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Cilostazol protects vessels against hyperglycemic injury and accelerates healing after implantation of drug-eluting stent in a type 1 diabetes mellitus rat aorta stent model

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ABSTRACT

Objective: Cilostazol, a selective phosphodiesterase-3 (PDE-3) inhibitor, can effectively suppress platelet activation and attenuate the increase in carotid intima-media thickness in diabetes mellitus (DM) patients. Therefore, we investigated whether cilostazol had effects on the healing process after implantation of a drug-eluting stent (DES) in a rat model of type 1 DM.

Methods and results: Streptozotocin-induced DM rats were divided into 2 groups in which cilostazol (30 mg/kg/day; DM-Cilostazol) or vehicle (DM-Vehicle) was orally administered. Age-matched rats treated with the vehicle were used as a control group (NDM-Vehicle). After 4 weeks, cilostazol changed the expression of vascular cell adhesion molecule and intercellular adhesion molecule and the apoptotic cell ratio of the media (DM-Vehicle: 53.5 ± 9.8%, DM-Cilostazol: 26.4 ± 8.3%, $p < 0.05$) in the aortic wall. Also, in a modified aortic ring test, cilostazol preserved the angiogenic potential of the aorta ([height of the sprouting tubes] DM-Vehicle: 0 ± 0 μm, DM-Cilostazol: 344.6 ± 236.8 μm, $p < 0.05$). After implantation of paclitaxel-eluting stents (PES) in rats treated with cilostazol or vehicle, thrombus formation, deposition of fibrin, and infiltration of inflammatory cells were attenuated by cilostazol. In particular, the re-endothelialization by von Willebrand factor expression in the DM-PES-Cilostazol group was enhanced compared with that in the DM-PES-Vehicle group.

Conclusion: Cilostazol has potential for protecting vessels against hyperglycemic injury and for accelerating the healing process after implantation of DES.

1. Introduction

Diabetes mellitus (DM) is a risk factor for cardiovascular disease (CVD). Patients with both type 1 and type 2 DM develop atherosclerosis at a significantly accelerated rate compared with non-diabetic patients [1–3]. DM is associated with a two- to four-fold higher risk for cardiovascular events [4], and nearly 80% of DM-associated deaths are caused by CVD [5]. As such, DM is regarded as a coronary heart disease risk equivalent, which means that the risk for CVD in a patient with DM is the same as in an individual with a previous CVD event [6].

Drug-eluting stents (DES) are more effective for reducing in-stent restenosis (ISR) than are bare metal stents. In the setting of DM, hyperglycemia is involved with the inflammatory reaction in the occurrence or progression of atherosclerosis. Thus, despite the efficacy of DES in reducing neointimal proliferation and ISR, stent failure and ISR still occur and develop more frequently in the setting of DM [7–9]. In addition to ISR, concern has arisen about the potential for late stent thrombosis or very late stent thrombosis after DES implantation, and this concern has led to extended-duration dual anti-platelet therapy. The occurrence of late or very late stent thrombosis has been attributed to delayed re-endothelialization and inhibition of vascular repair [10].

Cilostazol, a selective phosphodiesterase-3 (PDE-3) inhibitor, can effectively suppress platelet activation by increasing intracellular cyclic adenosine monophosphate (cAMP) [11] and can induce vasodilation by increasing cyclic guanosine monophosphate (cGMP) in vascular smooth muscle cells (SMCs) [12]. Also, cilostazol is suggested to attenuate the increase in carotid intima-media

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thickness in DM patients [11] and to prevent the progression of symptomatic intracranial arterial stenosis [13]. Generally, the pleiotropic effects of cilostazol are explained by a regulation of SMC proliferation, an anti-oxidant effect, an anti-apoptotic effect, and an increase of high-density lipoprotein cholesterol in patients and in animal models of type 2 DM [11,13]. These effects are mainly through the regulation of inflammatory cytokines such vascular cell adhesion molecule (VCAM) [14] or intercellular adhesion molecule (ICAM) [15]. In previous studies, cilostazol caused the decreased expression of ICAM via nitric oxide (NO) production in human umbilical vein endothelial cells in a hyperglycemic condition [15]. In a streptozotocin-induced DM nephropathy rat model, cilostazol decreased the activity of reactive oxygen species and could improve the levels of serum cholesterol, triglyceride, and low-density lipoprotein cholesterol [16]. The pathogenic mechanism and histologic evidence of the effects of cilostazol on CVD are not completely known in type 1 or type 2 DM, however.

We showed previously that streptozotocin-induced type 1 DM delays the re-covering or re-endothelialization in a novel rat aorta stent model and that the phenomenon was related to an increase in both inflammatory cytokines and inflammatory cells [17]. In the present study, we investigated the underlying mechanism and the histologic evidence of vascular protection by cilostazol in a type 1 DM rat aorta stent model.

2. Methods

2.1. Experimental animal model

All experiments were conducted in accordance with the institutional guidelines for the use and care of laboratory animals, which conform with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Also, animal protocols were approved by the Chonnam National University Animal Care and Use Committee (No. CNU IACUC-H-2009-18).

Type 1 DM was induced by intra-peritoneal injection of streptozotocin (STZ) (65 mg/kg, Sigma–Aldrich Inc, St. Louis, MO, USA) in male Sprague Dawley (SD) rats (Samtako Inc., Daejeon, Korea) as previously described [17]. Rats with stable blood glucose levels of 300 mg/dL with insulin administration (2 units/kg/day) for 1 consecutive week were considered diabetic. Age-matched non-diabetic rats made up the control group (NDM). STZ-induced DM rats were divided into two groups. One group (DM-Cilostazol, $n = 3$) was orally administered cilostazol (30 mg/kg/day) and the other group (DM-Vehicle, $n = 3$) was orally administered the vehicle for 4 weeks. NDM rats received vehicle treatment for 4 weeks (NDM-Vehicle, $n = 3$).

The stent implantation was conducted as previously described [17]. The DM and NDM rats were anesthetized with an

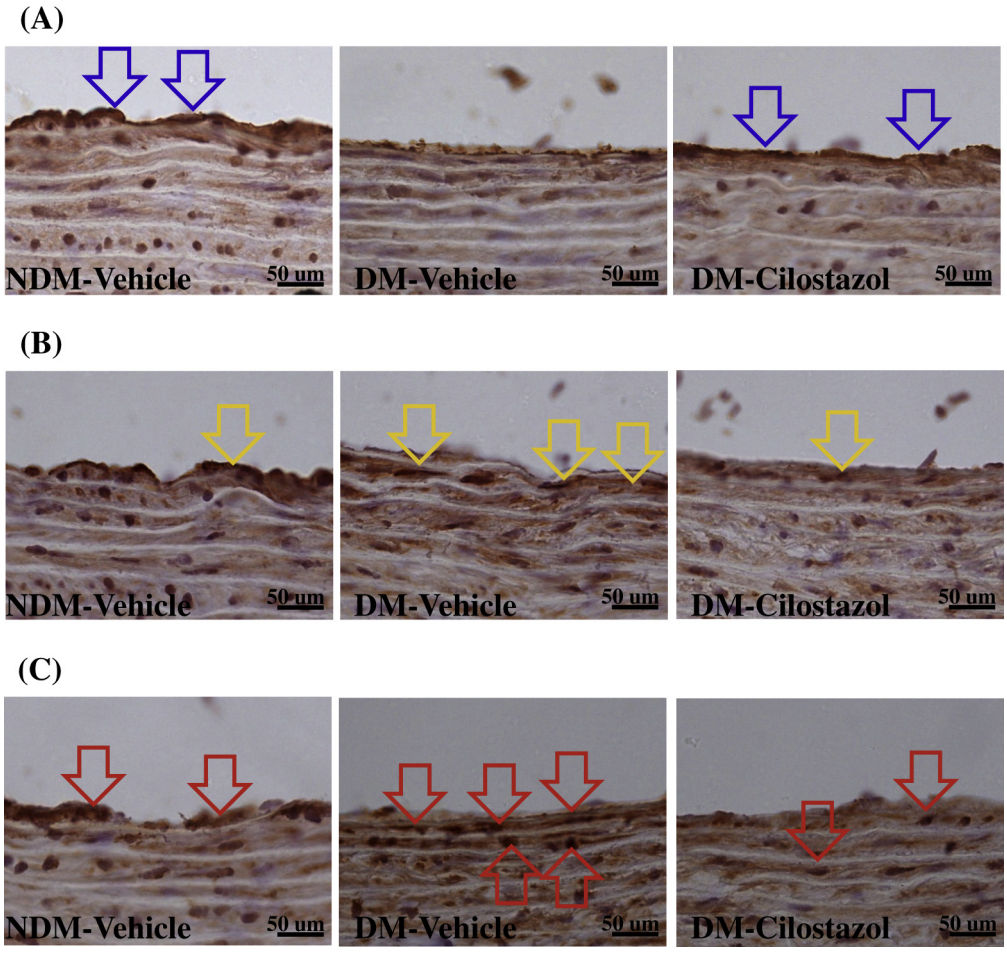

Fig. 1. Cilostazol preserved endothelial cells and regulated the expression of VCAM and ICAM in type 1 DM. (A) Representative picture of endothelial cells (vWF-positive cells, brown color, blue arrows), which were detected in both the NDM-Vehicle group and the DM-Cilostazol group but not in the DM-Vehicle group. (B) VCAM expression. The expression of VCAM (brown color, yellow arrows) was detected mainly at the intima in the NDM-Vehicle and DM-Cilostazol groups but at the media in the DM-Vehicle group. (C) ICAM expression. The expression of ICAM (brown color, red arrows) was detected mainly at the intima in the NDM-Vehicle and DM-Cilostazol groups but at the media in the DM-Vehicle group. VCAM: Vascular cellular adhesion molecule, ICAM: Intercellular adhesion molecule, DM: Diabetes mellitus, NDM: Non-DM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)