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Original Article

The Preventive Effect of 5-Iodo-6-Amino-1,2-Benzopyrone on Apoptosis of Rat Heart-derived Cells induced by Oxidative Stress

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Ischemia-reperfusion results in excess reactive oxygen species (ROS) that affect myocardial cell damage. ROS production inhibition is effectively proposed in treating cardiovascular diseases including myocardial hypertrophy. Studies have shown that oxidizing cultured cells in *in vitro* experiments gradually decreases the permeability of mitochondrial membranes time- and concentration-dependent, resulting in increased mitochondrial membrane damage due to secondary ROS production and cardiolipin loss. However, recent studies have shown that 5-iodo-6-amino-1,2-benzopyrone (INH_2BP), an anticancer and antiviral drug, inhibited peroxynitrite-induced cell damage in *in vitro* and alleviated partial or overall inflammation in animal experiments. Therefore, in this paper, we studied the preventive effect of INH_2BP on H9c2 cells derived from mouse heart damaged by oxidative stress using 700 μM of hydrogen peroxide. As a result of oxidative stress to H9c2 cells by hydrogen peroxide whether the treatment of INH_2BP or not, hydrogen peroxide caused serious damage in H9c2 cells. These results were confirmed with cell viability and Hoechst 33342 assays. And this damage was through cell death. However, it was confirmed that H9c2 cells pretreated with INH_2BP significantly reduced cell death by hydrogen peroxide. In addition, measurements with DCF-DA assay to determine whether ROS is produced in H9c2 cells treated with only hydrogen peroxide produced ROS significantly, but H9c2 cells pretreated with INH_2BP significantly reduced ROS production by hydrogen peroxide. Taken together, it is believed that INH_2BP can be useful for the prevention and treatment of cardiovascular diseases induced through oxidative stress such as heart damage caused by ischemia/reperfusion.

Key Words: 5-iodo-6-amino-1,2-benzopyrone, ROS, Apoptosis, Hoechst 33342 assay, DCF-DA assay

INTRODUCTION

Cardiovascular diseases included the diseases of heart and vessels and classified into primary and secondary diseases. In those, the secondary cardiovascular diseases included the ischemic heart disease, heart failure, coronary heart disease, myocardial disease, hypertensive heart disease, heart valve disease, and cardiac arrhythmia.

In particular, ischemic heart diseases developed by the out of balances among the supply of blood contents in the coronary arteries which carried the nutrients and oxygen, and the oxygen demands of myocardium.

In general, the causes of cardiovascular diseases are ath-

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erosclerosis and hypertension, however, they are developed by another various reasons.

In other words, atherosclerosis could be advanced by the factors of elderly, smoking, diabetes, hypercholesterolemia, and drinking which caused the damages of endothelium and acutely formed the thrombosis.

When these thrombi blocked the coronary arteries by more than 70%, myocardial infarction, the death of a portion of myocardium, developed. If not died, by preventing the blood from moving freely, angina developed and caused the severe chest pains.

Current treatment for the acute myocardial infarction is generally performed by the perfusion therapy such as surgery which largely based on the pharmacological methods, however, it can be said that these therapies are effective only when the ischemic injuries just begin (Hochman and Choo, 1987).

It is reported that the heart failure is occur in more than 40% of patients if the remodeling is delayed after the ischemic injury (Sutton and Sharpe, 2000).

In addition, the rapid inflow of oxygen-rich blood into the tissue results in a very harmful effect, so even after the treatment, secondary damage to the myocardium may occur due to the reperfusion, and this is called reperfusion injury (Cave and Garlick, 2000; Flaherty and Zweier, 1991).

Various therapies have been studied to protect the heart injuries from the ischemia-reperfusion during the past few decades and through these studies the decisive molecular mechanisms that can protect the myocardium have been revealed (Zhao et al., 2003).

According to the statistics of World Health Organization in 2008, among the top 10 major causes of death, the ischemic heart disease was 16% taking the first place in mortality (Lloyd-Jones et al., 2010).

According to the data of Statistics Korea in 2009, heart disease has been ranked the third leading cause of death over the past 10 years and is steadily increasing.

Recently, research on the prevention or treatment of cardiovascular diseases has been actively performed, and many studies have been taken out on drug development that is more effective and stable.

Ischemic reperfusion promotes the formation of reactive oxygen species (ROS) and is closely related to the formation of lipid peroxidation, protein oxidation, and DNA cleavage (Powers et al., 2002; Tacar et al., 2013).

Poly ADP ribose polymerase (PARP) is a kind of nucleic acid polymerase that is abundant in the nucleus. Its activity is caused by single-stranded DNA cleavage and can be induced by the various free radicals.

That is, PARP is rapidly activated by DNA damage, the intracellular nicotinamide adenine dinucleotide (NAD) is consumed to synthesize polymer of ADP-ribose (PAR) at the damaged site, and extensive DNA damage leads to the depletion of NAD.

The depletion of NAD dramatically decreases the intracellular ATP levels, and thus the excessive activity of PARP reduces the ability of cells to generate energy in the form of ATP, eventually leading to apoptosis (Ha and Snyder, 1999; Pieper et al., 2000).

Consequently, inhibition of PARP may enhance the recovery of various cells from oxidative injuries (Thiemermann et al., 1997).

The new PARP inhibitor, INH_2BP, has been developed for antiviral or anti-cancer therapy (Endres et al., 1998; Szabo et al., 1997), however, the recent studies have been shown that INH_2BP inhibited the oxidant-induced injuries of endothelial cells, and have an excellent prophylactic effect on cell damage in the inflamed animal cells (Franson et al., 1991; Szabo, 1996).

Therefore, this study was undertaken to know the preventive effect of INH_2BP on the apoptosis of the rat heart-derived H9c2 cells which were induced the oxidative stress using hydrogen peroxide.

MATERIALS AND METHODS

Materials and reagents

INH_2BP was purchased from Sigma-Aldrich (St. Louis, Mo, USA) and dissolved in 0.05% dimethyl sulfoxide (Sigma-Aldrich, St. Louis, Mo, USA), and further diluted in Dulbecco's modified Eagle's media (DMEM) (Thermo Fisher Scientific, Health, USA) without fetal bovine serum (FBS).

Reagents for cell culture, all antibodies and H_2O_2, and

general reagents were purchased from Thermo Fisher Scientific, Cell Signaling Technology Inc. (Beverly, Mass, USA), and Sigma-Aldrich, respectively.

Cell culture

H9c2 cells were purchased from the American Type Culture Collection (Rockville, Md, USA).

H9c2 cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin at 37℃ under 95% Air/5% CO_2 conditions.

Viability analysis

In order to evaluate the viability, $H9C_2$ cells were seeded in 12-well plate to 1×10^4 cells/well and cultured in DMEM for 24 hours at 37℃. After pretreatment in the different concentrations (10~250 μmol/L) of INH_2BP for 1 hour, the cells were cultured for 6 hours with 700 μmol/L of H_2O_2.

Then, the viabilities were analyzed using an optical microscope (Olympus Inc, Tokyo, Japan) connected to a platelet measuring device and a digital camera.

In addition, H9c2 cells were seeded in 96-well plates at a concentration of 1×10^4 cells/well and pretreated at the different concentrations of INH_2BP (10~250 μmol/L) for 1 hour. The cells were cultured for 6 hours with the addition of 700 μmol/L of H_2O_2 and 50 μL of XTT (2,3-bis [2,3-bis [2-methyloxy-4-nitro-5-sulphenfoyl]-2H-tetrazolium-5-carboxanilide) (Biological Industries Co. Beit Haemek, Israel) solution in each well.

To analyze the cell viabilities, the absorbance was measured at 460 nm using a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) after 2 hours of cultivation at 37℃ (Nowak et al., 2018).

Evaluation of apoptosis

Apoptotic myocardial cells were qualitatively analyzed using Hoechst 33342 staining method (BD Biosciences, Franklin Lakes, NJ, USA) which distinguishes between normal and apoptotic cells based on the condensation and the fragmentation of nuclear chromatin (Crowley et al., 2016).

H9c2 cells were seeded in 8-well chamber slides at a concentration of 1×10^4 cells/well and incubated in DMEM at 37℃ for 24 hours. The cells were pretreated at different concentrations of INH_2BP (10~250 μmol/L) for 1 hour, and then incubated for 6 hours with 700 μmol/L of H_2O_2.

After that, the cells were stained with 2 μg/mL of Hoechst 33342 for 15 minutes and washed with phosphate buffered saline (PBS) twice and then observed under a fluorescence microscope (FV-1000, Olympus, Tokyo, Japan).

Analysis of intracellular ROS production and scavenging effects

Using a green fluorescent probe, 6-carboxy-2',7'-dichlorofluoroscein diacetate (DCF-DA; Invitrogen, Rockville, MD, USA), the production of the intracellular ROS was analyzed (Pogue et al., 2012).

First, H9c2 cells were dispensed on 8-well cell culture plates (SPL, Seoul, Korea) at a concentration to 1×10^4 cells/well and cultivated in DMEM at 37℃ for 24 hours. The cells were further cultured for 6 hours with 700 μmol /L H_2O_2 after the pretreatment with a different concentration of INH_2BP (10~250 μmol/L).

Subsequently, they were stained with 10 μmol/L of DCF-DA for 30 minutes at 37℃ and observed under a fluorescence microscope system (FV-1000 spectral, Olympus, Tokyo, Japan).

In addition, H9c2 cells were aliquoted into black-welled 96-well plates to 1×10^4 cells/well, and stained with 10 μmol /L of DCF-DA at 37℃ for 30 minutes. ROS was measured using a fluorescence photometer (Victor 3, Perkin Elmer, Waltham, MA, USA excitation = 485 nm) according to the fluorescence measurement method.

Various radical scavenging activities measured by electron spin resonance (ESR)

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity: DPPH radical scavenging activity was measured as described by Nanjo et al. (Chen et al., 2001; Nanjo et al., 1996). Briefly, 60 μL of 1 mM INH_2BP was added to 60 μL of DPPH (St. Louis, Mo, USA) (60 mM) in a methanol solution. After the solution was mixed vigorously for 10 sec, it was transferred into a 100 μL Teflon capillary tube, and the scavenging activity of INH_2BP, with regard to DPPH radicals, was measured using an ESR spectrometer. The spin adduct was measured by the ESR spectrometer exactly 2 min

later. The experimental conditions were as follows; central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 5 mW; gain, 6.3×10^5; and temperature, 298 K.

Superoxide radical scavenging activity: Superoxide radicals were generated as a result of the ultraviolet irradiation of a riboflavin/ethlylenediaminetetraacetic acid solution. The reaction mixtures that contained 0.1 mL of 0.8 mM riboflavin, 0.1 mL of 1.6 mL EDTA, 0.1 mL of 800 mL DMPO, and 1 mL of 1 mM INH_2BP were irradiated for 1 min under an ultraviolet lamp at 365 nm. The measurement conditions were as follows; central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 1 mW; gain, 6.3×10^5; and temperature, 298 K.

Hydroxyl radical scavenging activity: Hydroxyl radicals were generated by an iron-catalyzed Haber-Weiss reaction (i.e., a Fenton-driven Haber-Weiss reaction), and the generated hydroxyl radicals rapidly reacted with nitrone spin-trap DMPO. The resultant DMPO-OH adduct was detected using an ESR spectrometer. Briefly, 0.2 mL of 1 mM INH_2BP was mixed with 0.2 mL of DMPO (0.3 M), 0.2 mL of $FeSO_4$ (10 mM), and 0.2 mL of H_2O_2 (10 mM) in a phosphate buffer solution (pH 7.2), and the resulting solution was transferred into a 100 μL Teflon capillary tube. After 2.5 min, the ESR spectrum was recorded on a JES-FA ESR spectrometer (JEOL Ltd., Tokyo, Japan). The experimental conditions were as follows; central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 1 mW; gain, 6.3×10^5; and temperature, 298 K.

Statistical analysis

Experimental results are expressed as means ± S.E.M. A one-way analysis of variance was used for multiple comparisons (GraphPad Prism version 4.00 for Windows, San Diego, CA, USA). Dunnett's test was applied if there was a difference among the treated groups. A $P < 0.05$ was considered significant.

RESULTS

Effect of INH_2BP on viability of H_2O_2-pretreated H9c2 cells

In order to determine the preventive effect of INH_2BP on the oxidative stress to myocardial cells, H9c2 cells pretreated

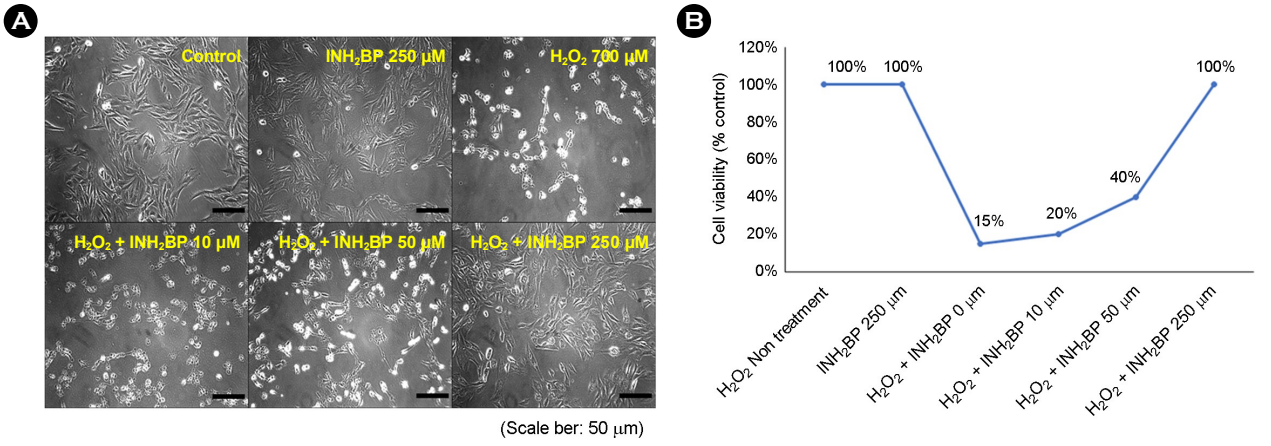

Fig. 1. (A) Cell morphology was observed after 6 h of H_2O_2 exposure. H9c2 cells were pre-cultured in serum-free medium in the presence or absence of INH_2BP (10, 50, and 250 μmol/L) for 1 h, and then stimulated further with 700 μmol/L H_2O_2 for an additional 6 h. H_2O_2 resulted in abnormal cell morphology, whereas INH_2BP pretreatment resulted in dose-dependent protection from the H_2O_2-induced morphological changes. Representative images were taken from three independent experiments (magnification, × 40). (B) Effect of INH_2BP on H_2O_2-induced cell death. H9c2 cells were pre-cultured in serum-free medium in the presence or absence of INH_2BP (10, 50, and 250 μmol/L) for 1 h, and then stimulated further with 700 μmol/L H_2O_2 for an additional 6 h, during which the XTT reagent was added at the end of hour 4, and the entire culture mixture was further incubated for 2 h. The absorbance was determined with an enzyme-linked immunosorbent assay reader at a wavelength of 460 nm. Data are means ± standard errors (N = 3). $^{\#\#}P < 0.01$ *vs*. untreated cells; $^{**}P < 0.01$ *vs*. H_2O_2 alone.

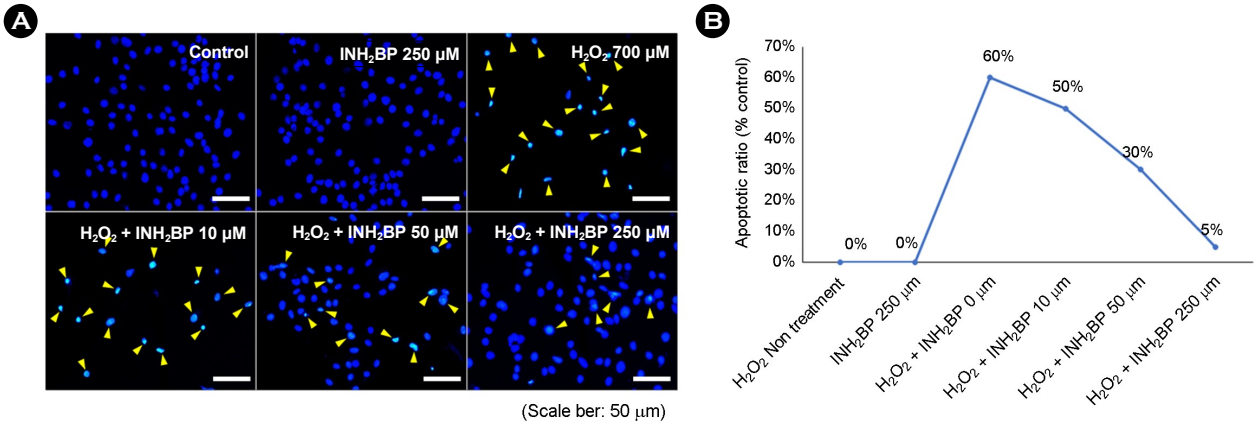

Fig. 2. (A) Effect of INH_2BP on H_2O_2-induced apoptosis in H9c2 cells. Apoptotic cells were examined under a fluorescence microscope at 200×magnification after Hoechst 33342 staining (scale bar = 50 μm). After 1 h of pretreatment with or without different concentrations of INH_2BP (10, 50, and 250 μmol/L), the cells were exposed to H_2O_2 and stained using Hoechst 33342, and then the cells were visualized under a fluorescence microscope. Apoptotic cells were identified as those with nuclei exhibiting brightly stained condensed chromatin (Hoechst-positive cells). Arrows indicate apoptotic cell nuclei (Hoechst-positive cells). Microscopic images are representative of three independent experiments. (B) Apoptotic rate on H_2O_2-induced apoptosis in H9c2 cells. After 1 h of pretreatment with or without different concentrations of INH_2BP (10, 50, and 250 μmol/L), the cells were exposed to 700 μmol/L H_2O_2 and stained using Hoechst 33342, and then the cells were visualized under a fluorescence microscope. The apoptotic rate was determined by calculating the percentage of Hoechst-positive cells over total cells. Data are means ± standard errors (N = 3). $^{\#\#}P < 0.01$ *vs*. untreated cells; $^{**}P < 0.01$ *vs*. H_2O_2 alone.

with H_2O_2 and/or INH_2BP were observed using the optical microscopy system. As a result, 85% of severe cell damage was identified in the group treated 700 μmol/L of H_2O_2 only.

However, about 80% and 60% of cell damage were observed at the concentration of 10 μmol/L and 50 μmol/L of INH_2BP, respectively. And no cell damage was observed at 250 μmol/L of INH_2BP (Fig. 1A).

In addition, XTT analyses showed that severe cell damage was induced at 700 μmol/L of H_2O_2 only- and at 10 μmol/L of INH_2BP-treated groups. And at 50 μmol/L and 250 μmol /L of INH_2BP-treated groups, the cell viabilities were increased by concentration-dependent manner (Fig. 1B).

As a result, it was confirmed that cell viability increased by concentration-dependent manner in the INH_2BP (10~ 250 μmol/L)-treated groups.

Preventive effect of INH_2BP on H_2O_2 induced cell apoptosis in H9c2 cells

To determine whether INH_2BP inhibited of H_2O_2 (700 μmol/L)-induced apoptosis in H9c2 cells, Hoechst 33342 staining was performed.

As marked with yellow arrow in Fig. 2A, more than 60% of apoptotic bodies were observed in only H_2O_2-treated group.

In 10 and 50 μmol/L of INH_2BP-treated groups, 50% and 60% of the apoptotic bodies were observed, respectively. However, almost no apoptotic bodies were observed in 250 μmol/L-treated groups.

In addition, by confirming of the ratio of Hoechst-positive cells to the total cells, the apoptosis was decreased in the concentration dependent manner by INH_2BP (Fig. 2B).

Scavenging effect of INH_2BP on H_2O_2-induced ROS in H9c2 cells

It has been generally accepted that the excessive H_2O_2 induces the cell apoptosis by producing ROS.

Therefore, to determine whether INH_2BP could remove intracellular ROS, H9c2 cells treated with H_2O_2 were stained by 10 μmol/L of DCF-DA and observed under a fluorescence microscopic system.

As a result of observation under the fluorescence microscope system, it was confirmed that the expression of ROS was increased as with the green fluorescence in the group treated with H_2O_2 only and reduced concentration-dependent manner in the INH_2BP-treated group (Fig. 3A).

In addition, the same result was obtained in the measure-

ment using a fluorescent spectrophotometer (Fig. 3B).

H_2O_2-induced intracellular ROS scavenging mechanism of INH_2BP using ESR

Several studies have suggested that PARP inhibitors can induce changes in intracellular ROS levels (Radnai et al., 2012; Kalai et al., 2009). Therefore, it was observed using a fluorescence image (Fig. 3A) and fluorescence assay (Fig. 3B) whether the PARP inhibitor, INH_2BP, abolished H_2O_2-induced ROS in H9c2 cells. As a result, ROS production was increased in H9c2 cells exposed only to H_2O_2, When H_2O_2 and INH_2B were treated together, it was confirmed

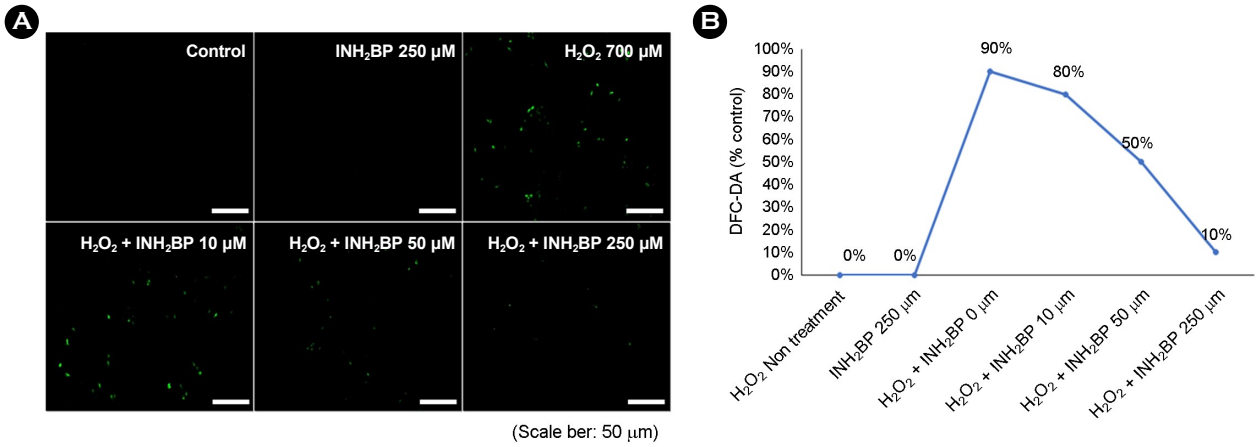

Fig. 3. (A) Effect of INH_2BP on intracellular reactive oxygen species (ROS) generation. After 1 h of pretreatment with or without INH_2BP (10, 50, and 250 μmol/L), the cells were exposed to 700 μmol/L H_2O_2 for 6 h and assayed for ROS generation using DCF-DA fluorescence. Fluorescence microscopy images of cells fluorescently stained with DCF-DA (magnification, ×40, scale bar = 50 μm). Microscopic images are representative of three independent experiments. (B) Effect of INH_2BP on intracellular reactive oxygen species (ROS) generation. After 1 h of pretreatment with or without INH_2BP (10, 50, and 250 μmol/L), the cells were exposed to 700 μmol/L H_2O_2 for 6 h and assayed for ROS generation using DCF-DA fluorescence. Fluorescence was measured with a fluorometer (excitation = 485 nm, emission = 535 nm). Data are representative of three independent experiments and means ± standard errors (N = 3). $^{\#\#}P < 0.01$ *vs*. untreated cells; $^{**}P < 0.01$ *vs*. H_2O_2 alone.

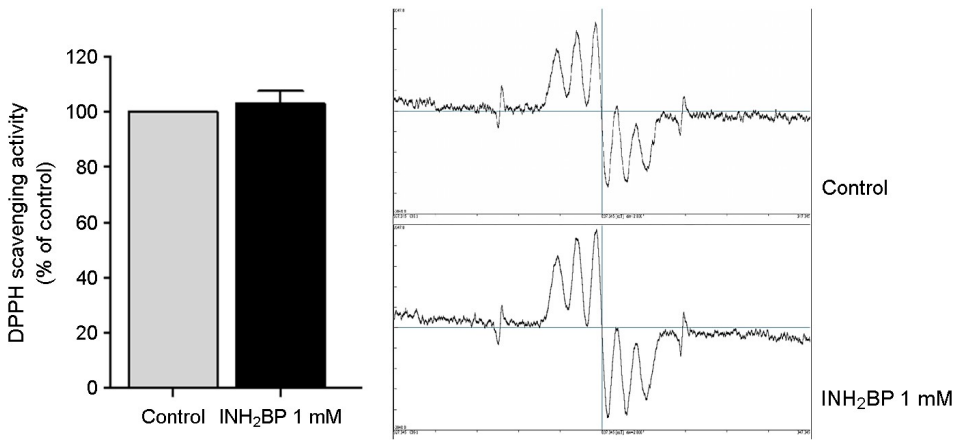

Fig. 4. Radical scavenging activity of INH_2BP assessed by ESR. DPPH radical scavenging activity was measured as described by Nanjo et al. (Nanjo et al., 1996). Briefly, 60 μL of 1 mM INH_2BP was added to 60 μL of DPPH (60 μM) in a methanol solution. After the solution was mixed vigorously for 10 sec, it was transferred into a 100 μL Teflon capillary tube, and the scavenging activity of INH_2BP, with regard to DPPH radicals, was measured using an ESR spectrometer. The spin adduct was measured by the ESR spectrometer exactly 2 min later. The experimental conditions were as follows; central field, 3,475 G; modulation frequency, 100 kHz; modu- lation amplitude, 2 G; microwave power, 5 mW; gain, 6.3×10^5; and temperature, 298 K. DPPH scavenging activity of INH_2BP of ESR spectra. Data are means ± standard errors (n = 3).

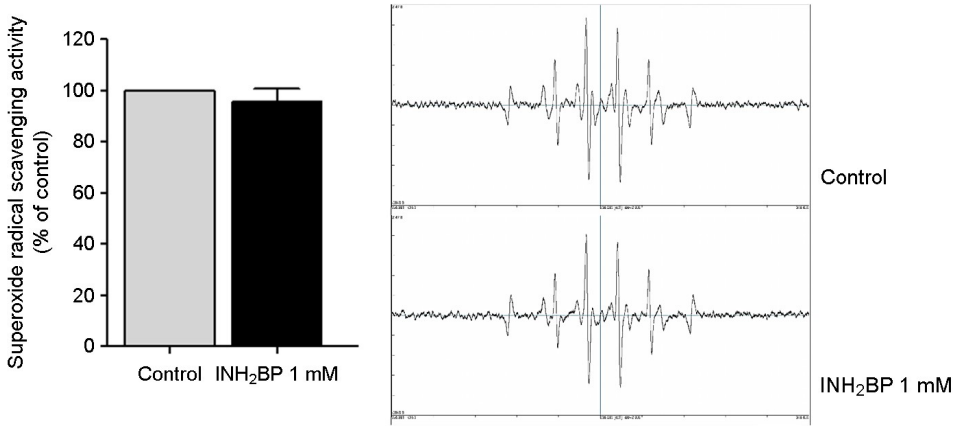

Fig. 5. Radical scavenging activity of INH_2BP assessed by ESR. Superoxide radicals were generated as a result of the ultraviolet irradiation of a riboflavin/ethlylenediaminetetraacetic acid solution. The reaction mixtures that contained 0.1 mL of 0.8 mM riboflavin, 0.1 mL of 1.6 mL EDTA, 0.1 mL of 800 mL DMPO, and 1 mL of 1 mM INH_2BP were irradiated for 1 min under an ultraviolet lamp at 365 nm. The measurement conditions were as follows; central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 1 mW; gain, 6.3×10^5; and temperature, 298 K.Superoxide radical scavenging activity of INH_2BP of ESR spectra. Data are means ± standard errors (n = 3).

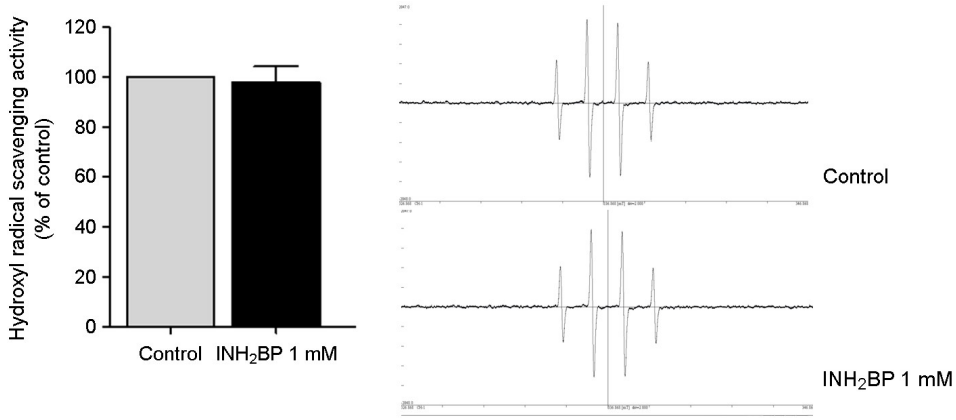

Fig. 6. Radical scavenging activity of INH_2BP assessed by ESR. Hydroxyl radicals were generated by an iron-catalyzed Haber-Weiss reaction (i.e., a Fenton-driven Haber-Weiss reaction), and the generated hydroxyl radicals rapidly reacted with nitrone spin-trap DMPO. The resultant DMPO-OH adduct was detected using an ESR spectrometer. Briefly, 0.2 mL of 1 mM INH_2BP was mixed with 0.2 mL of DMPO (0.3 M), 0.2 mL of $FeSO_4$ (10 mM), and 0.2 mL of H_2O_2 (10 mM) in a phosphate buffer solution (pH 7.2), and the resulting solution was transferred into a 100 μL Teflon capillary tube. After 2.5 min, the ESR spectrum was recorded on a JES-FA ESR spectrometer (JEOL Ltd., Tokyo, Japan). The experimental conditions were as follows; central field, 3,475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 1 mW; gain, 6.3×10^5; and temperature, 298 K.Hydroxyl radical scavenging activity of INH_2BP of ESR spectra. Data are means ± standard errors (n = 3).

that ROS production was reduced. Here, it was confirmed by using ESR whether INH_2BP directly acts on intracellular ROS to eliminate it. As a result of ESR experiment, INH_2BP did not directly scavenge DPPH, superoxide radical, and hydroxyl radical (Fig. 4, 5, 6). Therefore, INH_2BP does not directly eliminate intracellular ROS, but activates a mechanism that eliminates intracellular ROS.

DISCUSSION

INH_2BP has been reported to have various biological properties such as anti-cancer and anti-inflammatory (Endres et al., 1998; Szabo et al., 1997). However, the protective effect of cardiomyocytes has not yet been established. Cardiovascular disease is associated with increased production

of hydrogen peroxide from damaged cells, which has been reported to be a major cause of pathogenesis as well as apoptosis of cardiomyocytes (Aikawa et al., 2000). Therefore, it has been previously suggested that suppressing cardiomyocyte apoptosis in response to excessive ROS is the main therapeutic goal (Park et al., 2014). Recently, experimental evidence of improved cardiac function has been implicated in INO-1001 and poly(ADP-ribose) polymerase (PARP) inhibitors, whereas ROS-induced PARP activation results in cell death (Pacher et al., 2006; Giansanti et al., 2010). For this reason, the inhibitory role of PARP has been implicated in the development of cardioprotective medicine. Since poly ADP-ribosylation is regulated by PARP, properties related to essential processes such as gene expression and DNA replication have been reported (Giansanti et al., 2010). Therefore, in this study, we aimed to establish whether PARP inhibition could modulate cardiomyocyte apoptosis in ROS conditions by the potential efficacy of INH_2BP. Excessive ROS can induce heart enlargement or loss of function, and is the most common cause of cardiovascular disease. ROS triggers a specific mechanism that induces changes in the permeability of the mitochondrial membrane and disrupts the mitochondrial membrane. ROS can also be formed by a lack of ATP or an excess of viability Ca2+ ions (He et al., 2017; Sun et al., 2022). This ROS is phosphorylated or modified at the activation site of the protein, and not only regulates the protein related to signal transduction, but also inhibits the activity of the hydrolase to cause cell damage. In this study, as a result of light microscopy and XTT analysis, the cell viability of H9c2 cells subjected to oxidative stress by H_2O_2 was decreased due to severe cell damage, but the cell viability of H9c2 cells treated with INH_2BP increased in a concentration-dependent manner, and INH_2BP 250 μmol /L No cell damage was observed at the concentration ($P < 0.01$). Ischemic reperfusion promotes ROS formation and is closely related to lipid peroxidation, protein oxidation, and formation of DNA breaks. Poly ADP ribose polymerase (PARP) is a nucleic acid polymerase that is abundant in the nucleus, and it has been reported that when single strand DNA break occurs, PARP becomes active and can be induced by various environmental stimuli and free radicals (Gilad et al., 1997; Cuzzocrea et al., 2001). That is, PARP is activated in DNA damage, leads to continuous depletion of intracellular NAD, and depletion of NAD results in a sharp drop in intracellular ATP level. Therefore, excessive PARP activity leads to ATP depletion and eventually cell death (Ha and Snyder, 1999; Pieper et al., 2000). Hoechst 33342 staining was observed to confirm whether INH_2BP inhibited apoptosis, and more than 60% of apoptosis was observed in H9c2 cells subjected to oxidative stress by H_2O_2, and it was confirmed that the concentration-dependent decrease in the INH_2BP-treated group ($P < 0.01$). In addition, several studies have reported that PARP inhibitors reduce intracellular ROS levels (Radnai et al., 2012; Kalai et al., 2009), reported that it can enhance the recovery of various cells from oxidative damage (Franson et al., 1991). In order to confirm that INH_2BP, a strong inhibitor of PARP, eliminates intracellular ROS, DCF-DA was stained and confirmed with a fluorescence microscope and fluorescent spectrophotometer. As a result, ROS production was increased in the group treated with only H_2O_2, and ROS production was generated in the group treated with INH_2BP. It was confirmed that this concentration-dependently decreased ($P < 0.01$). ROS scavenging enzymes such as SOD (superoxide dismutase) and CAT (catalase) have a protective function against cell damage caused by ischemia/reperfusion (Zweier and Talukder, 2006). Although it was confirmed by using ESR whether INH_2BP acts directly on intracellular ROS scavenging, INH_2BP did not directly act on ROS scavenging, and it is thought that ROS scavenging will be activated by activating intracellular ROS scavenging enzymes.

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None.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

Aikawa R, Nawano M, Gu Y, Katagiri H, Asano T, Zhu W, et al. Insulin prevents cardiomyocytes from oxidative stress-induced apoptosis through activation of PI3 kinase/Akt. Circulation.

2000. 102: 2873-2879.

Cave A, Garlick P. Re: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. J Mol Cell Cardiol. 2000. 32: 1759-1760.

Crowley LC, Marfell BJ, Waterhouse NJ. Analyzing cell death by nuclear staining with Hoechst 33342. Cold Spring Harb Protoc. 2016. 2016.

Chen CF, Wang D, Hwang CP, Liu HW, Wei J, Lee RP, et al. The protective effect of niacinamide on ischemia-reperfusion-induced liver injury. Journal of Biomedical Science. 2001. 8: 446452.

Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacological Reviews. 2001. 53: 135-159.

Endres M, Scott GS, Salzman AL, Kun E, Moskowitz MA, Szabo C. Protective effects of 5-iodo-6-amino-1,2-benzopyrone, an inhibitor of poly(ADP-ribose) synthetase against peroxynitrite-induced glial damage and stroke development. Eur J Pharmacol. 1998. 351: 377-382.

Franson RC, Rosenthal MD, Regelson W. Mechanism(s) of cyto-protective and anti-inflammatory activity of PGB1 oligomers: PGBx has potent anti-phospholipase A2 and anti-oxidant activity. Prostaglandins Leukot Essent Fat Acids. 1991. 43: 63-70.

Flaherty JT, Zweier JL. Role of oxygen radicals in myocardial re-perfusion injury: experimental and clinical evidence. Klinische Wochenschrift. 1991. 69: 1061-1065.

Giansanti V, Dona F, Tillhon M, Scovassi AI. PARP inhibitors: new tools to protect from inflammation. Biochemical Pharmacology. 2010. 80: 1869-1877.

Gilad E, Zingarelli B, Salzman AL, Szabo C. Protection by inhibition of poly(ADP-ribose) synthetase against oxidant injury in cardiac myoblasts *In vitro*. Journal of Molecular and Cellular Cardiology. 1997. 29: 2585-2597.

Ha HC, Snyder SH. Poly(ADP-ribose) polymerase is a mediator of necrotic cell death by ATP depletion. Proc Natl Acad Sci USA. 1999. 96: 13978-13982.

He F, Wu Q, Xu B, Wang X, Wu J, Huang L, Cheng J. Suppression of Stim1 reduced intracellular calcium concentration and attenuated hypoxia/reoxygenation induced apoptosis in H9c2 cells. Biosci Rep. 2017. 37: BSR20171249.

Hochman JS, Choo H. Limitation of myocardial infarct expansion by reperfusion independent of myocardial salvage. Circulation. 1987. 75: 299-306.

Kalai T, Balog M, Szabo A, Gulyas G, Jeko J, Sumegi B, et al. New poly(ADP-ribose) polymerase-1 inhibitors with antioxidant activity based on 4-carboxamidobenzimidazole-2-ylpyrroline and -tetrahydropyridine nitroxides and -tetrahydropyridine nitroxides and their precursors. Journal of Medicinal Chemistry. 2009. 52: 1619-1629.

Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. Circulation. 2010. 121: e46-e215.

Nanjo F, Goto K, Seto R, Suzuki M, Sakai M, Hara Y. Scavenging effects of tea catechins and their derivatives on 1,1-diphenyl-2-picrylhydrazyl radical. Free Radical Biology & Medicine. 1996. 21: 895-902.

Pacher P, Liaudet L, Mabley JG, Cziraki A, Hasko G, Szabo C. Beneficial effects of a novel ultrapotent poly(ADP-ribose) polymerase inhibitor in murine models of heart failure. International Journal of Molecular Medicine. 2006. 17: 369-375.

Park ES, Kang JC, Jang YC, Park JS, Jang SY, Kim DE, et al. Cardioprotective effects of rhamnetin in H9c2 cardiomyoblast cells under H(2)O(2)-induced apoptosis. Journal of Ethnopharmacology. 2014. 153: 552-560.

Pieper AA, Walles T, Wei G, Clements EE, Verma A, Snyder SH, et al. Myocardial postischemic injury is reduced by poly ADP ripose polymerase-1 gene disruption. Mol Med. 2000. 6: 271-282.

Pogue AI, Jones BM, Bhattacharjee S, Percy ME, Zhao Y, Lukiw WJ. Metal-sulfate induced generation of ROS in human brain cells: detection using an isomeric mixture of 5- and 6-carboxy-2',7'-dichlorofluorescein diacetate (carboxy-DCFDA) as a cell permeant tracer. Int J Mol Sci. 2012. 13: 9615-9626.

Powers SK, Lennon SL, Quindry J, Mehta JL. Exercise and cardioprotection. Curr Opin Cardiol. 2002. 17: 495-502.

Radnai B, Antus C, Racz B, Engelmann P, Priber JK, Tucsek Z, et al. Protective effect of the poly(ADP-ribose) polymerase inhibitor PJ34 on mitochondrial depolarization-mediated cell death in hepatocellular carcinoma cells involves attenuation of c-Jun N-terminal kinase-2 and protein kinase B/Akt activation. Mol Cancer. 2012. 11: 34.

Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. Circulation. 2000. 101: 2981-2988.

Sun L, Wang H, Yu S, Zhang L, Jiang J, Zhou Q. Herceptin induces ferroptosis and mitochondrial dysfunction in H9c2 cells. Int J Mol Med. 2022. 49: 17.

Szabo C. DNA strand breakage and activation of poly-ADP ribosyltransferase: a cytotoxic pathway triggered by peroxynitrite. Free Radic Biol Med. 1996. 21: 855-869.

Szabo C, Wong H, Bauer P, Kirsten E, Oconnor M, Zingarelli B, et al. Regulation of components of the inflammatory response by 5-iodo-6-amino-1,2-benzopyrone, an inhibitor of poly (ADP-ribose) synthetase and pleiotropic modifier of cellular signal pathways. Int J Oncol. 1997. 10: 1093-1101.

Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013. 65: 157-170.

Thiemermann C, Bowes J, Myint FP, Vane JR. Inhibition of the activity of poly(ADP ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle. Proc Natl Acad Sci USA. 1997. 94: 679-683.

Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton RA, et al. Inhibition of myocardial injury by ischemic post-conditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol. 2003. 285: H579-588.

Zweier JL, Talukder MA. The role of oxidants and free radicals in reperfusion injury. Cardiovascular Research. 2006. 70: 181-190.

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