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Inhibitory Effects of Resveratrol Analogs on Unopsonized Zymosan-Induced Oxygen Radical Production

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ABSTRACT. Resveratrol, a natural hydroxystilbene, has been reported to have anti-inflammatory and anticarcinogenic activities. Inhibitory effects of resveratrol and its analogs on reactive oxygen species (ROS) production in unopsonized zymosan-stimulated murine macrophage Raw264.7 cells, human monocytes, and neutrophils were analyzed to investigate if the anti-inflammatory and anticarcinogenic activities of resveratrol are related to the inhibition of ROS production. Resveratrol was a potent inhibitor of ROS production in both unopsonized zymosan-stimulated Raw264.7 cells and human monocytes and neutrophils. Resveratrol exhibited 50% inhibition values (IC_{50}) of 17 μM in activated Raw264.7 cells, 18 μM in human monocytes, and 23 μM in human neutrophils. 3,5-Dihydroxy-4′-methoxystilbene or 3,4′-dimethoxy-5-hydroxystilbene exhibited IC_{50} values of 63 or 73 μM in Raw264.7 cells, 51 or >100 μM in human monocytes, and 10 or 37 μM in human neutrophils, respectively. Trimethylresveratrol, piceid, and 3,5-dihydroxy-4′-methoxystilbene-3-O-β-D-glucoside were weak inhibitors of ROS production. Thus, resveratrol was identified as a potent inhibitor of ROS production, which might be one biochemical mechanism related to its anti-inflammatory and anticarcinogenic activities. The number and position of hydroxy substituents in resveratrol analogs seem to play an important role in the inhibitory potency of ROS production. BIOCHEM PHARMACOL **57**;6:705–712, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. resveratrol analog; ROS production inhibitor; unopsonized zymosan; murine macrophages Raw264.7; human monocytes; human neutrophils

The respiratory burst of phagocytes involves a striking increase in oxygen consumption accompanied by activation of the hexose monophosphate shunt, which generates ROS‖ including superoxide, hydrogen peroxide, and hydroxy radicals [1]. The release of ROS is of major importance for host defense but can also induce tissue damage. Exact cellular mechanisms involved in the respiratory burst of phagocytes are not fully understood. However, the respiratory burst is known to rely on the activity of membrane-associated NADPH oxidase with multicomponent electron transport complex, including cytochrome b558, $p47^{phox}$, $p67^{phox}$, rac-1, and rap1a [2, 3]. The NADPH oxidase complex of phagocytes catalyzes the transfer of electrons from NADPH to molecular oxygen, which generates superoxide [1]. Most of the superoxides are oxidized and reduced spontaneously or by superoxide dismutase to form molecular oxygen and hydrogen peroxide [4]. Myeloperoxidase catalyzes the oxidation of halides by hydrogen peroxide to form hypohalous acids [5]. Metal-catalyzed reactions between superoxide and hydrogen peroxide generate hydroxy radicals [6].

The NADPH oxidase complex is dormant in resting cells and is rapidly activated by a variety of soluble mediators and by particulate stimuli interacting with cell surface receptors [7]. Various stimuli are used for activation of NADPH oxidase *in vitro*. In particular, phagocytosis of zymosan particles by leukocytes leads to the production of ROS [8]. Platelet-activating factor, complement fragment C5a, leukotriene B_4, and certain cytokines known as chemotactic factors are activators of the respiratory burst [7, 9–11]. Bacterial lipopolysaccharide, ionophore A23187, and phorbol myristate acetate can activate the NADPH oxidase of leukocytes to generate ROS. Even though these species are not physiological mediators, they are often used under experimental conditions [12, 13].

In addition to the host-defensive mechanism of inflammation, ROS play an important role in aging and carcinogenesis, and may also play a role in the etiology of certain ocular, neurological, and psychiatric diseases in humans

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‖ *Abbreviations:* ROS, reactive oxygen species; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium.

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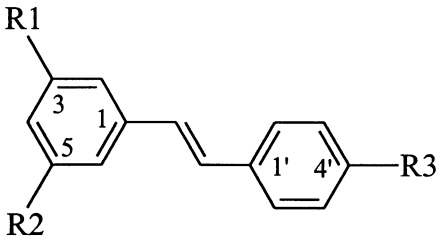

Compound	Substituents		
	R1	R2	R3
Resveratrol	OH	OH	OH
3,5-Dihydroxy-4'-methoxystilbene	OH	OH	OCH_3
3,4'-Dimethoxy-5-hydroxystilbene	OCH_3	OH	OCH_3
Trimethylresveratrol	OCH_3	OCH_3	OCH_3
Piceid	*O*Glc	OH	OH
3,5-Dihydroxy-4'-methoxystilbene-3-*O*-β-D-glucoside	*O*Glc	OH	OCH_3

FIG. 1. Chemical structures of resveratrol and its analogs used in this study. The substituents are hydroxy (OH) and methoxy (OCH_3) groups, and O-β-D-glucose (OGlc).

[14]. Therefore, controlling the respiratory burst may be useful for therapeutic intervention in many human diseases.

Resveratrol is a natural hydroxystilbene with anti-inflammatory and anticarcinogenic activities [15]. In this study, inhibitory effects of resveratrol and its analogs on ROS production in unopsonized zymosan-stimulated murine macrophages Raw264.7 and in human monocytes and neutrophils were analyzed. Resveratrol was a potent inhibitor of ROS production, which might be one biochemical mode of its anti-inflammatory and anticarcinogenic activities.

MATERIALS AND METHODS

Materials

DMEM, Hanks' balanced salts, luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), histopaque (d = 1.08), histopaque (d = 1.12), lucigenin (bis-*N*-methylacridinium nitrate), and zymosan A from *Saccharomyces cerevisiae* were purchased from Sigma Chemical Co. FBS and HEPES were obtained from GIBCO BRL, and benzylpenicillin potassium and streptomycin sulfate from Wako Pure Chemical Industries.

Resveratrol and Its Analogs

Resveratrol and some analogs (Fig. 1) were isolated from herbal extracts or obtained by chemical modifications as described in a previous work [16]. Resveratrol was isolated from *Veratrum album* var. *grandiflorum* Maxim. Both 3,5-dihydroxy-4′-methoxystilbene and 3,5-dihydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside were isolated from *Rheum undulatum* L. and piceid from *Polygonum cuspidatum* Sieb. *et* Zucc. 3,4′-Dimethoxy-5-hydroxystilbene was obtained from piceid by methylation followed by an acid hydrolysis, and trimethylresveratrol by methylation of resveratrol.

Culture of Murine Macrophage Raw264.7 Cells

Murine macrophage Raw264.7 cells were grown in DMEM (10 mg/mL Dulbecco's modified Eagle's medium, 24 mM $NaHCO_3$, 10 mM HEPES, 143 units/mL benzylpenicillin potassium, 100 μg/mL streptomycin sulfate, pH 7.1) containing 10% FBS with 5% CO_2 at 37°. When grown to confluence, the macrophages were detached from the culture dish (Nunc) by using a cell scraper, centrifuged at 250 *g* for 10 min at room temperature, and washed once with Hanks' solution (9.8 mg/mL Hanks' balanced salts, 4 mM $NaHCO_3$, pH 7.1). The Raw264.7 cells were resuspended

in a small volume of DMEM containing 10% FBS for passage or of HEPES-buffered 0.1% BSA (10 mM HEPES, 5.5 mM glucose, 1 mM $MgCl_2$, 5 mM KCl, 145 mM NaCl, 4 mM $NaHCO_3$, 1 mM $CaCl_2$, 0.1% BSA) for chemiluminescence assay, and counted after trypan blue exclusion. Viability of the cells was >95% in all preparations. The Raw264.7 cells were diluted to 2×10^6 cells/mL with the same buffer. For passage, 1 mL of the diluted Raw264.7 cells and 9 mL of fresh DMEM containing 10% FBS were added to a culture dish (100-mm diameter, Nunc), and then incubated at 37° with 5% CO_2.

Isolation of Monocytes and Neutrophils from Human Blood

Human monocytes and neutrophils were isolated from 0.3% sodium citrate-treated venous blood of healthy adult donors which was supplied by a local blood bank (Cheongju, Korea). Six mL of the venous blood was layered on a two-step discontinuous gradient of 3 mL each of histopaque (d = 1.08) and histopaque (d = 1.12), and centrifuged at 700 *g* for 30 min at room temperature. Monocyte-enriched fraction banded at the surface of the high density concentration and neutrophil-enriched fraction at the interface of the two density concentrations were collected [17]. Regardless of the visible contamination of erythrocytes, the monocyte- or neutrophil-enriched fraction was subjected to a hypotonic lysis. After washing once with PBS (140 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4), monocytes or neutrophils were resuspended in a small volume of HEPES-buffered 0.1% BSA and counted after trypan blue exclusion. Viability of the cells was >95% in all preparations. The monocytes or neutrophils were diluted to 2×10^6 cells/mL with HEPES-buffered 0.1% BSA.

Chemiluminescence Assay

Unopsonized zymosan A from *Saccharomyces cerevisiae* was used as a challenger. Luminol or lucigenin was used as an enhancer. Fifty μL of 2×10^6 cells/mL of Raw264.7 or human monocytes or neutrophils, and 50 μL of 0.4 mM luminol or 0.1 mM lucigenin were added per well of a white 96-well microplate (EG & G Berthold). After incubation at 37° in the dark for periods ranging from 15 to 30 min, 50 μL of the unopsonized zymosan and 50 μL of HEPES-buffered 0.1% BSA, with or without sample, were mixed thoroughly. Chemiluminescence emitted from each of the wells was immediately measured as relative light units (RLU) at 37° in the dark for 120 min at 5-min intervals using a luminometer (EG & G Berthold).

Oxygen Consumption Assay

An initial rate of oxygen consumption was measured in a 3-mL reaction mixture of 1×10^6 Raw264.7 cells and 0.3 mg/mL unopsonized zymosan in HEPES-buffered 0.1% BSA, with or without sample, using an oxygen monitor (Yellow Spring Instruments).

Statistical Analysis

The chemiluminescence response was quantitated as an integrated area (IA) below the resulting chemiluminescence curve during a period of 0 to 120 min. Inhibitory effects of samples on the chemiluminescence response or the oxygen consumption are represented as % inhibition [1 - (sample IA/control IA)] × 100, and significance was analyzed by the Student's *t*-test.

RESULTS

Inhibitory effects of resveratrol and its analogs (Fig. 1) on ROS production in murine macrophage Raw264.7 cells, human monocytes, and neutrophils stimulated with unopsonized zymosan were estimated. The amount of ROS generated was analyzed by chemiluminescence assays with luminol or lucigenin as an enhancer, where luminol interacts with all ROS including superoxide, hydrogen peroxide, and hydroxy radicals, and lucigenin predominantly with superoxide [18]. Zymosan is a carbohydrate-rich cell wall preparation derived from the yeast *Saccharomyces cerevisiae* and consisting of two major polysaccharide components of α-D-mannan and β-D-glucan, activating leukocytes to release ROS through the pathway involving cell surface complement receptors [8, 19].

Inhibitory Effect on ROS Production in Raw264.7 Cells

When murine macrophage Raw264.7 cells were stimulated with 0.03–0.3 mg/mL unopsonized zymosan, ROS production was dose dependently increased. However, ROS production in the Raw264.7 cells stimulated with <0.01 mg/mL unopsonized zymosan was similar to basal levels. ROS production in Raw264.7 cells stimulated with >1 mg/mL unopsonized zymosan seemed to be decreased, as it was in the cells stimulated with 0.3 mg/mL unopsonized zymosan. Kinetically, ROS production in murine macrophage Raw264.7 cells peaked at 20 min after unopsonized zymosan treatment in the luminol-enhanced chemiluminescence assay, and peaked at 25 min in the lucigenin-enhanced chemiluminescence assay (Fig. 2, A and B).

In the luminol-enhanced chemiluminescence assay, resveratrol at >12.5 μM exhibited significant inhibitory effects on ROS production in a dose-dependent manner (Fig. 2A). The inhibitory effects were 79 ± 0.7% inhibition at 50 μM resveratrol, 65 ± 1.7% at 25 μM, and 48 ± 1.5% at 12.5 μM. Kinetically, ROS production in Raw264.7 cells treated with resveratrol peaked at the same time as the control, and returned to basal level after 70 min at 50 μM of the compound, after 100 min at 25 μM, and after 120 min at 12.5 μM.

In the lucigenin-enhanced chemiluminescence assay, resveratrol at >25 μM exhibited significant inhibitory

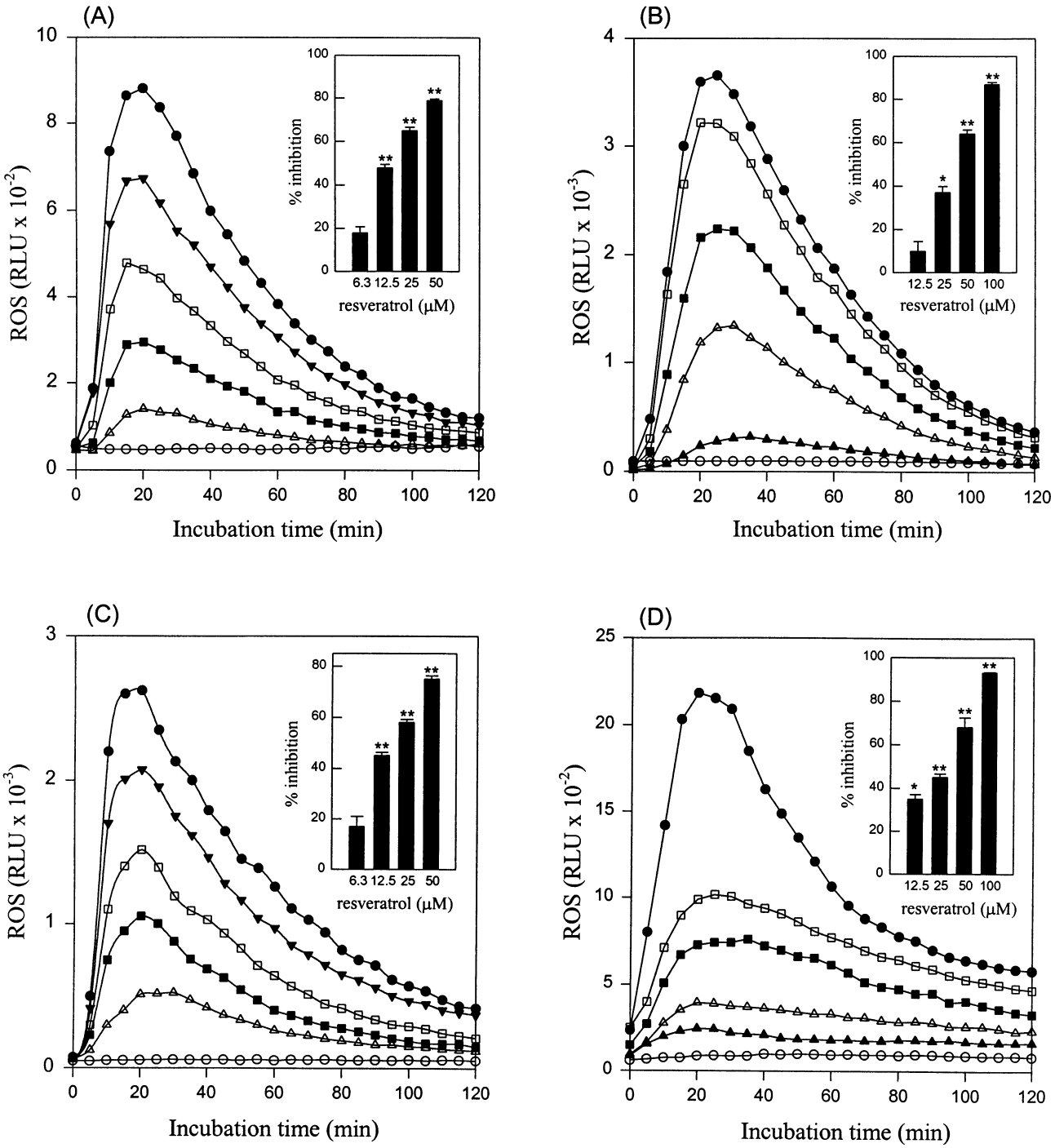

FIG. 2. Dose-dependent inhibitory effect of resveratrol on ROS production. ROS production in Raw264.7 cells is kinetically represented as relative light unit (RLU) values detected by luminol (A)-or lucigenin (B)-enhanced chemiluminescence assay, and that in human monocytes (C) and neutrophils (D) by luminol-enhanced chemiluminescence assay. ROS production in the cells treated with unopsonized zymosan only as the control (solid circle) or HEPES-buffered 0.1% BSA only as the blank (open circle) was measured during a period of 0 to 120 min at 5-min intervals. The cells were treated with resveratrol at 6.3 μM (solid inverted triangle), 12.5 μM (open rectangle), 25 μM (solid rectangle), 50 μM (open triangle), or 100 μM (solid triangle). The inhibitory effect on ROS production is represented as % inhibition, mean ± standard error of three independent tests. Significant differences from the control are $P < 0.001$ (**) and $P < 0.01$ (*).

effects on ROS production (Fig. 2B). The inhibitory effects were 87 ± 0.9% inhibition at 100 μM resveratrol, 64 ± 1.8% at 50 μM, and 37 ± 2.8% at 25 μM. Kinetically, ROS production upon treatment with 100 μM resveratrol peaked about 10 min later than the control and returned to basal level after 70 min, and that with 50 μM resveratrol

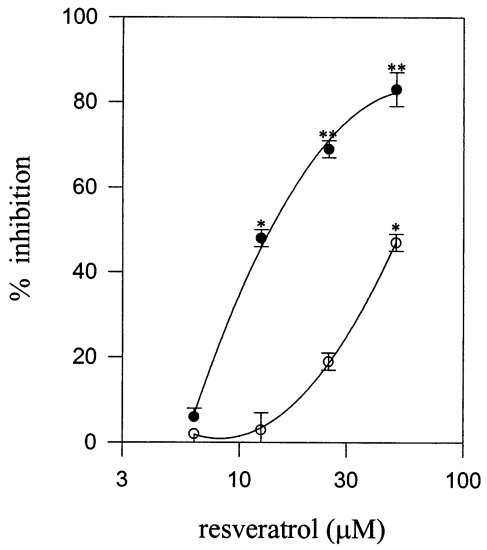

FIG. 3. Effect of resveratrol on oxygen consumption and ROS scavenging. An initial rate of oxygen consumption was measured in Raw264.7 cells stimulated by unopsonized zymosan with or without resveratrol. The inhibitory effect on oxygen consumption is represented as % inhibition, mean ± standard error of three independent tests (solid circle). Resveratrol was added to Raw264.7 cells at 20 min after unopsonized zymosan stimulation, and the amount of ROS was then measured by luminol-enhanced chemiluminescence assay. The ROS scavening effect is represented as % inhibition, mean ± standard error of three independent tests by comparison with the control amount of ROS (open circle). Significant differences from the control are $P < 0.001$ (**) and $P < 0.01$ (*).

peaked about 5 min later than the control and returned to basal level after 110 min.

Resveratrol at >12.5 μM exhibited significant inhibitory effects on oxygen consumption in Raw264.7 cells stimulated by unopsonized zymosan (Fig. 3). The inhibitory effects were 83 ± 4.0% at 50 μM resveratrol, 69 ± 2.1% at 25 μM, and 48 ± 1.9% at 12.5 μM. The oxygen-scavening effect by resveratrol was significant at 50 μM, but not at <25 μM (Fig. 3).

Resveratrol is a natural hydroxystilbene with hydroxy groups at the 3, 4′, and 5 positions (Fig. 1). The compound exhibited 50% inhibition values (IC_{50}) on ROS production at 17 μM in the luminol-enhanced chemiluminescence assay, and 36 μM in the lucigenin-enhanced chemiluminescence assay (Table 1). 3,5-Dihydroxy-4′-methoxystilbene, a resveratrol analog with a methoxy group instead of a hydroxy group at the 4′ position, exhibited IC_{50} values of 63 μM in the luminol-enhanced chemiluminescence assay, and 56 μM in the lucigenin-enhanced chemiluminescence assay. 3,4′-Dimethoxy-5-hydroxystilbene is a resveratrol analog with methoxy groups instead of hydroxy groups at the 3 and 4′ positions. 3,4′-Dimethoxy-5-hydroxystilbene exhibited IC_{50} values of 73 μM in the luminol-enhanced chemiluminescence assay, and 59 μM in the lucigenin-enhanced chemiluminescence assay. Trimethylresveratrol is a resveratrol analog with methoxy groups instead of hydroxy groups at the 3, 4′ and 5 positions. Piceid and 3,5-dihydroxy-4′-methoxystilbene-3-O-β-D-glucoside are glycosides of resveratrol and 4′-methoxy-5-hydroxystilbene, respectively. Trimethylresveratrol and piceid at a concentration of 100 μM exhibited 41 ± 2.7 and 37 ± 1.9% inhibition, respectively, in the luminol-enhanced chemiluminescence assay, but did not show significant inhibitory effects on ROS production in the lucigenin-enhanced chemiluminescence assay. 3,5-Dihydroxy-4′-methoxystilbene-3-O-β-D-glucoside at a concentration of 100 μM did not exhibit significant inhibitory effects on ROS production.

Inhibitory Effect on ROS Production in Human Monocytes

ROS production in unopsonized zymosan-stimulated human monocytes was analyzed using the luminol-enhanced chemiluminescence assay. The human monocytes were isolated from venous blood of healthy adult donors. ROS production in human monocytes stimulated with <0.003 mg/mL of unopsonized zymosan was similar to basal levels and was dose dependently increased when the monocytes were stimulated with 0.01–0.3 mg/mL unopsonized zymosan. Maximal ROS production in human monocytes was observed when the cells were stimulated by 0.3 mg/mL

TABLE 1. Inhibitory effects of resveratrol analogs on ROS production in murine macrophages Raw264.7

	Luminol		Lucigenin	
Compound	**Inhibition (%)**	**IC_{50} (μM)**	**Inhibition (%)**	**IC_{50} (μM)**
Resveratrol	88 ± 0.1*	17	87 ± 1.0*	36
3,5-Dihydroxy-4′-methoxystilbene	73 ± 1.8*	63	84 ± 2.6*	56
3,4′-Dimethoxy-5-hydroxystilbene	71 ± 3.3*	73	82 ± 2.4*	59
Trimethylresveratrol	41 ± 2.7†	>100	14 ± 9.7	>100
Piceid	37 ± 1.9†	>100	<0 ± 9.2	>100
3,5-Dihydroxy-4′-methoxystilbene-3-O-β-D-glucoside	13 ± 7.8	>100	<0 ± 5.5	>100

ROS production in Raw264.7 cells stimulated with 0.3 mg/mL unopsonized zymosan was measured by luminol- or lucigenin-enhanced chemiluminescence assay. The inhibitory effect on the ROS production by each sample at the concentration of 100 μM is represented as % inhibition compared with the control, mean ± standard error of three independent tests.

Significant differences from the control are $P < 0.001$ (*) and $P > 0.01$ (†).

TABLE 2. Inhibitory effects of resveratrol analogs on ROS production in human monocytes and neutrophils

Compound	Monocytes		Neutrophils	
	Inhibition (%)	IC_{50} (μM)	Inhibition (%)	IC_{50} (μM)
	90 ± 0.5*	18	93 ± 0.2*	23
3,5-Dihydroxy-4′-methoxystilbene	70 ± 2.1*	51	96 ± 0.1*	10
3,4′-Dimethoxy-5-hydroxystilbene	45 ± 2.9*	>100	63 ± 1.2*	37
Trimethylresveratrol	47 ± 1.5†	>100	46 ± 2.6†	>100
Piceid	33 ± 1.3†	>100	35 ± 2.2†	>100
3,5-Dihydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside	<0 ± 3.2	>100	29 ± 0.5†	>100

Human monocytes and neutrophils were isolated from venous blood of healthy donors, and ROS production in the cells stimulated with unopsonized zymosan was measured by luminol-enhanced chemiluminescence assay. The inhibitory effect on ROS production by each sample at the concentration of 100 μM is represented as % inhibition, mean ± standard error of three independent tests.

Significant differences from the control are $P < 0.001$ (*) and $P < 0.01$ (†).

unopsonized zymosan. Kinetically, ROS production in human monocytes was sharply increased up to 20 min after 0.3 mg/mL unopsonized zymosan treatment and then decreased gradually (Fig. 2C).

Resveratrol exhibited dose-dependent inhibitory effects on ROS production in human monocytes (Fig. 2C). The inhibitory effects of resveratrol on ROS production were 75 ± 1.2% inhibition at 50 μM, 58 ± 1.2% at 25 μM, and 45 ± 1.3% at 12.5 μM. However, resveratrol at a concentration of 6.3 μM did not show significant inhibition. Kinetically, ROS production in human monocytes treated with 50 μM resveratrol peaked 5–10 min later than the control, while those treated with 6.3–25 μM resveratrol peaked at the same time as the control.

Resveratrol at 100 μM exhibited 90 ± 0.5% inhibition, with an IC_{50} value of 18μM, on ROS production in unopsonized zymosan-stimulated human monocytes (Table 2). 3,5-Dihydroxy-4′-methoxystilbene exhibited 70 ± 2.1% inhibition at 100 μM, with an IC_{50} value of 51 μM. 3,4′-Dimethoxy-5-hydroxystilbene, trimethylresveratrol, and piceid at a concentration of 100 μM exhibited 45 ± 2.9, 47 ± 1.5, and 33 ± 1.3% inhibition, respectively, but 3,5-dihydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside, at the same concentration, did not inhibit.

Inhibitory Effect on ROS Production in Human Neutrophils

ROS production in unopsonized zymosan-stimulated human neutrophils was analyzed by the luminol-enhanced chemiluminescence assay. The human neutrophils were isolated from venous blood of healthy adult donors. Maximal ROS production in human neutrophils was observed when the cells were treated with 0.03 mg/mL unopsonized zymosan. Kinetically, ROS production in human neutrophils peaked at 20 min after unopsonized zymosan was added (Fig. 2D).

Resveratrol exhibited dose-dependent inhibitory effects on ROS production in unopsonized zymosan-stimulated human neutrophils (Fig. 2D). Inhibitory effects on ROS production were 93 ± 0.2% inhibition at 100 μM resveratrol, 68 ± 4.5% at 50 μM, 45 ± 1.6% at 25 μM, and 35 ± 2.1% at 12.5 μM. Kinetically, ROS production in human neutrophils treated with 12.5–25 μM resveratrol peaked 5–15 min later than the control, and those treated with 50–100 μM resveratrol at the same time as the control.

Resveratrol exhibited an IC_{50} value of 23 μM on ROS production in human neutrophils (Table 2). 3,5-Dihydroxy-4′-methoxystilbene and 3,4′-dimethoxy-5-hydroxystilbene exhibited IC_{50} values of 10 and 37 μM, respectively. Trimethylresveratrol, piceid, and 3,5-dihydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside at 100 μM exhibited 29 to 46% inhibition.

DISCUSSION

In both luminol- and lucigenin-enhanced chemiluminescence assays, ROS production in murine macrophage Raw264.7 cells was maximal when the cells were stimulated with 0.3 mg/mL unopsonized zymosan, and kinetically peaked 20–25 min after unopsonized zymosan treatment (Fig. 2, A and B). In the chemiluminescence assay, luminol is known to interact with all ROS, including superoxide, hydrogen peroxide, and hydroxy radicals, and lucigenin predominantly with superoxide [18]. Relative light unit values in the luminol-dependent chemiluminescence assay corresponding to total ROS produced were less than those in the lucigenin-dependent chemiluminescence assay corresponding to superoxide produced in unopsonized zymosan-stimulated murine macrophage Raw264.7 cells (Fig. 2, A and B). This result may be ascribed to the differential sensitivity of luminol or lucigenin to amplify chemiluminescence emission after interaction with ROS or superoxide.

ROS production in murine macrophage Raw264.7 cells stimulated with 0.03–10 μg/mL phorbol myristate acetate was significantly increased, which compared with the basal level, but much less than that with unopsonized zymosan (data not shown). However, ROS production in murine macrophage Raw264.7 cells stimulated with 0.001–1 μg/mL platelet-activating factor, 0.01–10 μM formyl-Met-

Leu-Phe peptide, 0.001–1 μM ionophore A23187, 0.001–1 ng/mL rat chemokine CINC-1, or 2.5–2500 units/mL human interleukin-1β was similar to the basal level (data not shown). Therefore, unopsonized zymosan was a potent challenger to release ROS from murine macrophage Raw264.7.

To our knowledge, ROS production in unopsonized zymosan-stimulated murine macrophage Raw264.7 cells was first demonstrated in this study. Several studies have reported that human monocytes, macrophages, and neutrophils released ROS when challenged with unopsonized zymosan [8, 20, 21]. In this study, ROS production in human monocytes or neutrophils was dose-dependent on unopsonized zymosan, and kinetically peaked at 20 min after the challenger was added (Fig. 2, C and D). Maximal ROS production occurred when human monocytes were treated with 0.3 mg/mL unopsonized zymosan and when human neutrophils were stimulated with 0.3 mg/mL. Thus, the number of complement receptors interacting with unopsonized zymosan or NADPH oxidase activities in monocytes and neutrophils from human venous blood might be significantly different.

Resveratrol is a natural product abundant in plants and known as a phytoalexin produced under adverse conditions such as environmental stress and pathogenic attack. Even though the physiological function of resveratrol in plants is not well defined, several biological activities of the natural compound have been identified. Resveratrol was reported to inhibit cyclooxygenase involved in eicosanoid metabolism and monoamine oxidase in catecholamine metabolism [15, 16, 22]. Resveratrol is also known to have anticarcinogenic activity in 7,12-dimethylbenz(lite(a)anthracene (DMBA)- and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced mouse skin tumorigenesis, and to show anti-inflammatory effects in carrageenin-induced rat paw edema [15]. Resveratrol was previously reported to inhibit free radical generation in human promyelocytic leukemia HL-60 cells stimulated with TPA, which was suggested as a biomarker of its anticarcinogenic activity [23].

Inhibitory effects of resveratrol analogs on ROS production in unopsonized zymosan-stimulated murine macrophage Raw264.7 cells, human monocytes, and neutrophils were analyzed by chemiluminescence assay in order to study whether anti-inflammatory and anticarcinogenic activities of the hydroxystilbenes are related to inhibitory effects on ROS production. Resveratrol inhibited, in a dose-dependent manner, ROS production in murine macrophage Raw264.7 cells stimulated with unopsonized zymosan (Fig. 2, A and B), and exhibited IC_{50} values of 17 and 36 μM in the luminol- and lucigenin-enhanced chemiluminescence assays, respectively (Table 1). Thus, the inhibitory effect of resveratrol on total ROS production was about 2-fold stronger than that on superoxide production. Resveratrol exhibited an IC_{50} value of 18 μM on oxygen consumption in unopsonized zymosan-stimulated Raw264.7 cells, and its ROS scavening effect was significant at 50 μM, but not at $<$25 μM (Fig. 3).

3,5-Dihydroxy-4′-methoxystilbene, 3,4′-dimethoxy-5-hydroxystilbene, trimethylresveratrol, piceid, and 3,5-dihydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside were used as resveratrol analogs in this study (Fig. 1). All of these analogs exhibited stronger inhibitory effects on superoxide production than total ROS production in unopsonized zymosan-stimulated murine macrophage Raw264.7 cells (Table 1). Inhibitory potency of resveratrol and its analogs on ROS production in murine macrophage Raw264.7 and human monocytes stimulated with unopsonized zymosan was in the same order of resveratrol $>$3,5-dihydroxy-4′-methoxystilbene $>$ 3,4′-dimethoxy-5-hydroxystilbene $>$ trimethylstilbene $\cong$ piceid $>$ 3,5-hydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside (Tables 1 and 2). However, resveratrol exhibited a weaker inhibitory effect on ROS production in human neutrophils than did 3,5-dihydroxy-4′-methoxystilbene, and a stronger inhibitory effect than the other analogs (Table 2). Thus, the number and position of hydroxy substituents seem to play an important role in the inhibitory potency of hydroxystilbenes on ROS production. The poor inhibitory effects of piceid and 3,5-hydroxy-4′-methoxystilbene-3-*O*-β-D-glucoside might be ascribed to steric hinderance by a glucose moiety.

Finally, resveratrol was identified as a potent inhibitor of ROS production, which might be one of the biochemical mechanisms of its anti-inflammatory and anticarcinogenic activities. ROS is known to be implicated in numerous pathophysiologies in addition to inflammatory and proliferative diseases [14]. However, at the present time, it is not known whether ROS production is the causal factor of illnesses or is a result of their progression.

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