# The Grape Component Resveratrol Interferes with the Function of Chemoattractant Receptors on Phagocytic Leukocytes

**Hengyi Tao[1, 6], Chunfu Wu[4], Ye Zhou[1], Wanghua Gong[3], Xia Zhang[2], Pablo Iribarren[1], Yuqing Zhao[5], Yingying Le[1] and Jiming Wang[1, 7]**

**Resveratrol (3, 5, 4'-trihydroxystilbene) (RV) is a constituent of grape seeds with anti-inflammatory and anti-oxidant activities. In this study, we examined the capacity of RV to modulate the function of G protein-coupled chemoattractant receptors, which play important roles in inflammation and immune responses. RV, over a non-cytotoxic concentration range, inhibited chemotactic and calcium mobilization responses of phagocytic cells to selected chemoattractants. At low micromolar concentrations RV potently reduced superoxide anion production by phagocytic leukocytes in response to the bacterial chemotactic peptide fMLF, a high affinity ligand for formylpeptide receptor FPR, and $A\beta_{42}$, an Alzheimer's disease-associated peptide and a ligand for the FPR variant FPRL1. In addition, RV reduced phosphorylation of extracellular signal-regulated kinase (ERK1/2) and the activation of nuclear factor NF-κB induced by formylpeptide receptor agonists. These results suggest that the inhibition of the function of chemoattractant receptors may contribute to the anti-inflammatory properties of RV. Thus, RV may be therapeutically promising for diseases in which activation of formylpeptide receptors contributes to the pathogenic processes. *Cellular & Molecular Immunology*. 2004; 1(1):50-56.**

**Key Words:** resveratrol, chemoattractants, receptors, signaling, inflammation

## Introduction

Phagocytic leukocytes infiltrate the sites of inflammation, injury and bacterial infection presumably in response to locally produced chemotactic factors. Over the past decades, a number of exogenous and host tissue-derived chemoattractants have been identified. The "classical" chemoattractants include bacterial chemotactic formylpeptides represented by fMLF, activated complement components, and chemotactic lipids (1-3). There is also a superfamily of chemokines that induce the migration of selected cell population (4, 5). Both classical and chemokine family members use seven-transmembrane G protein-coupled cell surface receptors to chemoattract and activate leukocytes. Recently, emerging evidence suggests that in addition to mediating leukocyte chemotaxis, chemoattractants play essential roles in hematopoiesis, development, immune responses, HIV infection, and the progression of malignant tumors. Thus, development of molecules capable of interacting with chemoattractants or their receptors may have important significance in regulating host-responses in health and disease states.

A grape seed component Resveratrol (RV) has raised considerable interest due to its beneficial effects in many disease states. RV is a polyphenolic compound with three hydroxyl groups (6) and possesses a variety of biological activities including inhibition of inflammatory responses, anti-oxidation, induction of tumor cell death, modulation of lipid and lipoprotein metabolism, and prevention of platelet aggregation (6-11). These activities of RV are believed to be beneficial for improving human health. The purpose of this study is to examine the capacity of RV to affect the function of G protein-coupled chemoattractant receptors, which are involved in a variety of proinflammatory and immunological diseases. Here we report that RV is capable of interfering with the signaling of selected chemoattractant receptors, notably the receptors for the bacterial formyl-

[1]Laboratory of Molecular Immunoregulation, [2]Laboratory of Experimental Immunology, Center for Cancer Research, and [3]Basic Research Program, SAIC-Frederick Inc., National Cancer Institute at Frederick, Frederick, Maryland 21702, USA.

[4]Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, China.

[5]Research Center for Modernization of Chinese Materia Medica of Liaoning Province, Shenyang 110000, China.

[6]Department of Nautical Medicine, Shanghai Changzheng Hospital, Shanghai, China.

[7]Corresponding to: Dr. Jiming Wang, Laboratory of Molecular Immunoregulation, Center for Cancer Research, National Cancer Institute at Frederick, Building 560, Room 31-40, Frederick, Maryland 21702; Tel: 301-846-6979. Fax: 301-846-7042, E-mail: wangji@mail.ncifcrf.gov.

Received for publication Jan 28, 2004. Accepted for publication Feb 12, 2004.



*Abbreviation:* RV, resveratrol; fMLF, formylpeptide; AD, Alzheimer's disease; SOA, stimulus-onset asynchrony; FPR, formyl peptide receptor; ETFR, epitope-tagged formyl peptide receptor; ERK, extracellular signal-regulated kinase.

peptides and for the 42 amino acid β amyloid peptide ($A\beta_{42}$) associated with Alzheimer's disease (AD) (3).

## Materials and Methods

*Reagents and cells*

RV was purified at the Research Center for Modernization of Chinese Materia Medica of Liaoning Province, P.R. China. The RV preparation at the highest concentration tested did not contain detectable levels of bacterial endotoxin. For studies of its effects on chemoattractant receptors, RV was dissolved in DMSO at a 100mM stock solution and frozen at -20°C. The bacterial chemotactic peptide fMLF was purchased from Sigma-Aldrich (St. Louis, MO). The chemotactic peptide MMK-1 (12) was synthesized and purified at the Department of Biochemistry, Colorado State University (Fort Collins, CO), according to the published sequence (13). IL-8 and other chemokines were purchased from PeproTech (Rocky Hill, NJ). The AD associated 42 aa form of $A\beta_{42}$ peptide was from California Peptide Research (Napa, CA). Antibodies against phosphorylated ERK1/2 and total ERK1/2 were purchased from Cell Signaling Technology (Beverly, MA). Human peripheral blood phagocytes were isolated from leukopacks obtained from the Transfusion Medicine Department, National Institutes of Health Clinical Center, Bethesda, MD. Rat basophilic leukemia cell line (RBL-2H3) transfected with epitope-tagged human FPR (ETFR), a high-affinity receptor for the bacterial chemotactic peptide fMLF, was a kindly gift from Drs. H. Ali and R. Snyderman (Duke University, Durham, NC).

*Chemotaxis assays*

Chemotaxis assays for human phagocytes and ETFR cells were performed with 48-well chemotaxis chambers (Neuro-Probe, Gaithersburg, MD) (14). Different concentrations of stimulants were placed in wells of the lower compartment of the chamber. The cell suspension ($1\times10^6$/ml in RPMI1640, 1% BSA) was seeded into wells of the upper compartment, which was separated from the lower compartment by polycarbonate filters (GE Osmonics Labstore, Minnetonka, MN; 5μM pore size for phagocytes; 10μM pore size for ETFR cells). The filters for migration of ETFR cells were precoated with 50μg/ml of collagen type I (Collaborative Biomedical Products, Bedford, MA) to favor cell attachment. After incubating the chemotaxis chambers at 37°C (90 min for monocytes, 60 min for neutrophils, and 270 min for ETFR cells), the filters were removed and stained, and the numbers of cells migrating across the filters were counted by light microscopy after coding the samples. Results are presented as the chemotaxis indexes (CIs) representing the fold increase in the number of migrating cells in response to stimuli over the spontaneous cell migration (to control medium).

*Measurement of superoxide anion release*

Human phagocytes were suspended at $1\times10^6$ cells/ml in HBSS containing $Ca^{2+}$ and $Mg^{2+}$. Aliquots of 50μl cells ($5\times10^4$) were distributed into individual chemiluminescence cuvettes (Pharmingen, San Diego, CA) and mixed with different concentrations of RV. The cells were incubated at 37°C for 5 min. Superoxide-specific chemiluminescence indicator reagent Diogenes (National Diagnostics, Atlanta, GA), chemoattractants or control agents were then added to the cells and chemiluminescence was measured in a luminometer (Analytical Luminescence, San Diego, CA). The results are expressed as integrated luminescence in relative light units (RLU).

*Western immunoblotting*

Human neutrophils or ETFR cells were treated with RV for different time periods followed by fMLF (100nM) for 5 min. The cells were lysed with 150μl ice-cold lysis buffer (62.5mM Tris-HCl [pH6.8], 2% SDS, 10% glycerol, and 50mM DTT), sonicated for 3 s, and boiled for 5 min. After centrifugation (12,000 rpm at 4°C for 10 min), the protein concentration in the supernatant of the lysates was measured by BCA protein assays (Pierce, Rockfold, IL). The cell lysates containing 80μg proteins were electrophoresed on 10% Tris-Glycine precast gels (Invitrogen, Carlsbad, CA) under reducing conditions, then were transferred onto Immobilon™-P membranes (Millipore, Billerica, MA). The membranes were blocked with 3% non-fat milk, 0.1% Tween-20 TBS for 2h at room temperature (RT) and then were incubated with specific primary antibodies overnight at 4°C. After incubation with a horseradish peroxidase conjugated secondary antibody, the protein bands were detected with a Super Signal Chemo-luminescent Substrate Stable Peroxide Solution (Pierce) and BIOMAX-MR film (Eastman Kodak, Rochester, NY).

*$Ca^{2+}$ mobilization assays*

Cells ($1x10^7$ cells/ml) suspended in DMEM containing 10% FBS were loaded with 5μM Fura-2 AM (Molecular Probes, Eugene, OR) at 24°C for 30 min in the dark. The dye-loaded cells were washed and resuspended at $5\times10^5$ cells/ml in saline buffer (138mM NaCl, 6mM KCl, 1mM $CaCl_2$, 10mM HEPES, 5mM glucose, and 1% BSA, pH7.4). The cells in a 2ml volume were placed in quartz cuvettes in a luminescence spectrometer (Perkin-Elmer, Beaconsfield, UK). Stimulants at different concentrations were added in a 20μl volume to the cuvettes and $Ca^{2+}$ mobilization was measured by recording the ratio of fluorescence emitted at 509nm under excitation at 340nm and 380nm wavelengths.

*Measurement of NF-κB activity*

ETFR cells were seeded in 12-well plates at a density of $0.5\times10^6$ cell/well. The cells were transfected with 1μg/well of p-NF-κB (X3)-luc (as a NF-κB reporter plasmid), which contains 3 copies of NF-κB consensus binding sites upstream of a TATA element derived from apoA-I gene in a pGL3-basic construct (Promega, Madison, WI) (15). As controls, 10ng/well of pRL-null (Promega) plasmid were included to correct transfection and harvesting variations. After 16h, the cells were cultured in RPMI1640 with 5% charcoal-dextran-treated FBS (Hyclone, Logan, UT) in the presence of stimulants for additional 16h. The cells were then washed with PBS, lysed, and measured for firefly and Ranilla luciferase activities using dual-luciferase assays (Promega). Each treatment was performed at least in triplicate and the mean value of the luciferase activity was presented as fold increase relative to the control samples in the absence of stimulants.

*Statistical analysis*
All experiments were performed at least three times with results from representative experiments shown. Where applicable, the significance of the difference between test and control groups was analyzed using the Student's *t* test.

## Results

*Attenuation of $Ca^{2+}$ mobilization in phagocytes in response to chemoattractants by RV.*
The effect of RV on $Ca^{2+}$ mobilization as an indicator of the activation of many G protein-coupled chemoattractant receptors of phagocytic leukocytes was evaluated. Several chemoattractants that use different receptors were tested in the experiments. We tested the bacterial chemotactic peptide fMLF that binds the formylpeptide receptor FPR with high affinity (5), the synthetic peptide MMK-1 that is an agonist for the low affinity fMLF receptor, FPR-like 1 (FPRL1) (12), the chemokine IL-8 (CXCL8) that activates at least two receptors, CXCR1 and CXCR2, in neutrophils, and SDF-1α that uses CXCR4 (16). All chemoattractants at low nanomolar concentrations induced a robust but transient $Ca^{2+}$ mobilization in neutrophils (Figure 1). Interestingly, RV exhibited a biphasic effect on $Ca^{2+}$ mobilization induced by chemoattractants in neutrophils. At low micromolar concentrations (up to 20-50μM), neutrophils treated with RV consistently showed an increase in $Ca^{2+}$ flux in response to the chemoattractants. However, at concentrations equal to or greater than 100μM, RV exhibited a progressive inhibition on cell response to all chemoattractants. Very similar biphasic effects of RV were observed on monocytes stimulated with formylpeptide receptor agonists and chemokines (Figure 2). Nevertheless, monocyte response to the chemokine MCP-1, which mainly activates the receptor CCR2, was relatively resistant to high concentrations of RV as compared to other chemoattractants including the chemokine MIP-1α, which is an agonist for CCR1 and CCR5 in human monocytes/ macrophages (16). These results suggest that RV at higher concentrations possesses inhibitory effects on $Ca^{2+}$ mobilization response to chemotactic factors that use diverse receptors. RV at high concentrations (up to 500μM) did not reduce the viability of human phagocytes over a 24h incubation period (Tao, data not shown), therefore the inhibitory effects on chemoattractant receptors in phagocytes were unlikely to be due to the toxic effect of RV.

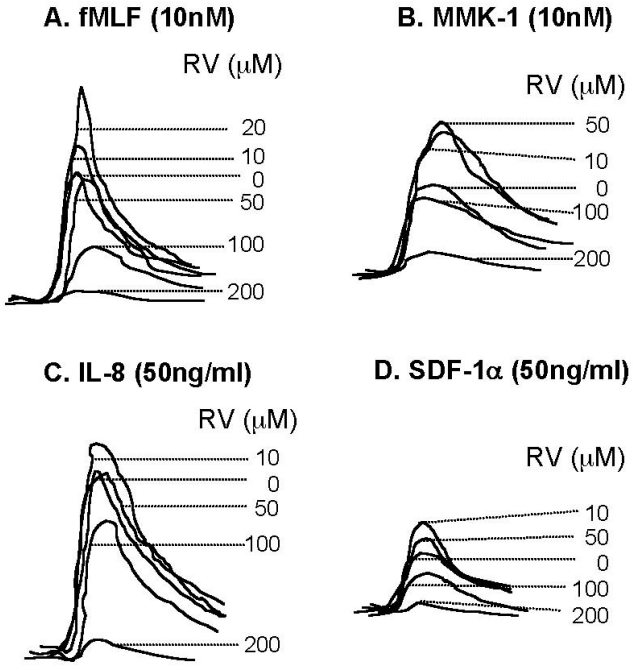


**Figure 1.** The effect of RV on chemoattractant-induced $Ca^{2+}$ flux in human neutrophils. Human neutrophils loaded with Fura-2 were treated with different concentrations of RV for 5 min before the cells were stimulated with fMLF (10nM, A), MMK-1 (10nM, B), IL-8 (50ng/ml, C), or SDF-1α (50ng/ml, D). The fluorescence was then monitored.

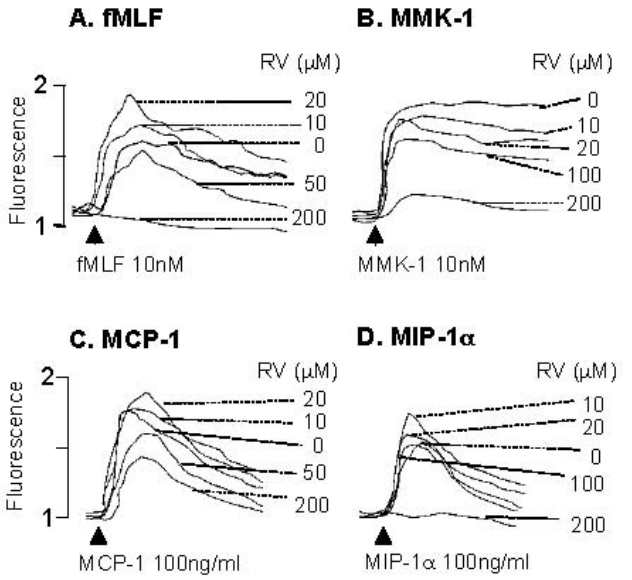


**Figure 2.** The effect of RV on chemoattractant-induced $Ca^{2+}$ flux in human monocytes. Human monocytes loaded with Fura-2 were treated with different concentrations of RV for 5 min then were stimulated with fMLF (10nM, A), MMK-1 (10nM, B), MCP-1 (100ng/ml, C) or MIP-1α (100ng/ml, D).

*RV selectively inhibits phagocyte migration mediated by formylpeptide receptors.*
Since phagocytic leukocytes constitute the first line host defense by rapidly infiltrating the sites of inflammation and infection, we next examined the effect of RV on phagocyte chemotaxis induced by a variety of chemoattractants. Preincubation with RV potently inhibited neutrophil chemotaxis induced by fMLF (Figure 3A) and the chemotaxis of RBL cells transfected with the high affinity fMLF receptor FPR (Figure 3B). RV also significantly reduced neutrophil chemotactic response to the FPRL1 agonist peptide MMK-1 (Figure 3C). Dose-response experiments revealed that significantly lower concentrations of RV were required to cause the reduction of neutrophil chemotaxis induced by fMLF rather than by MMK-1, suggesting that FPR in neutrophils is more sensitive to the effect of RV than FPRL1. On the other hand, RV failed to show any inhibition on neutrophil chemotaxis induced by the chemokine IL-8 (CXCL8) (Figure 3D). In monocytes,

RV similarly showed preferential inhibition of cell migration induced by fMLF and MMK-1 but not by chemokines MCP-1 and MIP-1α. In addition, monocytes preexposed to RV significantly reduced their migration in response to $A\beta_{42}$ (Tao, data not shown), a peptide associated with the lesions in AD and a chemotactic agonist for the receptor FPRL1 (17). Thus, RV appears to selectively interfere with the capacity of formylpeptide receptors in phagocytic leukocytes to mediate cell migration induced by their agonists.

*RV inhibits SOA production by phagocytes stimulated with formylpeptide receptor agonists.*

The production of SOA is a hallmark of phagocyte activation in inflammation and bacterial infection. While SOA is considered to be essential for the killing and elimination of invading microorganisms by the host, its over production also may cause undesirable inflammatory responses and tissue damage. We next examined the capacity of RV to reduce SOA production by activated phagocytes. Human neutrophils and monocytes failed to produce measurable SOA after activation with a wide concentration range of chemokines IL-8 (CXCL8) or MCP-1 (CCL2) that use specific receptors on either cell types (Tao, data not shown). In contrast, both neutrophils and monocytes rapidly released SOA in response to stimulation by the bacterial peptide fMLF (Figure 4A and 4B) and the effect of fMLF was abrogated by a brief pre-exposure of the cells to RV. Compared with its effect on formylpeptide receptor mediated cell migration, the concentrations of RV required to inhibit SOA release by fMLF-activated phagocytes were significantly lower, suggesting RV is markedly more efficient in inhibiting formylpeptide receptor mediated SOA responses in phagocytes. Interestingly, RV also potently inhibited SOA release by $A\beta_{42}$-activated monocytes (Figure 4C), a cell response attributable to the activation of the receptor FPRL1 (18). These results indicate a potentially beneficial role of RV in preventing superoxide mediated tissue damage during inflammation and bacterial infection in which agonists for both FPR and FPRL1 are elevated (5).

*RV reduces ERK phosphorylation and NF-κB activation induced by formylpeptide receptor agonists.*

Having observed that RV preferentially inhibited selected functions of the formylpeptide receptors in phagocytic

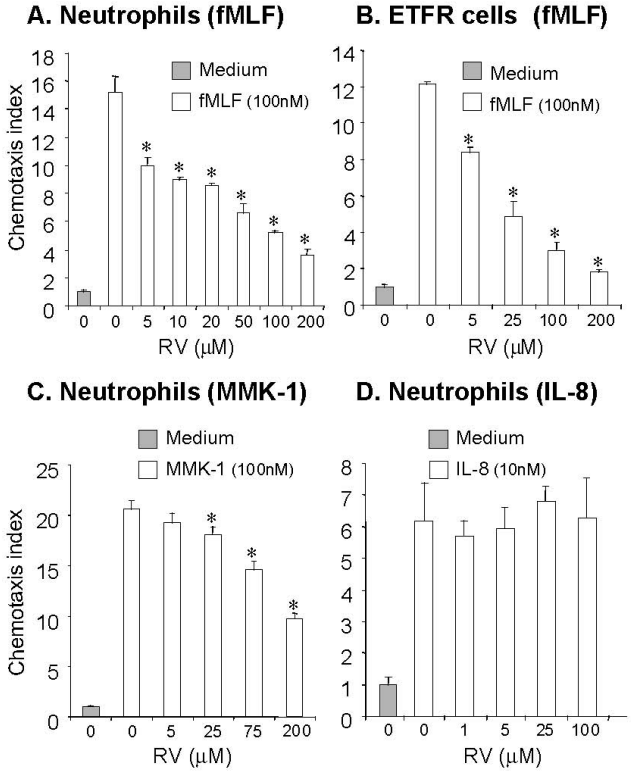


**Figure 3.** The effect of RV on cell chemotaxis. Human neutrophils or RBL cells transfected with FPR (ETFR) were preincubated with different concentrations of RV for 5 min then were measured for cell migration to different chemoattractants. (A) Neutrophil migration to 100nM fMLF; (B) ETFR cell migration induced by fMLF; (C) Neutrophil migration in response to MMK-1; (D) Neutrophil migration induced by IL-8. * indicates significantly reduced migration of cells treated with RV compared with cells treated with medium alone in response to chemoattractants.

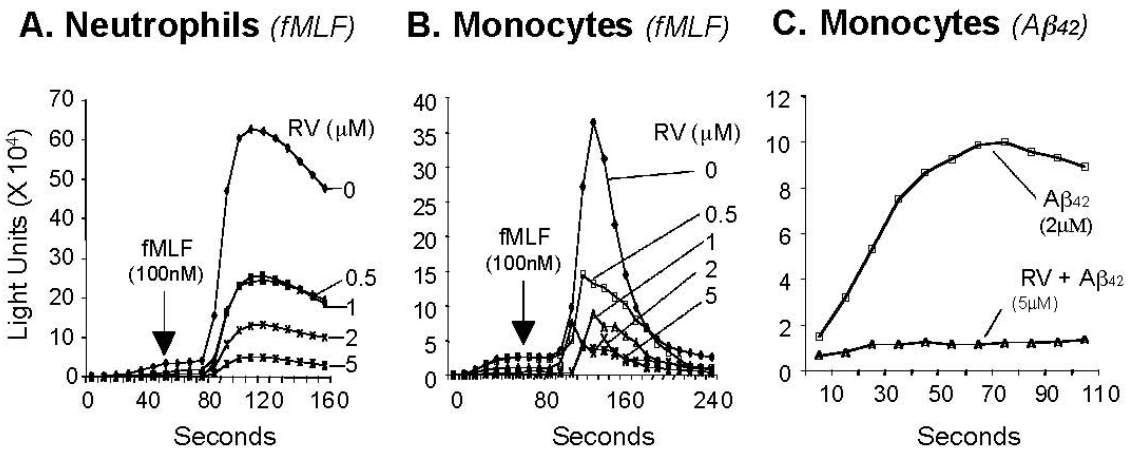


**Figure 4.** The effect of RV on superoxide anion production by phagocytes in response to chemoattractants. The cells were pretreated with RV for 5 min then were measured for their response to chemoattractants by using chemiluminescence indicator Diogene. (A) Neutrophil response induced by fMLF; (B) Monocyte response to fMLF; (C) Monocyte SOA release induced by $A\beta_{42}$.

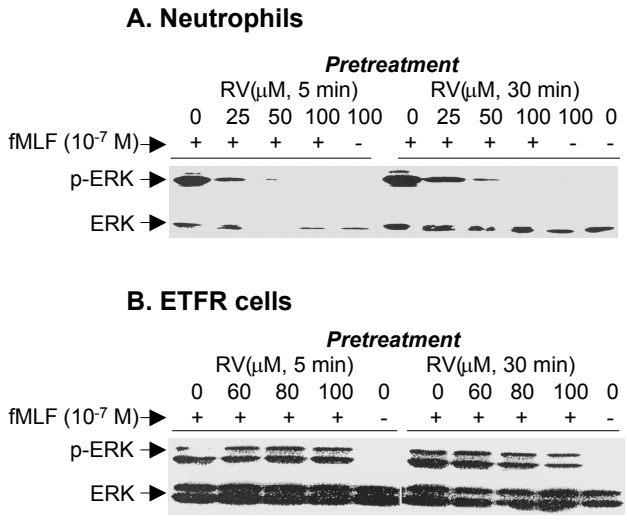


**Figure 5.** The effect of RV on ERK phosphorylation induced by fMLF. Human neutrophils (A) or ETFR cells (B) were pretreated with different concentrations of RV for 5 min or 30 min. The cells were then lysed and measured for ERK1/2 phosphorylation by Western blotting using an anti-phospho-ERK1/2 antibody. Total ERK1/2 was examined as a loading control.

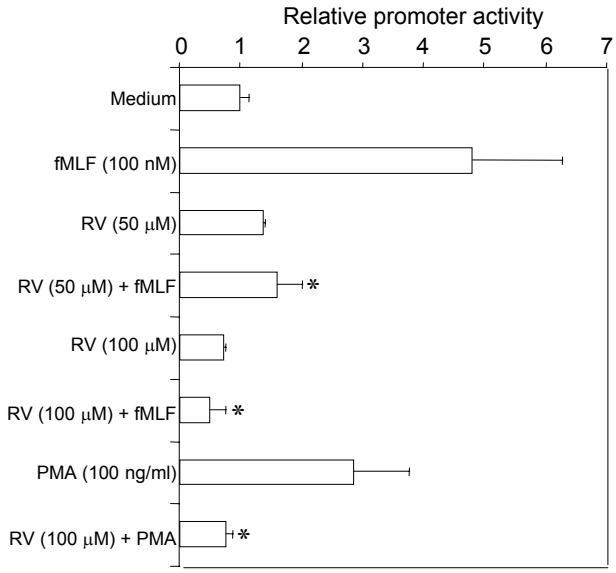


**Figure 6.** Inhibition of fMLF-activated NFκB by RV. ETFR cells were transfected with an NF-κB reporter plasmid. After preincubation with RV for 5 min, the cells were stimulated with fMLF and the luciferase activity was measured 16h later. PMA was used as a control. * indicates significantly reduced reporter activity measured in RV treated cells as compared with cells treated with stimulants alone. RV by itself does not induce the luciferase activity at any concentration tested.

leukocytes, we then explored the potential mechanisms by which RV exerted its effects, by examining the capacity of RV to regulate formylpeptide receptor mediated phosphorylation of ERK, which is crucial for a number of cell functions. In freshly isolated human neutrophils, RV treatment for 30 min significantly reduced the levels of ERK phosphorylation induced by fMLF. In rat basophil leukemia cells transfected with FPR (ETFR cells), preincubation with RV for 30 min also significantly reduced the level of ERK phosphorylation induced by fMLF (Figure 5A and 5B). RV by itself has no significant effect on ERK phosphorylation in either ETFR cells or neutrophils (Figure 5A, and data not shown). In addition to inhibiting FPR agonist stimulated ERK phosphorylation, RV also significantly reduced the level of ERK phosphorylation in monocytes induced by two FPRL1 agonists, the synthetic peptide MMK-1 and $A\beta_{42}$ (Tao, data not shown). These results suggest that RV is capable of interrupting formylpeptide receptor signaling cascade in primary cells and receptor transfected cells, leading to the impairment of agonist-induced cell activation. This conclusion was further supported by the capacity of RV to potently inhibit the formylpeptide receptor agonist-induced activation of NF-κB, a major transcription factor that regulates the expression of a number of genes. RV also reduced NF-κB activation by the phorbol ester PMA, a protein kinase C activator, suggesting that RV possesses broad regulatory effects on cell activation by multiple stimulants (Figure 6).

## Discussion

In this study, we demonstrated that RV at high micromolar concentrations attenuated phagocyte $Ca^{2+}$ mobilization induced by diverse chemoattractants, including chemokines and agonists for the classical formylpeptide receptors. However, at low micromolar concentrations, this grape component preferentially inhibited the chemotactic response of phagocytes to formylpeptide receptor agonists. In addition, RV potently abrogated the production of SOA by phagocytes triggered by the bacterial chemotactic peptide fMLF and AD-associated peptide $A\beta_{42}$, two agonists that activate the formylpeptide receptors FPR and its variant FPRL1, respectively (5). Our results thus suggest a novel function of RV and extend its potential as an anti-inflammatory agent by targeting G protein-coupled chemoattractant receptors.

The reason why RV selectively inhibits leukocyte chemotaxis induced by formylpeptide receptor agonists, but not chemokines, is unclear. RV did not down-regulate formylpeptide receptors from cell surface suggesting that this grape component is unlikely to compete with formylpeptide agonists for binding to the receptors. The expression of formylpeptide receptor genes in phagocytes was also not affected by overnight incubation of the cells with RV (data not shown). Thus RV may selectively block the signal transduction pathways that are essential for formylpeptide receptors to mediate phagocyte chemotaxis and SOA release. In fact, RV reduced formylpeptide receptor agonist induced phosphorylation of ERK1/2, a key molecule in the signaling cascade of the formylpeptide receptors and is required for directional cell migration (3). It is interesting to note that at low micromolar concentrations RV appears to potentiate $Ca^{2+}$ flux of phagocytic cells induced by various chemoattractants, suggesting that RV, which is considered as a general inhibitor, may induce active intracellular signaling events that are responsible for its broad biological effects. This notion was supported by

reports that RV may act as a low affinity ligand for an estrogen receptor in human epithelial cancer cells (19). However, our extensive experiments with phagocytes did not confirm the involvement of estrogen receptors in mediating the inhibitory effect of RV on formylpeptide receptor function (data not shown). Thus, the identity of membrane or intracellular molecules that serve as RV binding sites in phagocytic cells remains to be determined.

A number of biological effects have been reported for RV (20-25). At doses achievable by topical application, RV inhibits the growth of dermatophytes and fungal infection of skin (20). The growth of several clinically important bacterial strains, including *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Helicobacter pylori* are also susceptible to suppression by RV (21, 22). Interestingly, RV interferes with the release of inflammatory mediators by activated neutrophils and reduces the chemotaxis of rabbit neutrophils in response to the bacterial peptide fMLF (26). Studies of the mechanisms for RV action revealed that RV inhibited thrombin-induced $Ca^{2+}$ influx in human platelets (27, 28) possibly by directly blocking calcium channels on cell membrane. RV also down-regulates cyclooxygenase-2 (COX-2) pathway and the activity of nitric oxide synthase by suppression of NF-κB, a key nuclear factor that controls the transcription of many genes coding for proteins involved in inflammation, immune responses and cell growth (25). It is noteworthy that RV may activate different cellular components depending on the cell types. For instance, RV was able to induce phosphorylation of ERK1/2 in A375, but not in SK-mel28 melanoma cells lines (29), while both cell lines underwent apoptosis after treatment with RV. In vascular smooth muscle cells (30), RV diminished the cellular hypertrophy induced by angiotensin II, possibly by its interference with PI3K/Akt, p70 (S6K) and, to a lesser extent, ERK1/2 signaling pathway. The capacity of RV to inhibit ERK1/2 phosphorylation and NF-κB activation mediated by formylpeptide receptors in phagocytes shown in the present study suggests a broader and more complex pattern of the regulatory role of RV.

Our results showing RV to preferentially inhibit formylpeptide receptor mediated phagocytic chemotaxis and release of SOA may have important implications. FPR was defined as a high affinity receptor for the bacterial N-formylpeptide fMLF. The FPR variant, FPRL1, interacts with fMLF with low affinity. The primary function of these receptors has been suggested to promote the recruitment of innate immune cells to the sites of bacterial infection and tissue damage, where chemotactic formylpeptides are generated (3). However, accumulating evidence suggests that these receptors may act in a more complex manner. A number of novel, non-formylated peptide ligands for FPR and to a greater extent for FPRL1, have been identified (3). These new agonists include small peptides derived from peptide libraries (12, 31), peptide domains derived from HIV-1 envelope proteins (32, 33), and at least five host-derived molecules (17, 34-37), including $A\beta_{42}$. $A\beta_{42}$ is over produced in the brain tissues of AD and is a potent mediator of proinflammatory responses. The interaction of FPRL1 with $A\beta_{42}$ promotes the accumulation of monocytic phagocytes in the lesions of senile plaques in AD (17), and increases the cell production of SOA (18), one of the major neurotoxic mediators associated with AD (38). It is therefore plausible that RV may form a basis for the development of new therapeutic agents for AD to circumvent the undesirable consequences of FPRL1 activation by $A\beta_{42}$. As compared with some classical non-steroidal anti-inflammatory drugs that have shown beneficial effects by retarding the onset of AD (38), drugs based on RV may prove safer and with additional benefits for functions of other vital systems.

## Acknowledgement

The authors thank Dr. Joost J. Oppenheim for reviewing the manuscript, Ms. Nancy Dunlop for technical support and Ms. Cheryl Fogle and Ms. Cheryl Nolan for secretarial assistance. HT was also supported in part with Joint Key Research Project Funds from Second Military Medical University, Shanghai, People's Republic of China, under contract No. LH 2003-01. This project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract No. NO1-CO-12400.

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the USA Government. The publisher or recipient acknowledges right of the USA Government to retain a nonexclusive, royalty-free license in and to any copyright covering the article.

## References

1. Prossnitz ER, Ye RD. The N-formyl peptide receptor: a model for the study of chemoattractant receptor structure and function. Pharmacol Ther. 1997;74:73-102.
2. Murphy PM. The N-formyl peptide chemotactic receptors. In: Horuk R, eds. Chemoattractant ligands and their receptors. New York: CRC Press; 1996:269-299.
3. Le Y, Oppenheim JJ, Wang JM. Pleiotropic roles of formyl peptide receptors. Cytokine Growth Factor Rev. 2001;12: 91-105.
4. Le Y, Yang Y, Cui Y, et al. Receptors for chemotactic formyl peptides as pharmacological targets. Int Immunopharmacol. 2002;2:1-13.
5. Le Y, Murphy PM, Wang JM. Formyl-peptide receptors revisited. Trends Immunol. 2002;23:541-548.
6. Fremont L. Biological effects of resveratrol. Life Sciences. 2000;66:663-673.
7. Falchetti R, Fuggetta MP, Lanzilli G, Tricarico M, Ravagnan G. Effects of Resveratrol on human immune cell function. Life Sciences. 2001;70:81-96.
8. Brownson DM, Azios NG, Fuqua BK, Dharmawardhane SF, Mabry TJ. Flavonoid effects relevant to cancer. J Nutr. 2002; 132:3482-3489.
9. Manna SK, Mukhopadhyay A, Aggarwal BB. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-κB, activator protein-1, and apoptosis: potential role of reactive oxygen intermediates and lipid peroxidation. J Immunol. 2000;164:6509-6519.
10. Yu R, Hebbar V, Kim DW, Mandlekar S, Pezzuto JM, Kong AT. Resveratrol inhibits phorbol ester and UV-induced activator protein 1 activation by interfering with mitogen-activated protein kinase pathways. Mol Pharmacol. 2001;60: 217-224.
11. El-Mowafy AM, White RE. Resveratrol inhibits MAPK activity and nuclear translocation in coronary artery smooth

muscle: reversal of endothelin-1 stimulatory effects. FEBS Letters. 1999;451:63-67.
12. Hu JY, Le Y, Gong W, et al. Synthetic peptide MMK-1 is a highly specific chemotactic agonist for leukocyte FPRL1. J Leukoc Biol. 2001;70:155-161.
13. Klein C, Paul JI, Sauve K, et al. Identification of surrogate agonists for the human FPRL-1 receptor by autocrine selection in yeast. Nat Biotechnol. 1998;16:1334-1337.
14. Su SB, Gao J, Gong W, et al. T21/DP107, a synthetic leucine zipper-like domain of the HIV-1 envelope gp41, attracts and activates human phagocytes by using G-protein-coupled formyl peptide receptors. J Immunol. 1999;162:5924-5930.
15. Zhang X, Jiao JJ, Bhavnani BR, Tam SP. Regulation of human apolipoprotein A-I gene expression by equine estrogens. J Lipid Res. 2001;42:1789-1800.
16. Le Y, Zhou Y, Iribarren P, Wang JM. Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. Cell Mol Immunol. 2004;1:81-90.
17. Le Y, Gong W, Tiffany HL, et al. Amyloid $\beta_{42}$ activates a G-protein-coupled chemoattractant receptor, FPR-like-1. J Neurosci. 2001;21:RC123:1-5.
18. Tiffany HL, Lavigne MC, Cui YH, et al. Amyloid-β induces chemotaxis and oxidant stress by acting at formylpeptide receptor 2, a G protein-coupled receptor expressed in phagocytes and brain. J Biol Chem. 2001;276:23645-23652.
19. Gehm BD, McAndrews JM, Chien PY, Jameson JL. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. Proc Natl Acad Sci U S A. 1997;94:14138-14143.
20. Chan MM. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochem Pharmacol. 2002;63:99-104.
21. Docherty JJ, Fu MM, Tsai M. Resveratrol selectively inhibits Neisseria gonorrhoeae and Neisseria meningitidis. J Antimicrob Chemother. 2001;47:243-244.
22. Mahady GB, Pendland SL. Resveratrol inhibits the growth of helicobacter pylori *in vitro*. Am J Gastroenterol 2000;95:1849.
23. Holian O, Walter RJ. Resveratrol inhibits the proliferation of normal human keratinocytes in vitro. J Cell Biochem. 2001; Suppl 36:55-62.
24. Rotohdo S, Rajtar G, Manarini S, et al. Effect of trans-resveratrol, a natural polyphenolic compound, on human polymorphonuclear leukocyte function. Br J Pharmacol. 1998; 123:1691-1699.
25. Surh YJ, Chun KS, Cha HH, et al. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-κB activation. Mutat Res. 2001;480-481:243-268.
26. Zhong M, Guo Y, Deng J, Wang W, Cheng G. Effects of isorhapotigenin and resveratrol on function of peripheral blood polymorphonuclear leukocytes from rabbits. Yao Xue Xue Bao. 1998;33:812-815.
27. Dobrydneva Y, Williams RL, Blackmore PF. Trans-resveratrol inhibits calcium influx in thrombin-stimulated human platelets. Br J Pharmacol. 1999;128:149-157.
28. Dobrydneva Y, Williams RL, Morris GZ, Blackmore PF. Dietary phytoestrogens and their synthetic structural analogues as calcium channel blockers in human platelets. J Cardiovasc Pharmacol. 2002;40:399-410.
29. Niles RM, McFarland M, Weimer MB, Redkar A, Fu YM, Meadows GG. Resveratrol is a potent inducer of apoptosis in human melanoma cells. Cancer Lett. 2003;190:157-163.
30. Haider UGB, Sorescu D, Griendling KK, Vollmar AM, Dirsch VM. Resveratrol suppresses angiotensin II-induced Akt/protein kinase B and p70 S6 kinase phosphorylation and subsequent hypertrophy in rat aortic smooth muscle cells. Mol Pharmacol. 2002;62:772-777.
31. Le Y, Gong W, Li B, et al. Utilization of two seven- transmembrane, G protein-coupled receptors, formyl peptide receptor-like 1 and formyl peptide receptor, by the synthetic hexapeptide WKYMVm for human phagocyte activation. J Immunol. 1999;163:6777-6784.
32. Su SB, Gong WH, Gao JL, et al. T20/DP178, an ectodomain peptide of human immunodeficiency virus type 1 gp41, is an activator of human phagocyte N-formyl peptide receptor. Blood. 1999;93:3885-3892.
33. Deng X, Ueda H, Su SB, et al. A synthetic peptide derived from human immunodeficiency virus type 1 gp120 downregulates the expression and function of chemokine receptors CCR5 and CXCR4 in monocytes by activating the 7-transmembrane G-protein-coupled receptor FPRL1/LXA4R. Blood. 1999;94:1165-1173.
34. Su SB, Gong W, Gao JL, et al. A seven-transmembrane, G-protein-coupled receptor, FPRL1, mediates the chemotactic activity of serum amyloid A for human phagocytic cells. J Exp Med. 1999;189:395-402.
35. Yang D, Chen Q, Schmidt AP, et al. LL-37, the neutrophil granule-and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J Exp Med. 2000;192:1069–1074.
36. Resnati M, Pallavicini I, Wang JM, et al. The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. Proc Natl Acad Sci U S A. 2002;99:1359-1364.
37. Le Y, Yazawa H, Gong W, et al. The neurotoxic prion peptide fragment PrP (106-126) is a chemotactic agonist for the G protein-coupled receptor formyl peptide receptor-like 1. J Immunol 2001;166:1448-1451.
38. Cui Y, Le Y, Yazawa H, Gong W, Wang JM. Potential role of the formyl peptide receptor-like 1 (FPRL1) in inflammatory aspects of Alzheimer's disease. J Leukoc Biol. 2002;72: 628-635.