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Resveratrol-induced apoptosis is inhibited by nitric oxide in endothelial cells

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ABSTRACT

Resveratrol is a phenolic compound found in grapes and other food products. Previously, resveratrol was shown to pla yan important role in cell apoptosis for bovine aortic endothelial cells (BAECs). Here we examined whether nitric oxide (NO) is involved in endothelial cell apoptosis. In order to assess the role of NO in endothelial cell apoptosis, we monitored the endothelial cell apoptosis after treated cells with (+/-)-S-Nitroso-N-acetylpenicillamine (SNAP), an NO donor. When we treated cells with 0.1mmol/l SNAP, NO was produced up to 0.05-0.1 μmol/l . The enhanced amount of NO is likely to be physiologically controlled. Interestingly, NO was shown to inhibit the endothelial cell apoptosis. In conclusion, resveratrol-induced endothelial cell apoptosis was inhibited by NO physiologically controlled, indicating that NO can regulate the vessel wall remodeling.

Key words : resveratrol, endothelial cells, apoptosis, nitric oxide, cell signaling

Introduction

Resveratrol is a natural phytoalexin enriched in grapes, red wines and other food sources. Resveratrol is known to play an important role in anti-inflammatory and anti-oxidant processes. In addition, resveratrol has been suggested to have anti-cancer activity (1, 2). More interestingly, resveratrol has biphasic effects over its concentration. Low concentration (5 mM) of resveratrol appears cell-proliferative, whereas apoptosis is induced at 15 μM or higher concentration in various cancer cells (1).

Previously, we studied on the roles of resveratrol in normal endothelial cells to determine whether resveratrol can be utilized as a tumor growth-modulating biological response modifiers (BRMs) drug through the blockade of vascular functions. Recently, it was reported that resveratrol activates extracellular signal-regulated kinase (ERK), a family member of mitogen-activated protein kinase (MAPK) and endothelial nitric oxide synthetase (eNOS) at low concentration in endothelial cells (3). ERK and eNOS activities treated with resveratrol promote the endothelial cell proliferation.

Nitric oxide (NO) is a well-known regulator for vascular smooth muscle relaxation and angiogenesis. Defective NO production induces development of major cardiovascular diseases (arterial hypertension and dyslipidaemia) (4). On the other hand, vascular NO plays an important role in endothelial cell proliferation and survival (5). In this study, we determined whether NO acts as a resveratrol-induced endothelial cell apoptosis.

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Materials and Methods

Cell Culture and Drug Treatment: BAEC sobtained from descending thoracic aortas were maintained in a growth medium (DMEM (1 g/liter glucose, Life technologies, Inc.) con-

taining 20% fetal bovine serum (FBS, Atlanta Biologicals) without antibiotics) at 37°C and 5% CO_2 (6, 7). Cells used in this study were between passages 3 and 10 and grown on untreated culture dishes. Before drug treatment, BAECs were confluently grown in the growth media containing 50 μg/ml penicillin and streptomycin in a 5% CO2 incubator and then starved for 4-24 h in a starvation media (DMEM containing 0.5% fetal bovine serum with 50 mg/ml penicillin and streptomycin). For apoptotic analysis, confluent cells were incubated with 0.5% FBS-DMEM containing none or 100 mM resveratrol (RES, Sigma) for 36 h.

Measurement of Apoptosis: Confluent BAECs were starved for more than 4 h and then treated with indicated amounts of resveratrol. Then cells were incubated for additional hours (72 h) to execute time-course experiments. After additional incubations, we observed apoptotic cells (round shrink cells) under the microscope. For quantification, we counted apoptotic cells in the same visual field.

NO Measurement: Production of NO was examined by measurements of DAF fluorescence. Confluent cells were treated with indicated amounts of SNAP and 0.1 μmol/l DAF-2 (Calbiochem) for 5 min at 37°C. Stimulation was performed using variations of indicated resveratrol. Excitation wavelength and emission wavelength were used at 490 nm and 515 nm, respectively. NO amount was calculated by DAF fluorescence intensity (8).

Results

Resveratrol has a pro-apoptotic effect on endothelium. First, we tested an effect of resveratrol on the programmed cell death. The apoptotic activity was determined by counting the number of apoptotic cells that were decreased by pretreatment with resveratrol. In **Fig 1**, eighty percent of endothelial cells were apoptotic when cells were treated with 100 mM resveratrol, indicating that resveratrol has a strong pro-apoptotic effect on endothelial cells.

The SNAP treatment augments NO in endothelial cells. To increase NO in endothelial cells, we exposed endothelial cells to a nitric oxide donor, (+/-)-S-Nitroso-N-acetylpenicillamine (SNAP). We monitored NO amounts in extracellular and intracellular fractions (**Fig 2**). As shown in **Fig 2**, intracellular NO became higher at higher concentration of SNAP.

NO produced in endothelium can be regulated by a variety of stimuli including blood flow and VEGF (8). The enhanced amount of NO by SNAP is likely to occur in physiological conditions. Therefore, the effect of NO on vascular physiology can be assessed by experimental approaches with the exposure of SNAP.

The physiological amount of NO inhibits the resveratrol-dependent apoptosis. Nitric oxide production has significant effects on the endothelial physiology and pathophysiology (2, 3). Previously, we determined that eNOS is little modulated by high dose of resveratrol (9). On the contrary,

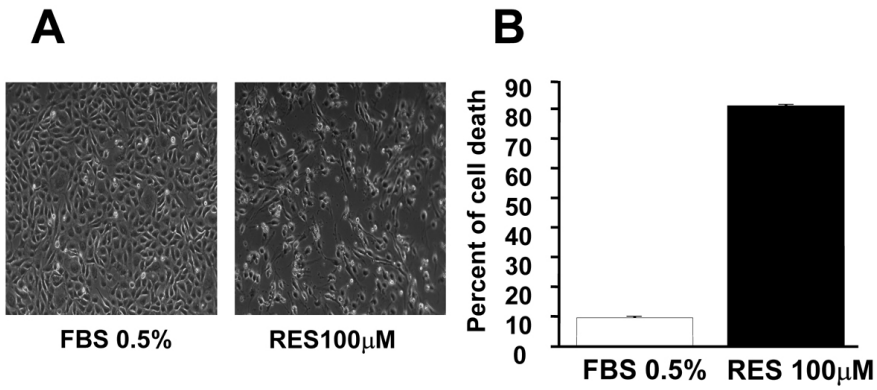

Fig 1. Resveratrol induces the endothelial cell apoptosis.
Confluent BAECs were starved for more than 4 hand then added with indicated doses of resveratrol (RES). Consecutively, cells were incubated for additional hours (72 h) along with indicated doses of resveratrol. Then, we counted apoptotic cells (round shrink cells) under the microscope (A). Bar graphs represent percents of apoptotic cells (means ± s.e.) (B). Experiments were independently performed at least three times.

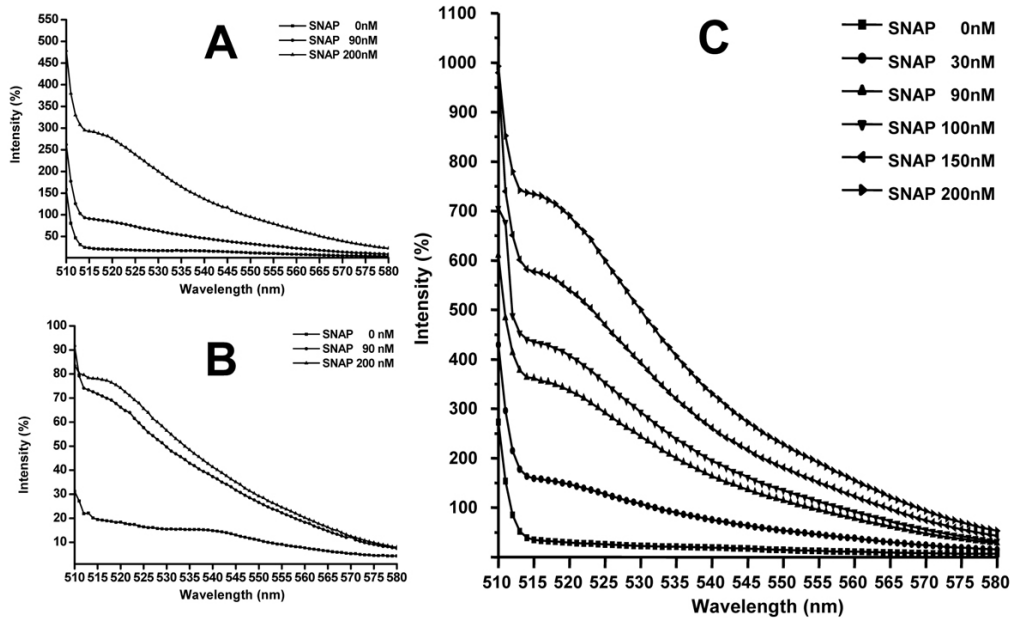

Fig 2. Endothelial NO is produced by SNAP.
Confluent cells were incubated with indicated amounts of SNAP in HEPES (HEPES 5 mM, pH 7.4, NaCl 140 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, glucose 5 mM). SNAP-exposed cells were loaded with 0.1 μmol/l DAF-2. Production of NO was examined by measurements of DAF fluorescence. Excitation wavelength and emission wavelength were used at 490 nm and 515 nm, respectively. (A: intracellular fractions, B: extracellular fractions, C: total)

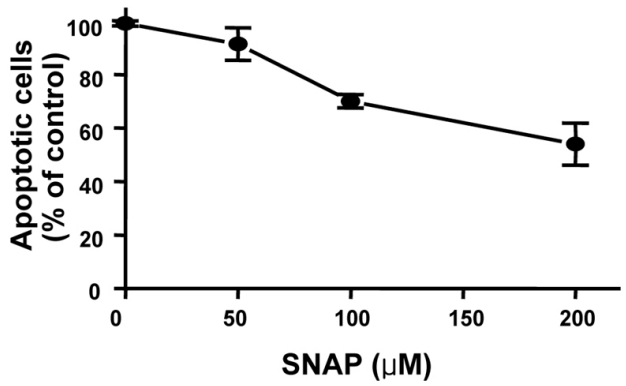

Fig 3. NO blocks the resveratrol-induced apoptosis.
Confluent cells were incubated with 0.5% FBS-DMEM containing none or 100 μM resveratrol for 36 h. Apoptosis was observed as described in Fig 1. As cells were exposed at higher amount of SNAP, apoptotic cells were more diminished. Line graphs represent means ± s.e. (n=3).

it was documented that NO production is enhanced by resveratrol in MCF breast cancer cells (2). Therefore, to more clarify the effect of NO in resveratrol-induced endothelial cell apoptosis, we examined whether SNAP has an important effect on resveratrol-induced endothelial cell apoptosis. As cells were exposed at higher amount of SNAP, apoptotic cells were more diminished (**Fig 3**), indicating that NO at physiological condition is highly possible to block resveratrol-induced endothelial cell apoptosis.

Discussion

Nitric oxide (NO) is a short-lived radical that has been known as an EDRF in endothelium. NO is mostly produced by endothelial nitric oxide synthetase (eNOS) in endothelial cells. eNOS can be activated by shear stress, VEGF and calcium ionophore (8). NO at high concentration induces cell apoptosis, whereas NO produced in low concentration exerts cytoprotective effects (4). Here, we also demonstrated that physiological NO blocks resveratrol-induced apoptosis. Therefore the current study provides an additional example of NO acting as a cytoprotective agent.

In addition, resveratrol has been tremendously studied to be developed as an anti-cancer drug. Previously we suggested

that resveratrol can be utilized as an anti-angiogenic drug (9). However, resveratrol has biphasic properties according to its concentration. For instances, in androgen-sensitive prostate cancer cells, resveratrol has a proliferative activity at low dose (5 μM), whereas it has a pro-apoptotic activity at high dose (15 μM or higher) (2). In endothelial cells, resveratrol activates eNOS at low dose (~nM) and inhibits eNOS at high concentration (100 μM). In addition, the efficacy of resveratrol remains still debated because of its multiplicity of targets and its contradictory functions depending upon concentration and cell types. Moreover, roles of resveratrol in endothelium were less known. However, this study provides a significant insight on the availability of resveratrol in endothelial conditions, because NO can partially antagonize the role of resveratrol. For instance, if we use NO modulators with resveratrol, the efficacy of resveratrol can be improved without side effects.

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