Journal of Pharmacological Sciences 127 (2015) 474–480

HOSTED BY

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Percutaneous carbon dioxide mist treatment has protective effects in experimental myocardial infarction

Takehiro Yamaguchi [a], Takanori Yamazaki [a, b], Yasuhiro Nakamura [a, c], Masayuki Shiota [d], Kenei Shimada [a], Katsuyuki Miura [e], Hiroshi Iwao [f], Minoru Yoshiyama [a], Yasukatsu Izumi [d, *]

[a] Department of Cardiovascular Medicine, Osaka City University Medical School, Osaka 545-8585, Japan
[b] Department of Cardiology, Asakayama General Hospital, Sakai 590-0018, Japan
[c] Department of Cardiology, Izumi Municipal Hospital, Izumi 594-0071, Japan
[d] Department of Pharmacology, Osaka City University Medical School, Osaka 545-8585, Japan
[e] Applied Pharmacology and Therapeutics, Osaka City University Medical School, Osaka 545-8585, Japan
[f] Department of Education, Shitennoji University, Habikino 583-8501, Japan

ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form
9 March 2015
Accepted 25 March 2015
Available online 1 April 2015

Keywords:
Animal model
Carbon dioxide
Preconditioning
Mist
Myocardial infarction

ABSTRACT

Percutaneous treatment with carbon dioxide (CO_2) mist, CO_2 gas dissolved in water, contributes to improved cardiac function after myocardial infarction (MI). In this study, we investigated the effects of repeated pretreatment with CO_2 mist on cardiac dysfunction after MI. The CO_2 mist was generated by a dry mist production unit. The whole body of rats below the axilla was wrapped in a polyethylene bag, which was sealed and filled with the CO_2 mist in the draft cabinet for 30 min daily for 7 days. MI was induced by ligation of the coronary artery in untreated (UT), CO_2 gas-pretreated (CG), and CO_2 mist-pretreated (CM) rats. The infarct size and the increase in oxidative stress due to MI were significantly smaller in the CM rats than in the UT rats. Furthermore, the expression of inflammation-related genes, such as monocyte chemoattractant protein-1, and fibrosis-related genes, such as transforming growth factor-β1, was significantly suppressed in the CM rats. The CM rats had a better left ventricular ejection fraction than the UT rats 7 days after MI. These parameters in the CG rats were the same as in the UT group. Thus, CO_2 mist preparative treatment may be potentially useful for the reduction of MI.

1. Introduction

The cardioprotective effect of ischemic preconditioning was reported in 1986 (1). Ischemic preconditioning resulted in infarct sizes that were approximately 25% of those observed in untreated hearts. The original study not only demonstrated the cardioprotection from ischemia/reperfusion (I/R) injury, but also provided a model with which to study ways to protect the ischemic heart. There have been many subsequent reports of the cardioprotective effects of remote ischemic preconditioning (2–4), in which brief and repeated non-lethal ischemia and reperfusion of a remote organ or tissue can increase the heart's tolerance of acute IR injury.

Balneotherapy in hot springs containing a high concentration of carbon dioxide (CO_2) has long been applied clinically to treat a variety of diseases. The effect of CO_2-enriched water on the cutaneous circulation depends primarily on the vasodilatation effects elicited by the CO_2 that diffuses into the subcutaneous tissue through the skin layers (5,6). Previous studies have reported that CO_2-enriched water induces peripheral vasodilatation, which increases cutaneous blood flow (6–8). Thus, bathing in CO_2-enriched water may be a useful therapeutic method for patients with cardiovascular diseases. However, no artificial CO_2-enriched water bathing system is widely available, probably because of the high setup cost and the difficulty of maintaining a constant artificial CO_2 concentration.

Recently, instead of a CO_2 bathing system, a new and simple device was developed to generate CO_2 mist, i.e., water containing a

* Corresponding author. Department of Pharmacology, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan. Tel.: +81 6 6645 3731; fax: +81 6 6646 1980.
E-mail address: izumi@msic.med.osaka-cu.ac.jp (Y. Izumi).
Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2015.03.009

high concentration of dissolved CO_2 molecules. We have previously reported that percutaneous treatment with CO_2 mist produced by this device improved the development of cardiac remodeling after myocardial infarction (MI) (9). Treatment with CO_2 mist also accelerated angiogenesis in a mouse model of peripheral arterial disease (10). However, it is still unclear whether pretreatment with CO_2 mist has a cardioprotective effect against myocardial ischemic injury. In the present study, we investigated whether pretreatment with CO_2 mist can reduce the infarct size in an experimental animal model of MI.

2. Materials and methods

2.1. Animals and experimental design

All procedures were performed in accordance with Osaka City University animal care guidelines, which conform to 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). The 8-week-old male Wistar rats weighing 260–290 g were purchased from CLEA Japan, Inc (Osaka).

The principal aim of the present study was to determine whether repeated pretreatment with CO_2 mist can attenuate cardiac dysfunction after MI. After 24 h of the last treatment, MI was induced by permanent ligation of the left coronary artery (9,11,12) in rats that were either untreated (UT), or had undergone pretreatment with CO_2 gas (CG) or CO_2 mist (CM) daily for 7 days. Untreated and sham-operated rats were used as a control (CON) group. Forty-eight hours after MI induction, the infarct size was quantified by the Evans blue method (13). Three days after MI induction, the blood pressure and heart rate of the conscious rats were measured by the tail-cuff method (BP98A; Softron, Tokyo); a blood sample was then collected and the heart weight was measured. Seven days after MI induction, a transthoracic echocardiographic study was performed, and the rats were then sacrificed. The ventricle was separated into the upper and lower portions, and then the upper portion of the left ventricle was divided into the marginal and non-infarcted zones. The specimens obtained were immediately frozen in liquid nitrogen and stored at −80 °C until use. The lower portion of the left ventricle was fixed in 10% formaldehyde overnight and embedded in paraffin.

2.2. CO_2 gas or CO_2 mist treatment procedure

The CO_2 mist was generated by a dry mist production unit (ACP JAPAN Co., Ltd., Tokyo) (9,10). In brief, 100% concentrated CO_2 was compounded and compressed with water through dual fluid nozzles at 4 atm. The rat was placed on a heating plate at 37 °C, under anesthesia with sodium pentobarbital (40 mg kg^{-1}, ip), and the whole body below the axilla was encased in a sealed polyethylene bag. The bag was then filled with CO_2 mist or CO_2 gas in the draft cabinet (9,10). The UT/MI and CON rats were kept on a heating plate at 37 °C for 30 min once a day under anesthesia.

2.3. Echocardiographic study

Transthoracic echocardiography was performed using a Xario ultrasound device (Toshiba Medical Systems, Tokyo) with a 6-MHz cardiac transducer, according to previously described methods (12,14). In brief, rats were anesthetized with pentobarbital. A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles. The left ventricular (LV) ejection fraction (LVEF) was calculated by measuring the LV end-diastolic volume (LVEDV) and the LV end-systolic volume (LVESV), by using a modified Simpson's method. Pulsed wave Doppler spectra (early rapid filling [E] wave and atrial contraction [A] wave) of mitral inflow velocities were recorded from the apical 4-chamber view, with the sample volume placed near the tips of the mitral leaflets and adjusted to the position at which velocity was maximum and the flow pattern was laminar, and the ratio of E wave velocity to A wave velocity (E/A) was calculated.

2.4. Histology and evaluation of oxidative stress

The area of interstitial fibrosis in the marginal area of the infarct was measured, as described previously (12). In brief, 4-μm-thick sections were cut and stained with hematoxylin-eosin stain.

At 3 days after MI induction, the serum levels of derivatives of reactive-oxygen metabolites (d-ROMs) were measured by the Free Radical Elective Evaluator (Diacron International, Grosseto, Italy) using commercial assay kits (Diacron International) (12). On the same day, cardiac oxidative stress was assessed by 8-hydroxydeoxyguanosine (8-OHdG) content, a marker of oxidative DNA damage, as described previously (15). Briefly, DNA from the LV tissues was extracted by NaI method using a DNA Extractor TIS Kit (Wako Pure Chemical Industries, Osaka, Japan). After 50 μg of DNA pellet was pretreated using 8-OHdG Assay Preparation Reagent Set (Wako Pure Chemical Industries), the 8-OHdG adducts were determined by use of an highly sensitive 8-OHdG enzyme-linked immunosorbent assay kit (Japan Institute for the Control of Aging, Shizuoka, Japan).

2.5. mRNA expression analysis

Total RNA in the marginal area of the infarct was extracted from tissues or exosomes with Isogen I (Nippon Gene, Toyama) (11,16). The concentration and quality of the RNA were assessed using a Nano Drop 2000 spectrophotometer (Thermo, Waltham, MA, USA). To quantify the gene expression levels, we used quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) with the TaqMan system (Applied Biosystems, CA, USA), as previously described. For normalization, the transcript levels were compared to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.6. Western blot analysis

Our detailed method has been described previously (10,12). Protein extracts were obtained from homogenized left ventricles with no treatment or CO_2 mist treatment for 7 days. After electrophoretic transfer to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Billerica, MA, USA), the membranes were probed with each primary antibody. Antibodies were obtained from the following sources: anti-GAPDH and heat shock protein 72 (Hsp72) antibodies from Santa Cruz Biotechnology (Dallas, TX, USA); anti-phospho endothelial nitric oxide synthase (eNOS) (p-Ser1177), anti-phospho Akt and Akt antibodies from Cell Signaling Technology (Beverly, MA, USA); anti-eNOS antibody from BD Biosciences (San Jose, CA, USA).

2.7. Statistical analysis

All data are presented as mean ± standard error of the mean. Differences among groups were compared by one-way analysis of variance followed by the Tukey–Kramer method, using StatView software (SAS Institute, Inc., Cary, NC, USA). Student's t test was used to assess differences between two groups when appropriate. The differences were considered statistically significant at a value of $p < 0.05$.