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Anagliptin, a dipeptidyl peptidase-4 inhibitor, decreases macrophage infiltration and suppresses atherosclerosis in aortic and coronary arteries in cholesterol-fed rabbits

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ABSTRACT

Introduction. Several studies have demonstrated suppression of aortic atherosclerosis by dipeptidyl peptidase-4 (DPP-4) inhibitors in hypercholesterolemic mice. However, it remains unknown whether DPP-4 inhibitors also exert anti-atherogenic effects in coronary arteries. We examined the effect of anagliptin, a DPP-4 inhibitor, on atherosclerosis development in the aorta and coronary arteries in a high-cholesterol diet-fed rabbits.

Methods. Japanese white rabbits were fed either normal chow (n = 8) or a diet containing 0.5% cholesterol (n = 34) for 14 weeks. Cholesterol-fed rabbits were given 0.3% anagliptin or not in drinking water (each n = 16 and 18) for 12 weeks.

Results. Dietary cholesterol intake markedly increased serum total cholesterol (TC) levels (1464 ± 150 mg/dL, mean ± SE), and the most striking increase was observed among the major lipoproteins in very low-density lipoprotein (VLDL) as determined by high-performance liquid chromatography. No significant changes were observed in body weight, water intake, hemoglobin A1c, or glucose response to intravenous glucose loading following anagliptin administration. Anagliptin decreased TC and VLDL-cholesterol as well as cholesterol absorption markers sitosterol and campesterol slightly, although not significantly. Serum DPP-4 activity was suppressed by 82%, and active glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide levels were increased 2- to 3-fold by anagliptin treatment. Severe hypercholesterolemia resulted in the development of atherosclerosis in the aorta, and the ratio of atherosclerotic lesions to the total aortic

Abbreviations: CV, cardiovascular; DPP, dipeptidyl peptidase; Apo, apolipoprotein; LDL, low-density lipoprotein; GLP, glucagon-like peptide; GIP, glucose-dependent insulinotropic polypeptide; ELISA, enzyme linked immunosorbent assay; LC–MS/MS/MS, liquid chromatography–tandem mass spectrometry; IVGTT, intravenous glucose tolerance test; HPLC, high-performance liquid chromatography; CM, chylomicrons; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; OHdG, oxidative stress markers 8-hydroxy-2′-deoxyguanosine; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; PAF-AH, platelet-activating factor acetylhydrolase; Q-RT-PCR, quantitative reverse transcription-polymerase chain reaction; TNF, tumor necrosis factor; IL, interleukin; MCP, monocyte chemoattractant protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HE, hematoxylin and Eosin HE; EVG, elastic van Gieson; SMA, smooth muscle actin; SEM, standard error of the mean; ANOVA, analysis of variance.

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surface area was 22 ± 2%. Anagliptin suppressed the lesion ratio to 9 ± 2% ($p < 0.001$). Atherosclerotic lesions were clearly observed in the coronary arteries, where the mean intima-media area was enlarged, and intimal formation was developed. Anagliptin treatment attenuated the intima-media area and the intimal area by 43%. Alpha-smooth muscle actin-positive and macrophage-positive areas in the coronary arteries were suppressed by 66 and 75%, respectively, after anagliptin treatment. The aortic lesion ratio and the coronary intima area were correlated with each other ($r = 0.506$, $p < 0.01$), and each lesion correlated with TC in the whole cholesterol-fed rabbits. Gene expression of the proinflammatory cytokines tumor necrosis factor-alpha and interleukin-6 in the carotid arteries was markedly reduced by approximately 90%, and vascular DPP-4 activity was reduced by 66% after anagliptin treatment.

Conclusions. We demonstrated for the first time that a DPP-4 inhibitor can substantially suppress plaque formation in coronary arteries with a marked reduction in macrophage accumulation likely via its anti-inflammatory properties.

1. Introduction

Patients with type 2 diabetes mellitus are at increased risk of cardiovascular (CV) disease [1]. Elevated plasma glucose levels lead to increased CV risk combined with associated co-morbidities such as obesity, hypertension, and dyslipidemia. Dipeptidyl peptidase (DPP)-4 inhibitors are a relatively new class of drugs that are used for type 2 diabetes treatment and recently have been widely used in clinical practice. DPP-4 inhibitors are expected to provide CV benefits because of amelioration of fasting and postprandial hyperglycemia without hypoglycemia or body weight gain [2,3]. Moreover, DPP-4 inhibitors potentially have lipid-lowering [4] and hypotensive effects [2,3]. There have been several preclinical studies demonstrating that DPP-4 inhibitors exert anti-atherosclerotic effects in hypercholesterolemic mice such as apolipoprotein (apo) E-null mice [5–10] and low-density lipoprotein (LDL) receptor-null mice [11]. Because these hypercholesterolemic mice are not diabetic, a direct effect of DPP-4 inhibitors to prevent atherosclerosis in a manner not related to glucose handling has been implicated. In contrast, recent clinical trials have failed to demonstrate favorable effect of DPP-4 inhibitors on suppression of CV outcomes in type 2 diabetic patients [12–14]. However, the majority of subjects enrolled in these trials already had CV diseases and were being treated with cardioprotective drugs such as statins. Additionally, the observation periods were only few years, which may be difficult to evaluate the anti-atherogenic potential of DPP-4 inhibitors, especially in early stages of atherosclerosis.

Most animal studies have used mice to explore the molecular mechanisms of anti-atherogenic effects of DPP-4 inhibitors. Unfortunately, mice are too small to clearly observe coronary arterial lesions, where the majority of CV events occur. Rabbits easily develop atherosclerosis when fed a high cholesterol diet, which is a classical atherosclerosis animal model [15,16]. Unlike mice, it is easy to observe coronary arterial lesions in rabbits [17,18]. Currently, there is no report examining the effect of DPP-4 inhibitors on coronary atherosclerosis in animal models. Anagliptin is a unique DPP-4 inhibitor that has a mild cholesterol-lowering effect in humans [19]. We here report that anagliptin can substantially suppress coronary arterial plaque formation as well as aortic atherosclerosis in cholesterol-fed rabbits without a significant cholesterol-lowering effect.

2. Methods

2.1. Animals

Japanese white rabbits (male, 12 w of age) were purchased from Kitayama Labes (Nagano, Japan). Following acclimation for 2 weeks, the rabbits were assigned to normal chow ($n = 8$) or 0.5% high cholesterol diet ($n = 34$). Two weeks after the assignment, the rabbits fed high cholesterol diet were divided into vehicle ($n = 18$) or anagliptin treatment ($n = 16$). Anagliptin was given to the rabbits via drinking water (0.3% *w/v*). Twelve weeks after the initiation of treatment, the rabbits were sacrificed under general anesthesia for sample collection. The study design was depicted in Supplemental Fig. A. This study design was approved by the animal care committee of Sanwa Kagaku Kenkyusho (Nagoya, Japan).

2.2. Measurements

Serum lipids and glucose concentrations were biochemically measured using an automatic analyzer (7180, Hitachi; Ibaraki, Japan). Glycohemoglobin levels were measured using an automated glycohemoglobin analyzer (HLC-723G8, Tosoh; Tokyo, Japan). Plasma active glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) levels were measured by enzyme linked immunosorbent assay (EGLP-35K; Merck Millipore, Tokyo, Japan) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) system consisted in Ultimate 3000 Series nano LC system (Dionex Softron GmbH; Germering, Germany) and an API QTRAP 5500 hybrid triple quadrupole/linear ion trap mass spectrometer (QqQ/LIT-MS; MDS Sciex, Ontario, Canada), respectively [20]. In the intravenous glucose tolerance test (IVGTT), 0.6 g/kg glucose was intravenously administered to rabbits, and a time course of blood glucose concentration changes was measured. Plasma and vascular DPP-4 activity was measured using the fluorescent substrate Gly-Pro-MCA (Peptide