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Mechanism of concentration-dependent induction of heme oxygenase-1 by resveratrol in human aortic smooth muscle cells

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Abstract

Resveratrol-mediated heme oxygenase-1 (HO-1) induction has been shown to occur in primary neuronal cultures and is thought to have potential neuroprotective action. Further, antioxidant properties of resveratrol have been reported to protect against coronary heart disease. We attempted to examine resveratrol's HO-1 inducing potency and its induction regulation in human aortic smooth muscle cells (HASMC). We showed that resveratrol-mediated HO-1 induction occurred in concentration- and time-dependent manners, but only at low concentrations (1–10 μM), and that it was modulated at both the transcription and translation levels. Additionally, the results of our study showed that nuclear factor-kappa B (NF-κB) inhibitors eliminated resveratrol-mediated HO-1 induction and promoter activity, and that deletion of NF-κB binding sites in the HO-1 promoter region strongly reduced promoter activity, suggesting involvement of the NF-κB pathway in HO-1 induction by resveratrol. Suppression of NF-κB activity by resveratrol at high concentrations (≥20 μM) has been reported to be attributed to its anti-inflammatory and anti-oxidative properties. Likewise, we showed that resveratrol at concentrations of ≥20 μM blocked the activity of NF-κB through suppression of I kappa-B alpha (IκBα) phosphorylation, which caused inhibition of HO-1 induction. Conversely, resveratrol in a range of 1–10 μM enhanced the phosphorylation and degradation of IκBα, a key step in NF-κB activation, resulting in HO-1 induction. Collectively, we suggest that resveratrol-mediated HO-1 expression occurs, at least in part, through the NF-κB pathway, which might contribute to resveratrol's vascular-protective effect at physiological concentrations after moderate red wine consumption.

Keywords: Human aortic smooth muscle cell; Heme oxygenase-1; Resveratrol; Nuclear factor-κB; I kappa-B alpha; Mitogen-activated protein kinases; Extracellular signal-regulated kinase 1/2; c-Jun N-terminal kinase

1. Introduction

Resveratrol (*trans*-3,4′,5-trihydroxystilbene), a polyphenolic compound, is abundant in red wine, and grape skin and seeds. It is present in *cis* and *trans* isoforms, of which the latter form is the biologically active one. Resveratrol-mediated HO-1 induction has been reported in neuronal cultures and has been implicated as having potential neuroprotective action [1]. Additionally, epidemiologic studies have reported that a low incidence of cardiovascular disease is associated with moderate red wine consumption in southern France; the so-called French paradox [2–4]. Exactly how resveratrol exerts its cardioprotective effects is not completely understood, but they have been ascribed to its ability to block platelet aggregation, inhibit oxidation of low-density lipoprotein, and induce NO production. Several studies within the last few years have shown that resveratrol protects against coronary heart disease due to its significant antioxidant properties [5–7].

Abbreviations: HASMC, human aortic smooth muscle cell; HO-1, heme oxygenase-1; NF-κB, nuclear factor-kappa B; IκBα, I kappa-B alpha; MAPKs, mitogen-activated protein kinases; ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-Jun N-terminal kinase; NBT, 4-nitroblue tetrazolium; BCIP, 5-bromo-4-chloro-3-indolyl-phosphate; Act D, actinomycin D; Cyclo, cycloheximide

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Additionally, several studies have reported that resveratrol at high concentrations possesses anti-inflammatory activity attributed to blockage of NF-κB activation by inhibiting phosphorylation and degradation of IκBα and thus preventing nuclear translocation of p65 and p50 [8,9].

Heme oxygenase (HO) is a rate-limiting catalyst in the degradation of heme to produce biliverdin, which is further converted to the antioxidant, bilirubin, by biliverdin reductase [10,11], free iron, and carbon monoxide (CO). Three HO isozymes have been identified as having distinct genes [12]. Among them, HO-1, a stress-response protein, can be induced by various oxidative-inducing agents, including heme, heavy metals, UV radiation, cytokines, and endotoxin [13,14]. Recently, numerous in vitro and in vivo studies have shown that the induction of HO-1 is an important cellular protective mechanism against oxidative injury [12]. We recently reported that adenovirus-mediated HO-1 gene transfer inhibits the development of atherosclerosis in apolipoprotein E-deficient mice [15].

Activation of the transcription of *HO-1* or other genes is mediated by a cascade of signal transduction pathways, mainly by modulating the activities of transcription factors. Mitogen-activated protein kinases (MAPKs) contribute to the major pathway for regulating numerous cellular processes such as cell proliferation, differentiation, stress response, and cell death. Three major classes of MAPKs have been described: extracellular signal-regulated kinase (ERK) [16], c-Jun N-terminal kinase (JNK) [17], and p38MAPK [18,19]. Generally, ERK modulates the responses of cell differentiation, whereas JNK and p38MAPK are activated by stress-associated stimuli, such as heat shock, inflammation, ultraviolet light, and irradiation [20].

Stress-related stimuli (sodium arsenite, cobalt chloride, hemin, and cadmium) are involved in MAPK signal transduction for activating the *HO-1* gene. But conflicting results exist in the literature: JNK and p38MAPK contribute to the transcription regulation of *HO-1* gene expression by sodium arsenite in rat hepatocytes, whereas ERK and p38MAPK mediate its regulation in a chicken hepatoma cell line [21,22]. Unlike ERK and JNK, p38MAPK mediates cadmium-dependent *HO-1* gene activation in MCF-7 mammary epithelial cells [23]. Therefore, the transcriptional regulation of HO-1 by MAPKs is tissue and species dependent. Furthermore, the redox-sensitive transcription factors of AP-1, Nrf2, and NF-κB have been found to be involved in the induction of HO-1 in tumor and human renal proximal tubule cells by heavy metals or curcumin (a major component of the spice turmeric) [22–25].

Red wine contains considerably varied amounts of *trans*-resveratrol of approximately 1–8 mg/l. Animal experiments have shown that moderate consumption of resveratrol-rich red wine (375–500 ml for 60 kg of body weight) may result in less than 1 μM resveratrol in the plasma, which is many times lower than the concentration of resveratrol which has been used in the in vitro studies [8,26,27]. In view of the relatively low physiological concentration after red wine consumption, we attempted to unravel the mechanism of resveratrol's cardiovascular protection associated with HO-1 induction within the range of physiological concentrations.

2. Materials and methods

2.1. Chemicals

Resveratrol, actinomycin D (Act D), cycloheximide (Cyclo), and curcumin were purchased from Sigma Chemical. U0126 and SB202190 were obtained from Tocris Cookson. Anti-phosphospecific IκBα, anti-IκBα, anti-p65, and anti-50 antibodies were purchased from Santa Cruz Biotechnology. [γ-^{32}P] dATP and [α-^{32}P] dCTP (6000 Ci/mmol) were purchased from Perkin-Elmer Life Science. F-12K medium, fetal bovine serum (FBS), and tissue culture reagents were obtained from Invitrogen. 4-Nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) were purchased from Kirkegarrd and Perry Laboratories. Protein assay agents were purchased from Bio-Rad.

2.2. Primary cell culture

Human aortic smooth muscle cells were obtained from the American Type Culture Collection (ATCC CRL-1999). Cells were cultured in F-12K medium with modifications according to the manufacturer's instructions and supplemented with 10% FBS. The purity and identity of cells were examined by immunostaining using a specific antibody against smooth muscle cell α-actin. Cells from passages 15 to 20 were used for the experiments.

2.3. RNA isolation and Northern blot analysis for HO-1

To examine the expression of HO-1 mRNA, HASMC were washed twice with ice-cold PBS, and RNA was extracted with the TRIzol method (Invitrogen). Ten micrograms of total RNA from each sample was separated by electophoresis on an agarose gel and transferred to a nylon membrane. Membranes were hybridized overnight with a ^{32}P-labeled human *HO-1* cDNA probe. The corresponding bands were visualized by autoradiograms. The equivalency of loading and transfer of RNA during the Northern blot analysis was assessed by the expression of 18S rRNA.

2.4. Constructs of plasmid variants and luciferase activity assay

The pGL3/h*HO-1* reporter plasmid, which contains a 3293 bp fragment, −3106 to +186 relative to the transcription start of the human *HO-1* gene, was amplified from the human BAC clone CTA-286B10 [28] using the primers

5′-AGAGAACAGTTAGAAAAGAAAG-3′ (sense) and 5′-TACGGGCACAGGCAGGATCAGAA-3′ (anti-sense). The PCR products were inserted into the pCR2.1-TOPO cloning vector (Invitrogen), and cut with KpnI/XbaI, such that the resulting PCR products contained the KpnI/XbaI site, and this was ligated in frame into the unique KpnI/NheI site present within the pGL3 plasmid (Promega). Therefore, we obtained a pGL3/h*HO-1* reporter construct containing about a 3300-bp region of human *HO-1* promoter driving luciferase gene expression. The luciferase reporter gene constructs pHO-2920 (BsiHKAI/BglII), pHO-2520 (HindIII/BglII), pHO-2405 (MfeI/BglII), and pHO-1890 (BstEII/BglII) were generated by deletion of the −3293/−2920 fragment of HO-2920, the −3293/−2520 fragment of HO-2520, etc. by the restriction enzymes indicated in parentheses. The Klenow enzyme was used to create a blunt end at the 5′-sequence, and BglII was used to generate a 3′-cohesive end. These fragments were ligated into the SmaI and BglII sites of pGL3. Similarly, the fragments of HO-2739 (SspI/BglII) and HO-2686 (BfrBI/BglII) were, respectively ligated into the SmaI and BglII sites of pGL3. The identities of the sequences were confirmed using an ABI PRISM 377 DNA Analysis System (Perkin-Elmer).

For the reporter activity assay, cells were seeded in six-well plates at a density of 1×10^5 cells/well. In brief, HASMC were transiently transfected with 3 μg of plasmid DNA containing 1 μg of the Renilla luciferase construct, phRL-TK (Promega), to control transfection efficiency and 2 μg of the appropriate *HO-1* promoter firefly luciferase (FL) construct. The next day, cells were transfected with pGL3/h*HO-1* and phRL-TK (internal control plasmid) using the LipofectAMINE 2000™ (Invitrogen). After transfection (12 h), the medium was replaced with complete medium, and incubation continued for another 36 h. Transfected cells were then treated with drugs for 12 h, and cell lysates were collected. Luciferase activities were recorded in a TD-20/20 luminometer (Turner Designs) using the dual luciferase assay kit (Promega) according to the manufacturer's instructions. Luciferase activities of reported plasmids were normalized to luciferase activities of the internal control plasmid.

2.5. Western blots

Western blot analysis was carried out using the following antibodies: HO-1, α-tubulin, P-IκBα, IκBα, p50, and p65. To prepare whole-cell lysates, cells were washed twice with ice-cold PBS, resuspended in ice-cold RIPA buffer containing 1 mM Na_3VO_4, 1 mM PMSF, 1 μg/ml leupeptin, and 1 μg/ml aprotinin, incubated on ice for 30 min, and vortexed every 10 min followed by centrifugation at 12,000 × *g* for 30 min at 4°. Whole-cell lysates (80 μg) were electrophoresed on 10% SDS-polyacrylamide gels and then transblotted onto Hybond-P membranes Pharmacia). Membranes were blocked in PBS containing 0.1% Tween-20 and 5% skim milk at room temperature for 30 min. Blots were incubated with the indicated antibodies (with dilutions used according to the manufacturer's instructions), in blocking buffer for 1 h at room temperature. After three washes with PBS containing 0.1% Tween-20, blots were incubated with alkaline phosphatase/peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG)/anti-mouse IgG (1:5000) for 1 h at room temperature, followed by another washing. Expression of protein was visualized by staining with NBT and BCIP or was detected using an enhanced chemiluminescence system.

2.6. Electrophoretic mobility shift assay

The electrophoretic mobility shift assay was performed as described previously [29]. To prepare nuclear protein extracts, HASMC in 10 cm^2 dishes after treatment with resveratrol for 8 h in various concentrations as indicated were washed twice with ice-cold PBS and scraped off into 1 ml PBS. After centrifugation of the cell suspension at 500 × *g* for 3 min, the supernatant was removed, and cell pellets were subjected to NE-PER™ nuclear extraction reagents (Pierce) with the addition of 0.5 mg/ml benzamidine, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 2 mM phenylmethylsulfonyl fluoride. The subsequent procedures for the nuclear protein extraction followed the manufacturer's instructions. The fraction containing the nuclear protein was used for the assay or was stored at −70° until use. The sequence of the oligonucleotide used was AGTTGAGGGGACTTTCCAGGC for NF-κB binding (Promega). The oligonucleotide was end-labeled with [γ-^{32}P] dATP. Extracted nuclear proteins (10 μg) were incubated with 0.1 ng of ^{32}P-labeled DNA for 15 min at room temperature in 25 μl of binding buffer containing 1 μg of poly (dI-dC). For competition with unlabeled oligonucleotide, a 100-fold molar excess of unlabeled oligonucleotide relative to the radiolabeled probe was added to the binding assay. Mixtures were electrophoresed on 5% non-denaturing polyacrylamide gels. Gels were dried and imaged by means of autoradiography.

2.7. Statistical analysis

Values are expressed as the mean ± S.D. The significance of the difference from the control group was analyzed by Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Concentration- and time-dependent induction of HO-1 expression

Induction of HO-1 by resveratrol in HASMC was analyzed at both the mRNA and protein levels. Northern blot

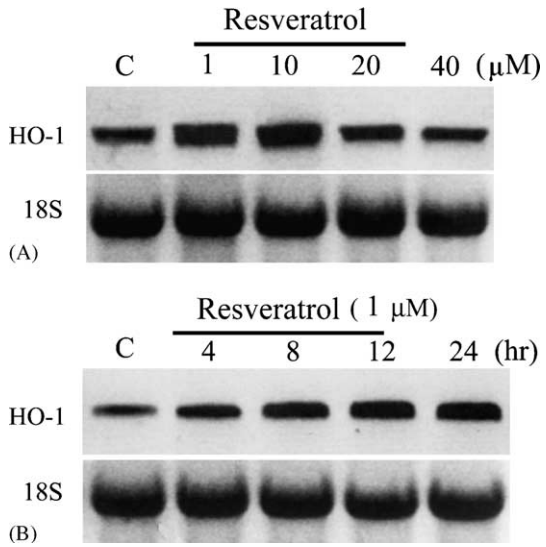

Fig. 1. Induction of HO-1 mRNA by resveratrol in human aortic smooth muscle cells. (A) Concentration-dependent induction of HO-1 mRNA by resveratrol. Total RNA was extracted and analyzed as described in "Section 2". Cells were treated with the indicated concentrations of resveratrol for 12 h. (B) Time-course induction of HO-1 mRNA by resveratrol. Cells were treated with resveratrol (1 μM) for 4, 8, 12, and 24 h. Results of three experiments are shown.

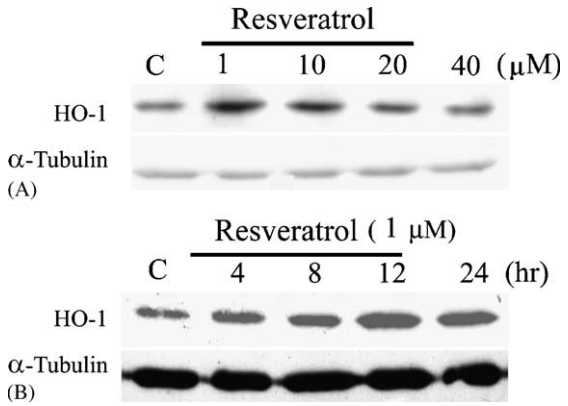

Fig. 2. HO-1 expression in human aortic smooth muscle cells treated with resveratrol. (A) Concentration-dependent induction of HO-1 protein by resveratrol. Cells were treated with the indicated concentrations of resveratrol for 12 h, and the expression of HO-1 was determined using α-tubulin as an internal control. (B) Time-course induction of HO-1 by resveratrol. Cells were treated with resveratrol (1 μM) for 4, 8, 12, and 24 h, and expression of HO-1 protein in cells was analyzed by Western blotting. Results of three experiments are shown.

analysis showed that resveratrol at the concentrations of 1 and 10 μM significantly induced HO-1 induction, but not at the concentrations of 20 and 40 μM (Fig. 1A). RNA was harvested from HASMC treated with 1 μM resveratrol for 4, 8, 12, and 24 h and was analyzed by Northern blotting. Induction of HO-1 mRNA by resveratrol was observed at 4 h and increased with time up to 24 h of treatment (Fig. 1B).

In protein preparations, HASMC were treated with various concentrations of resveratrol. After various lengths of time in culture, cell lysates were prepared, and protein levels of HO-1 in treated cells were assessed by Western blot analysis. In Fig. 2A, a 32 kDa protein band corresponding to HO-1 was highly expressed in cells exposed to resveratrol at the concentrations of 1 and 10 μM, but not at the concentrations of 20 and 40 μM, which agrees with the findings in Fig. 1A. Time-course experiments demonstrated that the expression of HO-1 was observed at 4 h after resveratrol treatment, and this was sustained until ≥24 h after treatment (Fig. 2A).

3.2. Transcriptional and translational regulation of HO-1 induction by resveratrol

To determine at which level the induction of HO-1 by resveratrol is regulated, HASMC were pretreated with Act D and Cyclo for 1 h prior to the addition of 1 μM resveratrol, followed by incubation for another 12 or 24 h. It is shown in Fig. 3A and B that Act D, a transcriptional inhibitor, alleviated HO-1 mRNA induction and that Cyclo, a translational inhibitor, abolished HO-1 protein expression following resveratrol treatment, as analyzed by Northern and Western blots, respectively. Cycloheximide also significantly reduced upregulation of HO-1 mRNA within 12 h and 24 h of exposure to resveratrol (Fig. 3A).

3.3. Induction of HO-1 promoter-driven luciferase expression by resveratrol

The luciferase reporter plasmid, flanked by ~3300 bp upstream of the 5′-untranslated region of the human *HO-1*

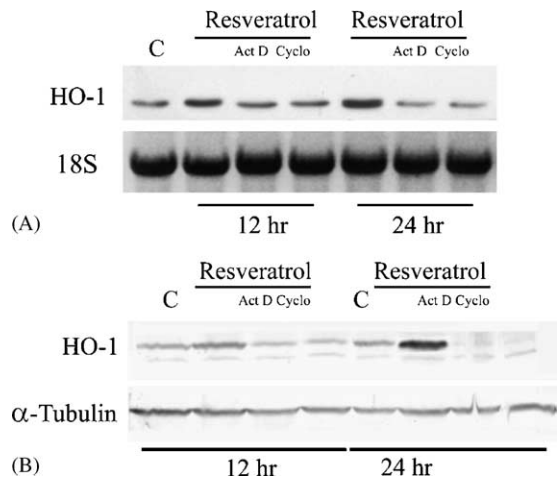

Fig. 3. Effect of actinomycin D (Act D) and cycloheximide (Cyclo) on the induction of HO-1 by resveratrol. (A) Northern blot of HO-1 induction by resveratrol after adding Act D and Cyclo. Human aortic smooth muscle cells grown in 10 cm^2 dishes were incubated in modified F-12K medium supplemented with 10% FBS. Cells were pretreated with Act D (1 μg/ml) or Cyclo (10 μg/ml) for 30 min prior to adding 1 μM resveratrol; RNA isolation and Northern analysis for HO-1 were as described in "Section 2". (B) Western blot of HO-1 induction by resveratrol after adding Act D and Cyclo. Cells were treated with 1 μM resveratrol for 12 h and 24 h and the expression of HO-1 was determined using α-tubulin as an internal control. Results are from three independent experiments.

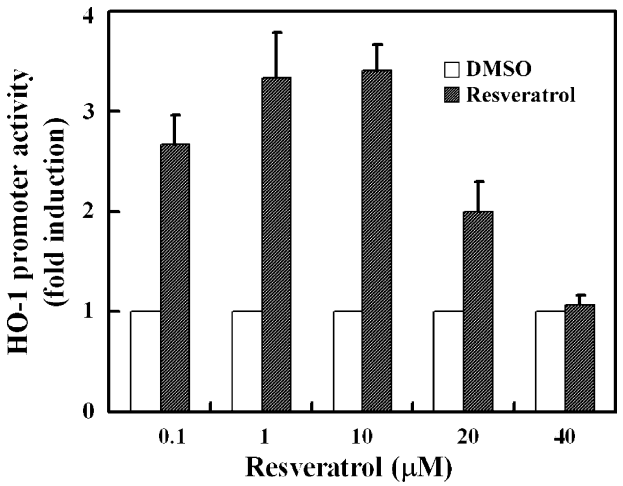

Fig. 4. *HO-1* promoter activity in relation to increasing concentrations of resveratrol. Human aortic smooth muscle cells were transiently transfected with pGL3/h*HO-1* and phRL-TK for 48 h, followed by treatment with increasing concentrations of resveratrol, as described in "Section 2". Luciferase activities of the reported plasmid were normalized to those of the internal control plasmid and are presented as the mean ± S.D. of three independent experiments.

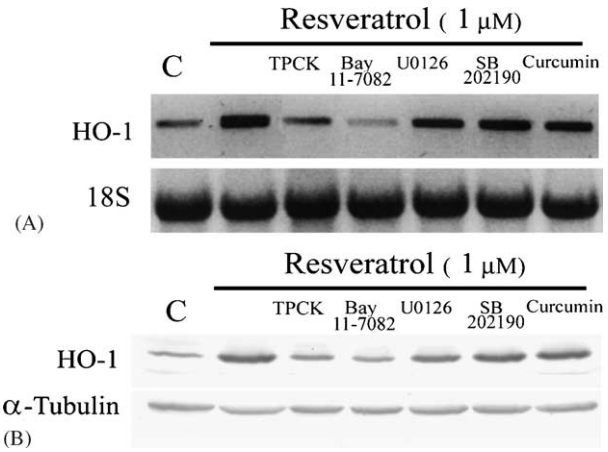

Fig. 5. Northern analysis (A) and Western blot (B) of the effect of MAPK and NF-κB inhibitors on human aortic smooth muscle cells overexpressing HO-1 by resveratrol. Human aortic smooth muscle cells were pretreated with the NF-κB inhibitors, TPCK (20 μM) and BAY 11-7082 (5 μM), and the MAPK inhibitors, U0126, SB202190, and curcumin (10 μM), for 1 h prior to adding 1 μM resveratrol, followed by incubation for another 12 h. Equal loading and transfer were confirmed using 18S in (A) and by incubating with an anti-α-tubulin antibody in (B). Representative results of three separate experiments are shown.

gene was constructed as described in "Section 2". This construct was transiently transfected into HASMC for 48 h followed by increasing concentrations of resveratrol of from 0.1 to 40 μM. After incubation for another 12 h, cells were harvested and analyzed for luciferase activity. As shown in Fig. 4, luciferase activity increased in response to the increased concentrations of resveratrol of from 1 to 10 μM. Conversely, the decline in *HO-1* promoter activity was shown in cells exposed to ≥20 μM resveratrol in agreement with the findings in Fig. 1A.

3.4. Inhibition of resveratrol-mediated HO-1 expression by the NF-κB inhibitor

To reveal the molecular mechanism of resveratrol-mediated HO-1 induction, MAPK and NF-κB inhibitors were employed. Fig. 5 shows that the level of resveratrol-induced HO-1 expression was attenuated by TPCK (a protease inhibitor that blocks activation of NF-κB) and BAY 11-7082 (an inhibitor of IκBα phosphorylation), but not by MAPK inhibitors including U0126, curcumin, and SB202190, which are inhibitors of Erk1/2, JNK, and p38 MAPK, respectively.

3.5. Inhibition of resveratrol-mediated HO-1 expression by deletion mutations

Another approach consisting of deletion mutations was used to confirm that the importance of the NF-κB binding site is attributable to resveratrol-mediated HO-1 induction. A luciferase reporter vector containing 3293 bp of the human *HO-1* promoter region was obtained. This construct was transiently transfected into HASMC for 48 h followed by treatment with MAPK and NF-κB inhibitors for 1 h prior to adding resveratrol (1 μM). After incubation for another 12 h, cells were harvested and analyzed for luciferase activity. Consistently, NF-κB inhibitors abolished the *HO-1* promoter activity induced by resveratrol, but not by MAPK inhibitors (Fig. 6A). NF-κB consensus binding elements are located at −2641 to −2632 and −2451 to −2442 bp in the *HO-1* promoter region. A series of deletion mutations in the *HO-1* promoter was carried out, and *HO-1* promoter activity was eliminated when the consensus binding sites for NF-κB were removed (Fig. 6B).

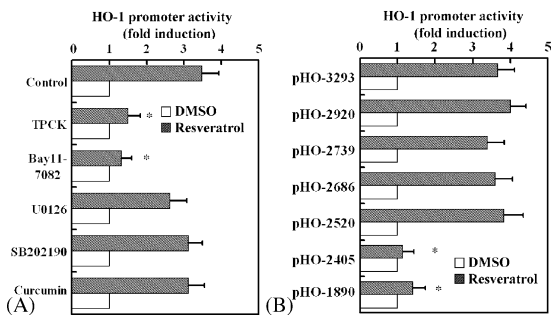

Fig. 6. *HO-1* promoter activity in relation to NF-κB and MAPK inhibitors (A), and deletion mutations in the *HO-1* promoter region (B). Human aortic smooth muscle cells were transiently transfected with pGL3/h*HO-1* variants and phRL-TK for 48 h, as described in "Section 2". Luciferase activities of the reported plasmid were normalized to those of the internal control plasmid and are presented as the mean ± S.D. of three independent experiments. $^{*}P < 0.01$ indicates a significant difference from the control group or from pHO-3293.

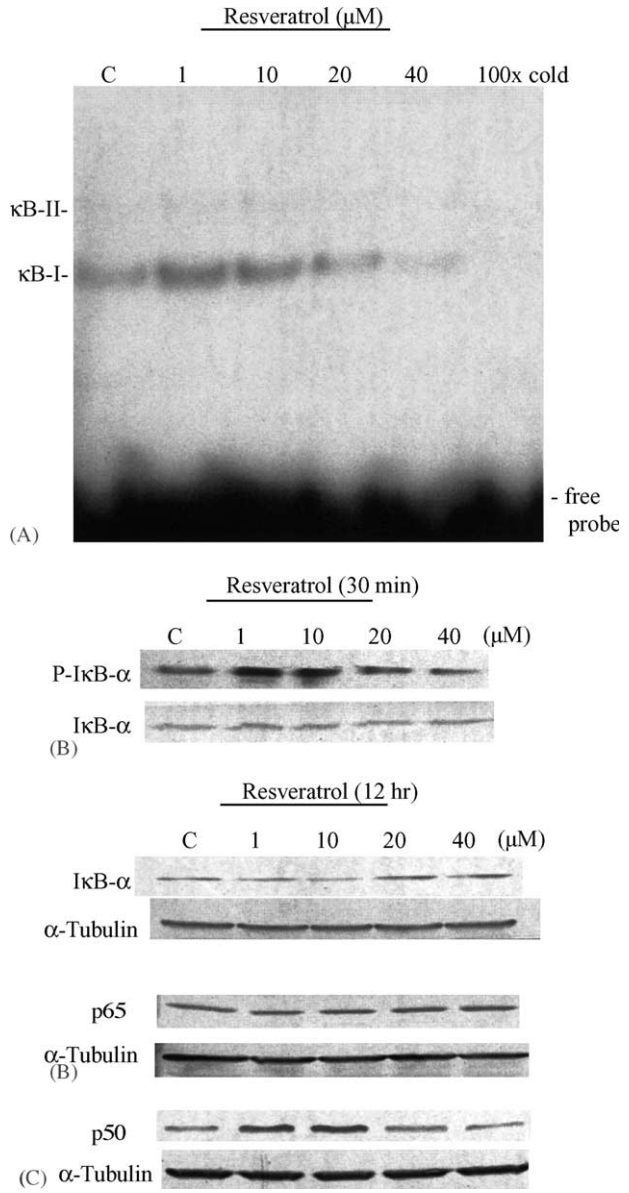

Fig. 7. Concentration-dependent effects of resveratrol on NF-κB activity. (A) Binding activity of NF-κB in human aortic smooth muscle cells exposed to resveratrol. Cells were cultured and treated with increasing concentrations of resveratrol as indicated. Nuclear proteins were assayed for NF-κB binding activity by EMSA. 100× cold denotes a 100-fold molar excess of unlabeled oligonucleotide relative to the ^{32}P-labeled probe; this was added to the binding assay for competition with the unlabeled oligonucleotide. The mobility of specific NF-κB complexes is indicated. (B) Effect of 30 min incubation of resveratrol on the phosphorylation of IκBα. (C) Effect of overnight incubation of resveratrol on cytoplasmic levels of IκBα, and on the nuclear translocation of p65 and p50 subunits of NF-κB. Cell lysates and nuclear extracts were prepared, and the levels of cytoplasmic IκBα nuclear p50 and p65 were determined by Western blot analysis. Representative results of three separate experiments are shown.

3.6. Resveratrol-mediated HO-1 induction modulated by NF-κB transcription activity

The electrophoretic mobility shift assay revealed that resveratrol within a range of 1–10 μM increased NF-κB binding activity, whereas resveratrol at concentrations ≥20 μM inhibited the DNA binding activity of NF-κB, as shown in Fig. 7A. To investigate the concentration-dependent effects of resveratrol on the transactivation of NF-κB. Fig. 7B shows that resveratrol at low concentrations induced a significant increase in IκBα phosphorylation after 30 min of incubation, but not resveratrol at concentrations of 20 and 40 μM. Fig. 7C shows that overnight incubation of resveratrol at low concentrations increased IκBα degradation and then nuclear p50 translocation. Unlike p50, the nuclear translocation of p65 was not affected by varying concentrations of resveratrol under the same conditions.

4. Discussion

HO-1, a heat shock protein, has been implicated in a cytoprotective mechanism to prevent tissues from oxidative damage in response to various insults [12,14,30,31]. The potential use of HO-1 on therapeutic targets in various diseases has been explored. For instance, adenovirus-mediated gene transfer of HO-1 in animal models can protect against hyperoxia-induced lung injury [32], reperfusion-induced injury of a transplanted liver [33], and atherosclerosis [15].

In the present study, we examined the HO-1-inducing potency of resveratrol and its molecular mechanism in HASMC. The results of our study showed that resveratrol-induced HO-1 expression occurred in concentration- and time-dependent manners. Resveratrol-mediated HO-1 induction occurred strongly at concentrations ≤10 μM in a concentration-dependent manner. Nevertheless, resveratrol at concentrations ≥20 μM diminished HO-1 expression as well as promoter activity. By using pharmacological inhibitors, we provide evidence showing no participation of ERK, JNK, or p38MAPK activations in the induction process.

In addition to the findings of resveratrol's induction in the transcriptional regulation of HO-1, we also demonstrated that not only Act D (a transcriptional inhibitor) but also Cyclo (a translational inhibitor) abolished resveratrol-mediated HO-1 mRNA and protein expressions. Based on our findings, we suggest that HO-1 is also subject to post-transcriptional and translational control in HASMC exposed to resveratrol. From our results, we suggest that resveratrol-mediated HO-1 induction may require de novo RNA and protein synthesis, and that the exact mechanism of translation regulation of HO-1 remains unclear. Further investigation of this issue is required.

The most significant finding in this study is the demonstration of the involvement of the NF-κB pathway in resveratrol-mediated *HO-1* gene induction. Evidence in support of this pathway was provided by two approaches. The NF-κB inhibitors, TPCK and BAY 11-7082, completely prevented resveratrol-induced HO-1 expression and the activity of the *HO-1* promoter, which indicated invol-

vement of the NF-κB pathway. The electrophoretic mobility shift assay revealed that resveratrol within a range of 1–10 μM indeed increased NF-κB binding activity. Additionally, serial mutants confirmed that NF-κB binding elements are responsible for resveratrol-mediated HO-1 induction. Furthermore, our study revealed that the transactivation of NF-κB by resveratrol within a range of 1–10 μM occurs by increasing the extents of IκBα phosphorylation and degradation. As a result, resveratrol facilitated the translocation of NF-κB into the nucleus and then modulated *HO-1* gene expression.

NF-κB is generally considered to be a redox-sensitive and proinflammatory transcription factor. Interestingly, the results of our study are in accord with a recent study showing that curcumin-induced HO-1 upregulation occurs through the NF-κB pathway [25]. Both resveratrol and curcumin are natural polyphenolic compounds possessing anti-tumor and anti-inflammatory properties. Thus, the structural and functional similarities of resveratrol and curcumin also exert similar molecular actions on HO-1 induction. It is intriguing that resveratrol, on the one hand, induces HO-1 via a pro-oxidative and proinflammatory transcription factor (NF-κB), but on the other hand, possesses antioxidant and anti-inflammatory properties, further emphasizing the functional importance of HO-1 as an adaptive response to oxidative stress and inflammation.

Given that resveratrol induces HO-1, it is tempting to postulate that the antioxidant and anti-inflammatory properties of resveratrol may, at least in part, be related to HO-1 induction. Although numerous in vitro studies have reported that resveratrol within a range of 20–100 μM inhibits tissue factor gene expression, and is involved in thrombotic disorders or any other cellular effects in vitro, the concentrations required to reach these effects are many-fold higher than physiological concentrations [8,9]. The molecular mechanism of these protective actions is that higher concentrations of resveratrol block the activation of NF-κB by various inducers via suppression of IκBα phosphorylation. Contrariwise, we found that physiological concentrations of resveratrol are able to induce NF-κB through increasing IκBα phosphorylation and degradation, resulting in the induction of the *HO-1* gene. Thus, it is likely that resveratrol exerts its vascular protective action through HO-1 induction with moderate red wine consumption.

To sum up, results from our study indicated that the activation of NF-κB is attributable to HO-1 induction by resveratrol at physiological concentrations after moderate red wine consumption. Recently, numerous in vitro and in vivo studies have shown that the induction of HO-1 is an important cellular protective mechanism against oxidative injury [5,6,12,14,30]. Therefore, resveratrol might be a potential dietary component for protecting cells and tissues against oxidative injuries. Further studies using resveratrol will clarify the possibility of developing this new "drug" for the prevention or treatment of cardiovascular disease.

Acknowledgments

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