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Research paper

SIRT1 inhibition causes oxidative stress and inflammation in patients with coronary artery disease

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ABSTRACT

Coronary artery disease (CAD) is the primary critical cardiovascular event. Endothelial cell and monocyte dysfunction with subsequent extravagant inflammation are the main causes of vessel damage in CAD. Thus, strategies that repress cell death and manage unsuitable pro-inflammatory responses in CAD are potential therapeutic strategies for improving the clinical prognosis of patients with CAD. SIRT1 (Sirtuin 1) plays an important role in regulating cellular physiological processes. SIRT1 is also thought to protect the cardiovascular system by means of its antioxidant, anti-inflammation and anti-apoptosis activities. In the present study, we found that the SIRT1 expression levels were repressed and the acetylated p53 expression levels were enhanced in the monocytes of patients with CAD. LOX-1/oxidative stress was also up-regulated in the monocytes of patients with CAD, thereby increasing pro-apoptotic events and pro-inflammatory responses. We also demonstrated that monocytes from CAD patients caused endothelial adhesion molecule activation and the adherence of monocytes and endothelial cells. Our findings may explain why CAD patients remain at an increased risk of long-term recurrent ischemic events and provide new knowledge regarding the management of clinical CAD patients.

1. Introduction

Coronary artery disease (CAD) is the primary critical cardiovascular event, causing high morbidity and mortality all over the world [1]. Coronary artery luminal obstructions and plaque cracks due to atherosclerosis are the most common causes of CAD, which is distinguished by endothelial damage, lipid aggregation and the generation of atherosclerotic plaques [2]. Apoptosis and necrosis of cardiomyocytes, endothelial cells and monocytes with subsequent extravagant inflammation are the main causes of vessel damage under CAD [3]. Thus, strategies that repress cell death and manage unsuitable pro-inflammatory responses under CAD are potential therapeutic strategies for improving the clinical prognosis of patients with CAD.

Oxidative stress is one complication that occurs when the generation of reactive oxygen species (ROS) exceeds antioxidant enzyme activity [4]. Oxidative stress is recognized as a key regulator of the progression of cardiovascular diseases. For example, previous studies have suggested that hyperlipidemia and diabetes mellitus (DM) are both associated with elevated oxidative stress, which may result in the development of atherosclerosis and CAD [5]. In addition to cardiovascular diseases, other systemic diseases, degeneration and aging are associated with oxidative stress and have been well reported by investigators [6]. In normal situations, cells are protected from ROS by antioxidant enzymes such as Superoxidase dismutase (SOD), glutathione peroxidase (GPx), and catalase [7]. SOD quickly catalyzes the chelation of O^- to H_2O_2·H_2O_2 is converted to O_2 and H2O by catalase or GPx [8]. Therefore, antioxidant enzyme activity is important for the normal redox balance in humans.

The chronic inflammation linked with CAD is currently well-known in clinical practice. The American Heart Association suggested that the blood C-reactive protein (CRP) level is a risk factor in coronary disease development [9]. In addition, the immune capacity in the human body plays a critical role in the induction and deterioration of atherosclerosis, with monocytes/macrophages playing critical roles in this

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action [10]. Monocyte participation in the progression of atherosclerotic plaques was demonstrated in the 1970s, with monocyte aggregation manifested in atherosclerotic lesions [11].

SIRT1 (Sirtuin 1) is recognized to play an important role in regulating cellular physiological processes, such as metabolism, cell degeneration, cell growth and cell survival. In human endothelial cells, SIRT1 regulates anti-aging in endothelial cells and protects against endothelial inflammation [12,13]. Some survival genes or stress-resistance related genes are targets of SIRT1, such as mTOR, PI-3K, PPAR-γ and p53 [14]. SIRT1 has also been shown to enhance antioxidant enzyme activity and inhibit free radical-mediated oxidative injuries via decreasing NADPH oxidase activation [15]. Furthermore, a previous study reported that the expression level and activity of SIRT1 were reduced in inflammatory endothelial cells [16]. Recently, SIRT1 has been recognized as a novel target in preventing human endothelial pathology. For example, SIRT1 protects against ionomycin-induced ICAM-1 expression in endothelial cells [17] and attenuates thrombomodulin down-regulation after particulate matter exposure [18]. Activating SIRT1 function via drugs has also been reported to reduce oxidative injury-induced endothelial cell death [19]. Moreover, the expression level of SIRT1 is shown to be decreased in inflammatory human endothelial cells [16]. SIRT1 influences the biological activity and signaling transduction of a number of proteins by modulating their deacetylation or through non-deacetylating reactions. LKB1 is one of the important targets of SIRT1. A previous study suggested that SIRT1 positively regulates endothelial cell proliferation and prevents senescence by targeting LKB1 [20]. SIRT1 has been well investigated in endothelial cells. However, there is only one study that has reported on monocyte SIRT1 repression in patients with CAD [21]. This study was designed to understand whether SIRT1 inhibition causes oxidative stress and inflammation in patients with coronary artery disease.

2. Materials and methods

2.1. Study patients

The study group included 30 patients diagnosed with CAD over 60 days and 30 cases with normal coronary arteries. The two groups were recruited from the National Cheng Kung University Hospital. All patients presented with stable clinical symptoms and syndromes. The basic parameters of the studied population are presented in Table 1. In detail, the clinical diagnosis of stable CAD was made according to a clinical evaluation, echocardiography, and angiography. Patients who satisfied the following inclusion criteria were included in the study: patients diagnosed with CAD without angina or clinical presentation and syndromes that remained stable for at least 60 days with no indication of new myocardial damage. Exclusion criteria were previous coronary bypass surgery, unstable angina, and new myocardial infarction. The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of National Cheng Kung University Hospital (Approval number A-ER-103-335), and each participant provided written informed consent.

Table 1
Characteristics of subjects [a].

Characteristics[b]	Control (n = 30)	CAD (n = 30)	p values[c]
Age (years)	52.2 ± 9.5	57.4 ± 11.2	*0.455*
Male/Female	14/16	17/13	*0.075*
BMI (kg/m^2)	24.4 ± 3.4	25.6 ± 4.7	*0.121*
BUN (mmol/L)	5.9 ± 1.4	6.0 ± 2.1	*0.335*
CK (U/dL)	130.5 ± 18.3	183.3 ± 25.1	*0.035**
Hs-crp (mmol/L)	1.2 ± 0.4	2.8 ± 2.1	*0.041**
TC (mmol/L)	4.1 ± 1.1	4.9 ± 1.3	*0.036**
TG (mmol/L)	1.7 ± 0.7	2.0 ± 1.1	*0.184*
LDL-C (mmol/L)	2.3 ± 0.7	3.3 ± 0.8	*0.042**
HDL-C (mmol/L)	1.2 ± 0.4	1.1 ± 0.4	*0.551*
HTN,n(%)	7 (23.3)	23(76.6)	*0.034**
DM,n(%)	6 (20)	19 (63.3)	*0.024**
Smoking habit,n(%)	14 (46.6)	17(63.3)	*0.412*

[a] BMI: body mass index; BUN: blood urea nitrogen; CK: creatine kinase; Hs-crp: high-sensitivity C-reactive protein; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; HTN: hypertension; DM: Diabetes mellitus.
[b] Parameters for age, BMI, Hs-crp, and lipid analysis are provided as mean ± SD.
[c] Control versus CAD.
* Bold indicates significant differences ($p < 0.05$).

2.2. Reagents

Fetal bovine serum (FBS), medium 199 (M199), and trypsin-EDTA were obtained from GIBCO (Grand Island, NY). EDTA, penicillin, and streptomycin were obtained from Sigma (St. Louis, MO). Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) staining kits were obtained from Boehringer Mannheim (Mannheim, Germany). The superoxide dismutase activity assay kit was purchased from Calbiochem (San Diego, CA). DCF-AM was obtained from Molecular Probes (Eugene, OR). 5,58,6,68-tetraethylbenzimidazolcarbocyanine iodide (JC-1) and anti-active caspase 3 were obtained from BioVision (Palo Alto, CA). Anti-vascular cell adhesion molecule-1 (VCAM-1), anti-intercellular adhesion molecule (ICAM), and anti-E-selectin were purchased from R & D Systems (Minneapolis, MN). SRT1720, anti-SIRT1 and anti-β-actin were obtained from Santa Cruz Biotechnology (CA, USA). Anti-acetyl-p53 and anti-p53 were obtained from Cell Signaling (MA, USA).

2.3. Laboratory data analysis and blood monocytes isolation

Blood samples were collected into vacuum tubes containing ethylenediamine tetraacetic acid (EDTA) for the measurement of blood profiles, cardiac markers and pro-inflammatory parameters. Blood was sampled for isolation of monocytes. Monocytes were isolated from heparinized blood from CAD subjects and control cases using Isopaque-Ficoll (Lymphoprep; Fresenius Kabi Norge AS, Oslo, Norway) gradient centrifugation. Monocytes were isolated for further biological tests. In some cases, isolated monocytes were cultured in RPMI with 10% FBS at a density of 5×10^6 cells/ml for transfection.

2.4. Total RNA isolation and real-time PCR reaction

Total RNA was isolated using TRIzol reagent. Reverse transcription was performed at 42 °C for 60 min, followed by incubation at 95 °C for 5 min. The reaction 20 mixture (20 μl of total volume) consisted of 2 μg of isolated total RNA, 1 mM dNTP, 1 unit/μl of recombinant RNasin ribonuclease inhibitor, 15 U/μg of avian myeloblastosis 22 virus (AMV) reverse transcriptase, 5× RT buffer, and 0.5 μg of oligo (dT)12 primer. The gene-specific primers used are listed in Table 2. Real-time PCR reactions were performed using the SYBR Green method in an ABI 7000 sequence detection system (Applied Biosystems, Foster City, CA) according to the manufacturer's guidelines. Primers were designed using the computer software Primer Express 2.0 (Applied Biosystems, Foster City, CA). The reactions were set by mixing 12.5 μl of the SYBR Green Master Mix (Applied Biosystems, Foster City, CA) with 1 μl of a solution containing 10-μM concentrations of both primers and 2 μl of cDNA solution. The Ct value was defined as the number of PCR cycles required for the fluorescence signal to exceed the detection threshold value. The relative amounts of mRNA for each gene were normalized based on the amount of the housekeeping gene β-actin.

2.5. Immunoblotting

Monocytes were lysed in RIPA buffer (in mM: HEPES 20, MgCl2 1.5, EDTA 2, EGTA 5, dithiothreitol 0.1, phenylmethylsulfonyl fluoride 0.1, pH 7.5). Proteins (30 μg) were separated by electrophoresis on an SDS-

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