tissue level of endothelin-1, aortas were isolated and stored at -80°C until the analysis.

2.2. Plasma lipid levels

Blood samples were taken and stored at -80°C to determine plasma lipid levels. Total cholesterol, triglycerides and phospholipids in plasma were measured by cholesterol oxidase-3,5-dimethoxy-*N*-ethyl-(2-hydroxy-3-sulfopropyl)-aniline sodium (DAOS), glycerol-3-phosphate oxidase-DAOS, and choline oxidase-DAOS methods, respectively, using commercially available kits (Wako, Osaka, Japan) (Mitani et al., 1996a,b).

2.3. Endothelin-converting enzyme activity

Endothelin-converting enzyme activity was determined by the production rate of endothelin-1 from big endothelin-1 (human, 1–38; Peptide Institute, Osaka, Japan) by previously reported methods (Minamino et al., 1997). All samples were cut into small pieces and homogenised by use of a Polytron homogenizer (Kinematica, Lucerne, Switzerland) in a 10-fold volume (1 ml/100 mg sample weight) of homogenisation buffer which consisted of 20 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 20 μM pepstatin A (acid protease inhibitor), 20 μM leupeptin (serine and cysteine protease inhibitor), and 50 μM (*p*-amidinophenyl) methanesulphonyl fluoride (serine protease inhibitor). These inhibitors at each concentration do not affect endothelin-converting enzyme activity (Xu et al., 1994). After the homogenates were centrifuged ($1000 \times g$, 4°C) for 10 min, 200 μl of the supernatant was taken for the assay of tissue endothelin-1 levels. The supernatant was further centrifuged ($100{,}000 \times g$, 4°C) for 45 min. Then, the resulting pellets were solubilized in homogenisation buffer containing 0.5% (w/v) Triton X-100 and centrifuged ($100{,}000 \times g$, 4°C) for 60 min.

The supernatant (25 μl) was mixed with assay buffer (100 μl), composed of 20 mM Tris–HCl (pH 6.8), 100 nM human big endothelin-1 (1–38), 0.1% (w/v) bovine serum albumin, 20 μM pepstatin A, and 20 μM leupeptin, and incubated for 120 min at 37°C. The enzyme reaction was terminated by the addition of an equal volume (125 μl) of 5 mM EDTA. The concentration of mature endothelin-1 (1–21) was determined by using commercially available ELISA kits (Biomedica, Divischgasse, Germany). The cross-reactivities of endothelin-1 (1–21), big endothelin-1 (1–38) and big endothelin-1 (22–38) are 100%, $< 1\%$ and $< 1\%$, respectively. There was a linear increase in product amount by enzyme reaction for 120 min. Protein concentration was measured by using a Micro BCA assay kit (Pearce, Rockford, IL). Tissue endothelin-converting enzyme activity was expressed as the amount of endothelin-1 generated per 100 mg tissue weight for 2 h.

2.4. Tissue and plasma endothelin-1 levels

Tissue and plasma endothelin-1 levels were also determined by using commercially available ELISA kits (Biomedica). The supernatants after the first centrifugation ($1000 \times g$, 4°C) of homogenate were directly used for the assay of tissue endothelin-1 level. Before the endothelin-1 assay of plasma, endothelin-1 was extracted according to the instructions of the ELISA kit. The level of endothelin-1 was expressed as femtomol per 100 mg tissue weight or per milliliter.

2.5. Atherosclerotic lesion area

Photographs of the intraluminal surface of aortic segments were computer-analysed (Mac Scope version 2.1, Mitani, Fukui, Japan). The computer-analysed images of atherosclerotic lesion area were assessed, and the coverage was then expressed as a percentage of the lesioned area to the total area in each morphological preparation as described previously (Mitani et al., 1996a).

2.6. Plasma lipid peroxide levels

After the blood sampling, plasma was separated briefly. The lipid peroxides in plasma were immediately measured after plasma-preparation as thiobarbituric acid-reactive substances using commercial kits (Wako) and the results are given in nanomol malondialdehyde-equivalents per milliliter (Mitani et al., 1996a).

2.7. Statistical analysis

Values are means ± S.E. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Lipid profiles in plasma

After 16 weeks, total cholesterol levels in plasma of the 0.5% and 1.5% cholesterol diet-fed group were significantly elevated to approximately 83- and 160-fold, respectively, compared to those in the normal diet-fed group (Table 1). Also, phospholipids in both of the groups fed with the high cholesterol diet were significantly increased 8.4- to 18-fold, while triglyceride levels were significantly elevated 15-fold only in the 1.5% cholesterol group, but not in the 0.5% cholesterol group (Table 1).

3.2. Tissue endothelin-converting enzyme activity

Vascular endothelin-converting enzyme activities in the aortic arch were significantly increased in the high cholesterol diet-fed rabbits, compared with those in the normal diet-fed rabbits (Fig. 1). The enzyme activity increased up to 4.4- and 3.5-fold in the 0.5% and 1.5% cholesterol diet groups, respectively (vs. normal diet group: 14 ± 2.7 fmol/2 h/100 mg tissue) (Fig. 1A). Endothelin-converting enzyme activity in the aortic arch of the 1.5% cholesterol diet group seemed to decrease, but not significantly, compared with that in the 0.5% cholesterol diet-fed rabbits. In the thoracic aorta, vascular endothelin-converting enzyme activity also increased up to 1.4- (not significant) and 2.0-fold in the 0.5% and 1.5% cholesterol diet groups, respectively (vs. normal diet group: 25 ± 2.9 fmol/2 h/100 mg tissue) (Fig. 1B).

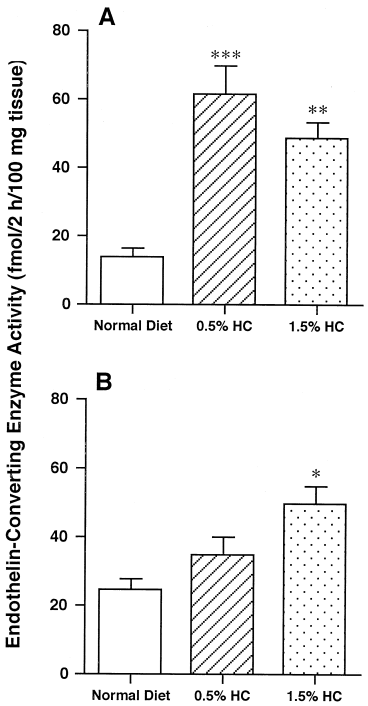

Fig. 1. Effects of atherogenic diet on vascular endothelin-converting enzyme activity in rabbit arterial segments. Rabbits were fed a normal (open bars) diet or high cholesterol (HC) [0.5% (hatched bars) and 1.5% (dotted bars)] diets for 16 weeks. The enzyme activity was assessed in the aortic arch (A) and thoracic aorta (B), respectively. Values are means ± S.E. of five to eight animals. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different from normal diet group.

In addition, endothelin-converting enzyme/neutral endopeptidase inhibitor, phosphoramidon (1 mM), inhibited this enzyme activity by 85% for the normal diet group, 89% for the 0.5% cholesterol diet-fed group, and 92% for the 1.5% cholesterol diet-fed group, respectively ($n = 3$–4, data not shown). On the other hand, neutral endopeptidase inhibitor, thiorphan (100 μM) did not affect the enzyme activity under this condition (data not shown).

3.3. Tissue and plasma endothelin-1 levels

Tissue endothelin-1 levels of aortas were determined in the high cholesterol diet-fed rabbits. Vascular endothelin-1 levels in the aortic arch (both 0.5% and 1.5% cholesterol

Table 1
Plasma lipid levels at 16 weeks
Rabbits were fed a normal diet or high cholesterol (HC, 0.5% and 1.5%) diets for 16 weeks. Values are means ± S.E. of five to eight animals.

Parameter	Normal diet	0.5% HC diet	1.5% HC diet
Total cholesterol (mg/dl)	21 ± 4	1752 ± 150[a]	3436 ± 298[a]
Triglycerides (mg/dl)	24 ± 2	51 ± 5	354 ± 126[b]
Phospholipids (mg/dl)	55 ± 4	459 ± 43[b]	972 ± 115[a]

[a] $P < 0.001$, significantly different from normal diet group.
[b] $P < 0.05$, significantly different from normal diet group.

diet groups) and the thoracic aorta (1.5% cholesterol diet group) were significantly increased in the high cholesterol diet group, compared with the normal diet group (Fig. 2). The increase of tissue endothelin-1 level in the aortic arch was 6.8- and 5.2-fold in the 0.5% and 1.5% cholesterol diet groups, respectively (vs. normal diet group: 3.4 ± 0.7 fmol/100 mg tissue) (Fig. 2A), and was 2.3-fold in the thoracic aorta of the 1.5% cholesterol diet group (vs. normal diet group: 2.9 ± 0.4 fmol/100 mg tissue) (Fig. 2B).

On the other hand, the plasma endothelin-1 level in the 0.5% cholesterol diet group did not change significantly, compared with the normal diet group (Fig. 3). In contrast, the plasma endothelin-1 level in the 1.5% cholesterol diet group was significantly elevated (2.7-fold vs. normal diet group: 0.43 ± 0.08 fmol/ml).

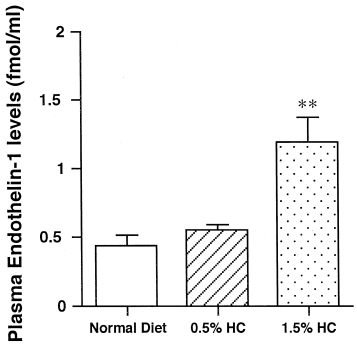

Fig. 3. Effects of atherogenic diet on plasma endothelin-1 level in rabbits. Rabbits were fed a normal (open bars) diet or high cholesterol (HC) [0.5% (hatched bars) and 1.5% (dotted bars)] diets for 16 weeks. Values are means ± S.E. of five to eight animals. $^{**}P < 0.01$, significantly different from normal diet group.

3.4. Atherosclerotic lesion area

The atherosclerotic lesion areas of aortic arch and thoracic aorta were significantly increased in high cholesterol diet-fed rabbits, compared with those in normal diet-fed rabbits (Fig. 4). The lesion area in the aortic arch was $84 \pm 4.8\%$ and $100 \pm 0.2\%$ in the 0.5% and 1.5% cholesterol diet groups, respectively (Fig. 4A), and in the thoracic aorta of 1.5% cholesterol diet group, it was $50 \pm 8.1\%$ (Fig. 4B), but no lesion area was demonstrated in either aorta of the normal diet group.

3.5. Plasma lipid peroxide levels

To determine the circulating oxidative stress, lipid peroxide levels in plasma were measured as thiobarbituric acid-reactive substances. The level of thiobarbituric acid-reactive substances was significantly increased up to 3.1-

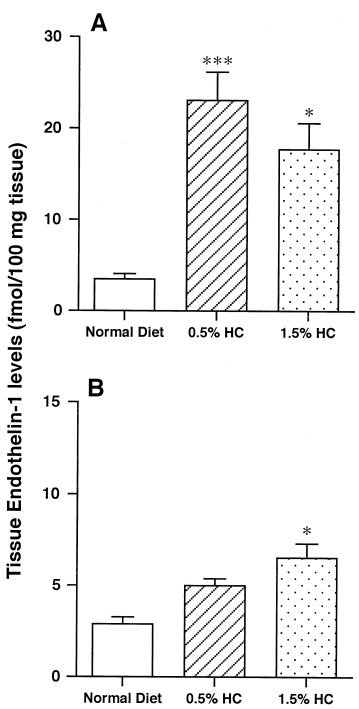

Fig. 2. Effects of atherogenic diet on tissue levels of endothelin-1 in rabbit arterial segments. Rabbits were fed a normal (open bars) diet or high cholesterol (HC) [0.5% (hatched bars) and 1.5% (dotted bars)] diets for 16 weeks. Tissue endothelin-1 levels were determined in the aortic arch (A) and thoracic aorta (B), respectively. Values are means ± S.E. of five to eight animals. $^{*}P < 0.05$, $^{***}P < 0.001$, significantly different from normal diet group.

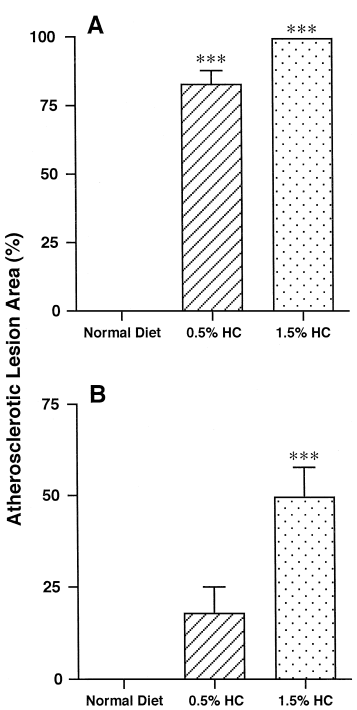

Fig. 4. Effects of atherogenic diet on atherosclerotic lesion area in rabbit arterial segments. Rabbits were fed a normal (open bars) diet or high cholesterol (HC) [0.5% (hatched bars) and 1.5% (dotted bars)] diets for 16 weeks. Atherosclerotic lesion area was assessed in the aortic arch (A) and thoracic aorta (B), respectively. Values are means ± S.E. of five to eight animals. $^{***}P < 0.001$, significantly different from normal diet group.

and 4.8-fold in the 0.5% and 1.5% cholesterol diet-fed rabbits, respectively, compared with that in the normal diet group (1.2 ± 0.05 nmol malondialdehyde-equivalents/ml) ($n = 5–8$, data not shown).

4. Discussion

The present study clearly demonstrated that vascular endothelin-converting enzyme activity was quantitatively increased by the high cholesterol diet for 16 weeks, particularly in the aortic arch, resulting in the corresponding increase of tissue endothelin-1 level, which was associated with the development of lesion formation. These findings suggest that the activation of tissue endothelin-converting enzyme may play a role in developing the atherosclerotic plaque in hyperlipidemia with a consequent increase in local endothelin-1 level. Our results are consistent with other reports indicating enhanced enzyme immunoreactivity in rabbit atherosclerotic lesion (Grantham et al., 1998) and the up-regulation of the functional enzyme activity indicated by the experimental results that the contractile responses to big endothelin-1 were increased in atherosclerotic human coronary arteries denuded of endothelium (Maguire and Davenport, 1998).

When different stages of atherosclerotic progression, in the present study, were induced by loading the two kinds of atherogenic diet, the extent of endothelin-converting enzyme activation associated with the increase in tissue endothelin-1 level and lesion formation in the aortic arches was different from those in the thoracic aortas in each atherogenic diet group. The lesions induced by hyperlipidemia were often seen adjacent to the ostia of branching vessels, for example, aortic arches and intercostal arteries that are part of the thoracic aorta (Stehbens, 1997), in which a disturbed flow is presumed. Exposure of the intimal surface to injurious factors, including disturbed flow in high cholesterol, may differ in the aortic portions. Alternatively, there may be region-related heterogeneity of endothelial cells for their vulnerability to atherosclerotic lesion-forming stimuli. On the other hand, the amount of solubilized membrane protein used for tissue enzyme activity assay was not different between the two arteries: for example, the protein was 0.239 ± 0.009 and 0.212 ± 0.007 mg/100 mg tissue weight for the aortic arch and the thoracic aorta, respectively, of the 0.5% cholesterol diet group. Thus, the amount of protein extracted does not seem attributable to the regional heterogeneity of increased endothelin-converting enzyme activity.

In what kinds of cells is vascular endothelin-converting enzyme stimulated in chronic hyperlipidemia? Because endothelin-converting enzyme is most abundantly found in vascular endothelial cells (Takahashi et al., 1995), and endothelial function in terms of endothelial-dependent relaxation is modulated by hyperlipidemia (Mitani et al., 1996b), it is conceivable that endothelial cells directly facing hyperlipidemia in circulation are responsible for the excessive activation of the enzyme. On the other hand, vascular smooth muscle cells and monocytes/macrophages as well as endothelial cells are known to synthesise and secrete endothelin-1 in vitro (Resink et al., 1990; Kowala, 1997) and to be critical factors in the formation of atherosclerosis (Ross, 1993). It is postulated that activated endothelin-converting enzyme after balloon injury is derived from neointimal smooth muscle cells (Minamino et al., 1997). Also, intense endothelin-converting enzyme immunoreactivity was present in both smooth muscle cells and macrophages in rabbit coronary atherosclerotic lesion (Grantham et al., 1998). Thus, it is conceivable that these cells are included as sources of increased endothelin-converting enzyme in the blood vessels.

Previous studies have demonstrated that the plasma endothelin-1 concentrations were increased in patients with hypercholesterolemia and coronary artery disease (Lerman et al., 1991; Arendt et al., 1993). In the present study, plasma endothelin-1 levels were increased in the higher (1.5%) cholesterol diet-fed rabbits associated with increased tissue endothelin-converting enzyme activity. In addition, increased circulating endothelin-1 has been demonstrated in full development atherosclerosis (Lerman et al., 1991). Our results in higher cholesterol diet rabbits may support the view that circulating endothelin-1 is activated in advanced disease. However, interestingly, plasma endothelin-1 levels in lower (0.5%) cholesterol diet-fed rabbits were not significantly elevated in spite of the increase of tissue enzyme activity and endothelin-1 level, especially in the aortic arch. Our findings in the present study suggest that local activation of endothelin-converting enzyme and endothelin-1 production may relate with atherosclerosis progression irrespective of change in plasma endothelin-1 level. Although the mechanism of local activation of the endothelin system remain unclear, it has been presumed that plasma endothelin levels underestimate the degree of endothelin synthesis in atherosclerotic arteries since in vitro, endothelial cells secrete endothelin-1 on the basolateral side (Wagner et al., 1992; Kowala, 1997). Another possible mechanism that the clearance of endothelin-1 by the irreversible binding to its receptors is included in endothelin metabolism, also plays a key role in maintaining low plasma endothelin-1 levels (Gray and Webb, 1996). Furthermore, our results are supported by another experiment in which aortic levels, but not plasma levels, of endothelin-1 was increased in a rabbit model of early atherosclerosis with intense enzyme immunoreactivity in the lesion (Grantham et al., 1998).

Oxidative stress is thought to be one of the major triggering factors of endothelial dysfunction with an impaired response to endothelium-dependent vasodilation (Stehbens, 1997). The alteration of endothelial function has been proposed to be related to either the expression or function of nitric oxide and endothelin-1. Moreover, oxidized LDL, which is a product from the reactive oxygen-

mediated chain reaction and a critical factor of atherosclerosis, but not native LDL, has been shown to stimulate endothelin-1 production and decrease nitric oxide production by the downregulation of endothelial nitric oxide synthase expression in endothelial cells in vitro (Hernandez-Perera et al., 1998). Also in our study, thiobarbituric acid-reactive substances as a marker of circulating oxidative stress (albeit non-specific), was increased in hyperlipidemic rabbits with the increase in vascular endothelin-converting enzyme activity. And there was a weak, but significant, correlation between plasma thiobarbituric acid-reactive substances and vascular enzyme activity ($r = 0.448$ for the aortic arch and $r = 0.514$ for the thoracic aorta, $P < 0.05$, data not shown). Taken together, circulating oxidative stress may attribute, at least in part, to the enzyme activation and endothelial dysfunction. In contrast, a variety of growth factors, including transforming growth factor-β, thrombin, interleukin-1, angiotensin II, and platelet-derived growth factor as well as oxidized LDL, which have been shown to be activated in atherosclerosis, modulate endothelin-1 production from endothelial cells, macrophages and smooth muscle cells in vitro (Resink et al., 1990; Maemura et al., 1992; Kowala, 1997). Although we have not addressed in this study what signal is delivered to the cells with altered tissue enzyme activity, it would be of interest to pursue what triggers increased enzyme activity among a variety of cytokines and growth factors in hyperlipidemia.

Endothelin-1 has atherogenic features such as the stimulation of proliferation, migration and matrix formation of smooth muscle cells (Komuro et al., 1988; Hahn et al., 1993; Ohlstein and Douglas, 1993). Therefore, it is hypothesised that the local activation of endothelin-converting enzyme in the vascular wall contributes to the development of atherosclerosis. In the present study, there was a significant correlation between atherosclerotic lesion area and tissue endothelin-converting enzyme activity in the thoracic aorta ($r = 0.780$, $P < 0.001$, data not shown). Also, early atherosclerotic progression has been decreased by treatment with endothelin ET_A receptor-selective antagonist in hyperlipidemic hamster (Kowala et al., 1995). Thus, it is conceivable that the activation of endothelin system in the blood vessel is involved in the progression of atherosclerosis, whereas the effects of endothelin-converting enzyme inhibitor on the atherosclerotic lesion formation in the present conditions were not presented.

Two types of isoenzymes, endothelin-converting enzyme-1 and -2, have been identified (Xu et al., 1994; Emoto and Yanagisawa, 1995); endothelin-converting enzyme-1 is associated with plasma membranes and has a neutral pH optimum, whereas endothelin-converting enzyme-2 is an intracellular enzyme with an acidic pH optimum. Since the enzyme activity was measured at neutral pH in the present study, it is postulated that endothelin-converting enzyme-1 activity increases in hyperlipidemia. In addition, endothelin-converting enzyme-1 has four subtypes, endothelin-converting enzyme-1α (= -1c), -1β (= -1a), -1b and -1d, which are encoded by the same gene and are produced through alternative splicing (Shimada et al., 1995; Valdenaire et al., 1995, 1999; Schweizer et al., 1997). Since the present enzyme activity assay did not distinguish between these two isoenzymes, it was not clear whether the increase in vascular enzyme activity in the present study was due to the enhanced endothelin-converting enzyme-1α (= -1c), -1β (= -1a), -1b and/or -1d. However, analysis of the endothelin-converting enzyme-1 gene structure has shown that the promoter region surrounding endothelin-converting enzyme-1α (= -1c), -1b and -1d, shows the features of a housekeeping gene promoter (Valdenaire et al., 1995, 1999; Schweizer et al., 1997; Turner and Tanzawa, 1997). In contrast, the features of endothelin-converting enzyme-1β (= -1a) promoter region suggest that this enzyme is induced in the pathological state, for example, under oxidative stress (Valdenaire et al., 1995, 1999; Schweizer et al., 1997; Turner and Tanzawa, 1997). The increase of plasma lipid peroxides in the present study suggests that the endothelium is exposed continuously to highly oxidative stress. Taken together, the changes of oxidative stress in a stage of atherosclerotic progression might result in the activation of endothelin-converting enzyme-1β (= -1a).

In conclusion, we demonstrated that vascular endothelin-converting enzyme activity in rabbit blood vessels was increased in the atherosclerotic lesion by an atherogenic diet. This increase coincides with the augmentation of tissue endothelin-1 level and is associated with development of lesion area. The observed heterogeneity of vascular endothelin-converting enzyme activation may be attributed to differing vascular susceptibility to atherogenic changes in each arterial region. Thus, local activation of endothelin-converting enzyme in the vascular walls may be involved in hyperlipidemia-induced atherogenesis through the increase in tissue endothelin-1 level.

Acknowledgements

The authors thank Ms. K. Oda-Omae and Ms. T. Kimura-Ninomiya for excellent technical assistance.

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