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A novel thiazolidinedione MCC-555 down-regulates tumor necrosis factor-α-induced expression of vascular cell adhesion molecule-1 in vascular endothelial cells

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Abstract

Thiazolidinediones (TZDs) are anti-diabetic agents that enhance insulin sensitivity through activating peroxisome proliferator-activated receptor (PPAR) γ. Besides their glucose-lowering effects, TZDs are shown to exhibit anti-inflammatory properties in vascular cells, although their precise molecular mechanisms are unknown. In the present study, we examined the effects of a novel TZD MCC-555, which has unique characteristics of ability to activate not only PPARγ but also PPARα and PPARδ on vascular cell adhesion molecule-1 (VCAM-1) expression in vascular endothelial cells (ECs). Human aortic ECs were treated with MCC-555, followed by stimulation with tumor necrosis factor (TNF)-α. Cell surface VCAM-1 protein expression and human monocytoid U937 cell adhesion to these cells were determined. MCC-555 efficiently inhibited TNF-α-stimulated VCAM-11expression and U937 cell adhesion. Transient transfection of bovine aortic ECs with a VCAM-1 promoter construct revealed that MCC-555 inhibited TNF-α-induced VCAM-1 promoter activity. Electrophoretic mobility-shift assay demonstrated that MCC-555 reduced the amount of nuclear factor-κB (NF-κB) bound to its recognition site on the VCAM-1 promoter. The considered PPARδ activator GW501516 and the considered PPARα activator fenofibrate also inhibited TNF-α-induced VCAM-1 expression, whereas pioglitazone and rosiglitazone did not. These results indicate that MCC-555 is a strong TZD agent to inhibit the cytokine-induced VCAM-1 expression in vascular ECs. This effect is exerted probably through activation of PPARα and/or PPARδ, rather than PPARγ, mediating down-regulation of NF-κB activity.

Keywords: Thiazolidinediones; Endothelium; Vascular biology; Adhesion molecules; Atherosclerosis

1. Introduction

Thiazolidinediones (TZDs) are pharmaceutical agents that enhance insulin sensitivity at a level distal to insulin receptor. The action mechanism of TZDs is not completely understood, but a number of evidence suggests their function as ligands for peroxisome proliferator-activated receptor (PPAR) γ [1]. PPAR is a member of nuclear receptor family molecules, and is known to consist of three subtypes (α, δ and γ) [2]. Of the potent TZDs, troglitazone, pioglitazone and rosiglitazone (BRL49653) are able to bind to PPARγ with a high affinity and to lower plasma glucose levels in vivo [2], and the rank order of TZD potency for PPARγ activation correlates with their rank order potency for in vivo glucose-lowering [3]. Besides their anti-diabetic property, these TZDs have been shown to exert anti-inflammatory effects on vascular cells. In monocytes/macrophages, TZDs inhibit the production of inflammatory cytokines and the expression of inducible nitric oxide synthase, gelatinase B and scavenger receptor A [4,5]; in vascular smooth muscle cells, they inhibit cell migration and the expression of matrix metalloproteinases [6]; in vascular endothelial cells (ECs), certain TZDs like troglitazone and

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ciglitazone inhibit the production of monocyte chemoattachment protein-1 [7] and the expression of adhesion molecules [8]. Furthermore, several clinical studies revealed that treatment with TZDs for relatively short periods in type 2 diabetic patients reduces intima-media thickness of carotid arteries [9]. Thus, there is a growing evidence that TZDs exert their effects directly on vascular walls. Such anti-inflammatory effects of TZDs on vascular cells are also thought to be mediated probably via activating PPARγ.

Vascular cell adhesion molecule-1 (VCAM-1) is an adhesion molecule expressed in atherosclerotic lesions, which is involved in mononuclear cell adhesion to the vascular endothelium [10]. The expression of VCAM-1, in addition, to that of other adhesion molecules and inflammatory cytokines, represents part of the inflammatory process affecting vascular walls [11]. A study using VCAM-1-deficient mice demonstrated that VCAM-1 plays a dominant role in the initiation of atherosclerosis [12]. Thus, to inhibit the expression of this adhesion molecule is one of the targets of anti-inflammatory drugs. Several nuclear receptor-activators such as estrogens, progestins, retinoic acids and fibric acid derivatives have been shown to inhibit the expression of VCAM-1 in cultured vascular ECs [13–17]. However, there are controversial results concerning the effects of the PPARγ-activators TZDs on VCAM-1 expression: troglitazone and ciglitazone inhibited VCAM-1 expression while rosiglitazone and pioglitazone did not [8,17]. Such differential effects of TZDs suggest the ligands-specificity of PPARγ function mediating VCAM-1 repression, or evoke questions as to which subtypes of PPARs are involved in this process.

MCC-555 is a novel TZD that has the potentials to improve metabolic status and insulin sensitivity in obese Zucker and Zucker Diabetic Fatty (ZDF) rats [18], and attenuate the development of overt diabetes in young ZDF rats [19]. It has greater anti-hyperglycemic potency than that of rosiglitazone, and pioglitazone in KK-A^y mice yet has lower affinity for PPARγ than rosiglitazone [20]. Moreover, the effect of MCC-555 on PPARγ transcriptional activity is highly context-specific such that it can function as a full agonist, partial agonist or antagonist that depends on the cell type or DNA binding site [20]. In addition, MCC-555 was found to have ability to efficiently activate not only PPARγ but also PPARα and PPARδ in vitro [21], whereas troglitazone, pioglitazone, and rosiglitazone are not potent to activate PPARα and PPARδ [22]. From these animal and in vitro studies, MCC-555 was found to have unique characteristics compared with other TZDs. We hypothesized that this novel TZD down-regulates VCAM-1 expression in cultured vascular ECs, and aimed to elucidate its molecular mechanism.

2. Materials and methods

2.1. Cell culture and materials

Human aortic endothelial cells (HAECs: Kurabo Industries, Osaka, Japan) were maintained in HuMedia-EG2 medium containing 5 ng/ml human basic fibroblast growth factor, 10 ng/ml human epidermal growth factor, 1 μg/ml hydrocortisone, 50 μg/ml gentamycin, 50 μg/ml amphotericin B and 10 μg/ml heparin (Kurabo Industries) supplemented with 2% FBS (JRH Biosciences, Lenexa, KS) (growth medium). Bovine aortic endothelial cells (BAECs; Cell Systems, Kirland, WA) were grown in Dulbecco's modified eagle's medium (DMEM; Sigma, St. Louis, MO) with 10% FBS. Human TNF-α was obtained from Dainippon Pharmaceutical (Osaka, Japan). MCC-555, pioglitazone, rosiglitazone, fenofibrate and GW501516 were obtained from Mitsubishi Pharma Corporation (Yokohama, Japan). Mouse anti-human VCAM-1 antibody and mouse anti-human E-selectin antibody were obtained from Genezyme (Cambridge, MA) and Upstate Biotechnology (Lake Placid, NY), respectively. Seapansy luciferase control plasmid was purchased from Toyo Beanet (Tokyo, Japan). SuperFect Transfection Reagent was from Qiagen (Valencia, CA).

2.2. Adhesion assays

For adhesion assays, we used human monocytoid U937 cells (American Tissue Culture Collection, Rockville, MD). These cells were used in the adhesion experiments of the previous studies [16,23,24], and thus seemed adequate for the data comparison. HAECs were plated on 6-well collagen-coated dishes at a density of 1.0×10^5 cells/well in the growth medium. After the cells were being subconfluent, FBS concentration in the culture medium was decreased to 0.4%. The cells were treated with test compounds or vehicle (0.1% DMSO) for 24 h, and then were stimulated with 20 ng/ml TNF-α for 4 h. Thereafter, U937 cells (3×10^5 cells/well) were added to each monolayer and incubated under rotating conditions (63 rpm) at room temperature. Ten minutes later, non-adhering cells were removed by gentle washing with phosphate-buffered saline, and monolayers were fixed with 1% paraformaldehyde. The number of adherent cells was counted in four different fields using an ocular grid (magnification area of 0.025 mm^2).

2.3. Determination of cell surface VCAM-1 expression

HAECs were plated onto 96-well collagen-coated dishes (2×10^4 cells/well) in the growth medium. The next day, when the cells were subconfluent, FBS concentration in the culture medium was reduced to 0.4%. Thereafter the cells were treated with test compounds or vehicle (0.1% DMSO) for 24 h, and then were stimulated with 20 ng/ml TNF-α for 4 h. ELISA for cell surface VCAM-1 was performed as described previously [14].

2.4. Transient transfection

To investigate the effect of test compounds on human VCAM-1 promoter activities, we transiently transfected BAECs with a VCAM-1 reporter construct. We used BAECs