行政院國家科學委員會專題研究計畫 成果報告

丁香醇和異丁香醇衍生物之體外細胞模式及體內內毒素模式之抗發炎活性評估與訊息傳遞路徑之探討
研究成果報告(精簡版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 98-2320-B-041-001-
執 行 期 間 ：98 年 08 月 01 日至 99 年 07 月 31 日
執 行 單 位 ：嘉南藥理科技大學藥學系

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報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 99 年 08 月 16 日

行政院國家科學委員會補助專題研究計畫■成果報告 □期中進度報告

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丁香醇和異丁香醇衍生物之體外細胞模式及體內內毒素模式之抗發炎活性評估與訊息傳遞路徑之探討

Anti-inflammatory activity and signal transduction pathways of eugenol and isoeugenol derivatives in lipopolysaccharide-stimulated RAW 264.7 macrophages and in vivo endotoxemic model

計畫編號：NSC 98－2320－B－041－001
執行期限：98 年 08 月 01 日至 99 年 07 月 31 日
計畫主持人：劉 淑 芬　　嘉南藥理科技大學藥學系
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一、中文摘要

本研究實驗的藥物是 4 種不同的 eugenol 和 isoeugenol 衍生物，這些衍生物已被本實驗室合成並研究。許多研究顯示 isoeugenol 和 eugenol 展現了抗發炎的作用，然而這些衍生物的抗發炎活性仍未被探討，因此本研究的目的為評估這些衍生物的抗發炎功效並進而探討其機轉。在實驗中，我們發現 eugenolol 能夠有效的抑制脂多醣體(LPS)刺激的老鼠巨噬細胞株中環氧化酶、誘導型一氧化氮合成酶表現和一氧化氮、腫瘤壞死因子-α、介白素-1 釋放量，且作用比 eugenol 更為顯著。我們也發現了 eugenolol 能藉著阻斷 Akt、inhibitor κB (IκB)α 、 extracellular signal-regulated kinase 1/2 (ERK1/2) 磷酸化來減少 LPS 誘導的 nuclear factor-κB 次單元 p65 的核轉移以及 NF-κB 的 DNA 結合活性，作用比 eugenol 更強。而在 isoeugenol 衍生物方面，我們發現 glyceryl-isoeugenol 的抑制 COX-2、iNOS、TNFα 以及 NO 表現活性是所有 isoeugenol 衍生物中最強的，而此作用主要是藉著阻斷 MAPK、Akt、IκBα 磷酸化以及接續的 p65 核轉移和 NF-κB 的 DNA 結合活性。除此之外，我們也探討了其他轉錄因子，像 AP-1、CREB、HIF 是否參與衍生物的抗發炎作用。我們發現 eugenolol 和 glyceryl-isoeugenol 能夠抑制 LPS 誘導的 HIF-1α 表現和 AP-1-DNA 結合活性。總結來說，eugenolol 和 glyceryl-isoeugenol 能夠藉著抑制 MAPKs 及 Akt/IκBα 磷酸化來阻斷下游的 NF-κB、AP-1 DNA 結合能力和 HIF-1α 蛋白表現，進而達到抗發炎的效果。

Abstract

Eugenol and isoeugenol, two components of clover oil, have been reported to possess several biomedical properties, such as anti-inflammatory, antimicrobial and antioxidant effects. A great deal of attention has been paid recently to improve the functional properties of eugenol and isoeugenol for different pharmacological applications. This study was aimed to examine anti-inflammatory effects of eugenol, isoeugenol and four related derivatives on lipopolysaccharide (LPS)-induced expression of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in mouse macrophages (RAW 264.7), and to investigate molecular mechanisms underlying these effects. We found that COX-2 expression was not affected by these agents. However, two derivatives, eugenolol and glyceryl-isoeugenol, had potent inhibitory effects on LPS-induced upregulation of nitrite levels, iNOS protein and iNOS mRNA. In addition, they both suppressed the release of tumor necrosis factor-α (TNF-α) and

interleukin-1β (IL-1β) induced by LPS. Moreover, they both attenuated the LPS-induced DNA binding activity of NF-κB and LPS-induced phosphorylation of inhibitory κBα (IκBα), resulting enhanced nuclear translocation of p65 protein. Furthermore, these two derivatives inhibited the LPS-induced HIF-1α expression and AP-1 binding activity. Finally, we demonstrated that glyceryl-isoeugenol suppressed the phosphorylation of ERK1/2, JNK and p38, whereas eugenolol suppressed the phosphorylation of ERK1/2. Taken together, these results suggest that that eugenolol and glyceryl-isoeugenol suppress LPS-induced iNOS expression by down-regulating NF-κB, HIF-1α and AP-1 through inhibition of MAPKs and Akt/IκBα signaling pathways. Thus, this study implies that eugenolol and glyceryl-isoeugenol may provide therapeutic benefits for inflammatory diseases.

Keywords: inflammation, derivatives, lipopolysaccharide, mitogen-activated protein kinases, transcription factor

Introduction

Pharmacological options for the treatment of chronic inflammatory diseases are often associated with severe side effects due to prolonged duration of treatment. Therefore, developing compounds that are less toxic yet equally efficacious would have important clinical implications. *o*-Methoxyphenol compounds such as eugenol and isoeugenol are components of clover oil, and are commonly used in cosmetic, food industry, dentistry, and traditional medicine for its pharmacological properties of antifungicidal, antibacterial (Li et al., 2006), anti-oxidant (Ko et al., 1995; Ito et al., 2005) and anti-inflammatory properties (Choi et al., 2007). Thus, there is growing research interest in the synthesis of new anti-inflammatory compounds derived from eugenol and isoeugenol. Previous studies have shown that several isoeugenol-derived compounds, including dehydrodiisoeugenol, demethyldiisoeugenol, and α-diisoeugenol, have valuable cytoprotective and anti-inflammatory effects (Murakami et al., 2005; Atsumi et al., 2005; Ko et al., 1995; Lin et al., 1994). Our previous studies have also demonstrated that some of these derivatives developed by our laboratory possess pleiotropic effects including vasodilatory, antioxidant, tracheal relaxant, and α- or β-adrenoceptor blockade properties (Chen et al., 1997; Lin et al., 1999; Yeh et al., 2000). However, their anti-inflammatory effects have not been examined yet.

In this study, we used the mouse macrophage (RAW 246.7 cells) treated by lipopolysaccharide (LPS) as a model of inflammatory response. The anti-inflammatory effects of eugenol, isoeugenol and four associated derivatives (Fig. 1A) were examined by investigating their effects on production of inflammatory mediators (NO, TNF-α, IL-1β), protein expression of regulatory enzymes (iNOS and COX-2), regulation of transcriptional factors (NF-κB, CREB and AP-1), and related signal transduction (Akt and MAPKs).

Methods

Cell culture

Macrophage RAW 264.7 cell line was purchased from the Bioresource Collection and Research Center in Taiwan. Cells were maintained in DMEM medium supplemented with 10% FBS, 2mM glutamine, 100 U/ml of penicillin G, 100 μg/ml streptomycin and 0.25 mg/ml amphotericin B at 37°C and 5% CO_2. All studies were performed with exponentially growing cells. Cells were seeded in cell culture plates or dishes. After 24 h, cells were treated with various concentrations of derivatives and incubated for the indicated times. Cells were scraped out and centrifuged at 13,000 rpm for 30 min at 4 °C.

Cell viability assay

Cell viability was assessed by the MTT assay. The assay was performed according to the manufacturer's instruction. After experiments, MTT (0.5 mg/ml) was added in the medium for 4 h. Then the culture medium was removed, and cells were dissolved in isopropanol and shaken for 10 min. The

amount of MTT formazan was quantified by determining the absorbance at 540 nm and 630 nm, using an ELISA reader (DYNEX Technologies, Germany). The viability was calculated as follows:

Viability (%) = $(OD_{540,\ sample}-OD_{630,\ blank})$ / $(OD_{540,\ control}-OD_{630,\ blank})\times 100$

Measurement of NO

The accumulation of nitrite, a stable oxidized product of NO, was determined in the cell culture media by Griess reagent (1% sulfanilamide and 0.1% *N*-(1-naphthyl) ethylenediamide in 5% phosphoric acid). The culture supernatants were collected and mixed with an equal volume of Griess reagent and incubated at room temperature for 10 min. The absorbance was measured at 540 nm (OD_{540}).

Measurement of TNF-α and IL-1β

RAW 264.7 cells (10^5 /ml) were seeded in 24 well plate. The cells were pretreated with various concentrations of eugenol and isoeugenol derivatives, and then were stimulated with 100 ng/ml LPS. After 24 hr LPS stimulation, the cell suspensions were collected, and the concentrations of TNF-α and IL-1β were measured using enzyme-linked immunosorbent assay (ELISA) kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's protocol.

Reverse-transcriptase polymerase chain reaction (RT-PCR)

Total RNA was extracted with Trizol reagent by a standard protocol (Invitrogen, Carlsbad, Calif). Reverse transcription was performed with 5 μg mRNA and random primer at 65°C for 5 min, then mixed with Moloney murine leukemia virus reverse transcriptase (RT) to react at 37°C for 1 h to obtain cDNA. Gene amplification was followed with RT-polymerase chain reaction (PCR). Primer sequence was as described: iNOS sense, 5'-CAGGAACCTACCAGCTCACTCT-3'; iNOS antisense, 5'-ACAAGATCAGGAGGGATTTCAA-3'; COX-2 sense, 5'-TGTATGAGCACAGGATTTGACC-3'; COX-2 antisense, 5'-TGAAAAACTGATGGGTGAAGTG-3'; GAPDH sense, 5'-AACTTTGGCATTGTGGAAGG-3'; and GAPDH antisense, 5'-CCCTGTTGCTGTAGCCGTAT-3'. Reaction cycles for iNOS, COX-2, and GAPDH includes 30 cycles of 94°C for 30 s, 61.4°C for 30 s, 72°C for 1 min, and a final incubation at 72°C for 7 min. Polymerase chain reaction products were analyzed on 1.5% agarose gel in the presence of 1 μg/m/ of ethidium bromide.

Western blot analysis

Cells were treated with various concentrations of eugenol and isoeugenol derivatives for the indicated times. Reactions were terminated by washing twice with cold PBS and then cells were harvested. Total cell extracts were prepared in lysis buffer (20 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol (DTT), 5 mM EGTA, 2 mM EDTA, 0.5 mM PMSF, 20 μM leupeptin, and 20 μM aprotinin. The cell lysate was centrifuged at 13,000 rpm for 30 min, and the supernatant fraction was collected for Western blot. Equivalent amount of protein was resolved by SDS-polyacrylamide gel electrophoresis (PAGE) (10-14%) and transferred to polyvinylidene difluoride membranes. After blocking for 1 h in 5% non-fat dry milk in Tris-buffered saline, the membrane was incubated with the desired primary antibody for 2 h. The membrane was then treated with appropriate horseradish peroxidase conjugated secondary antibody (1:1000 dilution, Chemicon), and the immunoreactive bands were detected by chemiluminescence (ECL) reagents (PerkinElmer Life Sciences Inc. Waltham, MA, USA).

Electrophoretic Mobility Shift Assay, EMSA

The protein content of the nuclear extracts of RAW 264.7 cells was determined using a Bio-Rad Protein Assay kit according to the manufacturer's instructions (Bio-Rad, Hercules, CA). The sequences of the double-stranded oligonucleotides used to

detect the DNA-binding activities of NF-κB, AP-1 and CREB are as follows: NF-κB, 5'-GATCTCAGAGGGGACTTTCCGAGA GA-3'; AP-1, 5'-CGCTTGATGAGTCAGCCGGAA-3', and CREB, 5'-AGAGATTGCCTGACGTCAGAGAGC TAG-3'. The double-stranded oligonucleotides were end-labeled with 3'-biotin. EMSA was performed using LightShift® Chemiluminescent EMSA kit (Pierce Biotechnology, Rockford, IL, USA), and the procedure was according to the protocol of the manufacturer. Nuclear proteins (10 μg) were incubated with 1X binding buffer, 50 ng/μl poly (dI-dC), 2.50 % glycerol, 0.05 % NP-40, and 5 mM $MgCl_2$ on the ice for 20 min. 20 fmol biotin-labeled DNA was then added and the mixture was allowed to proceed for another 20 min. The reaction mixture was analyzed using 8% nondenaturing polyacrylamide gel. The protein-DNA-biotin complexes were blotted onto Nylon membrane (Millipore, USA) followed by UV cross-linking. The complexes were revealed with blocking buffer, horseradish peroxidase-conjugated streptavidin, substract equibilium buffer, and then exposed to the X-ray film.

Statistical analysis

The results are expressed as mean ± SE. Statistical differences were estimated by one-way analysis of variance (ANOVA) followed by Dunnett's test. A *p* value of 0.05 was considered significant. Analysis of the data and plotting of the figures were performed with the aid of software (SigmaPlot Version 8.0 and SigmaStat Version 2.03, Chicago, IL) urn on an IBM-compatible computer.

Results

Effects on viability of RAW 264.7 cells

Potential cytotoxic effects of eugenol, isoeugenol and four associated derivatives (eugenolol, isoeugenolol, glyceryl-isoeugenol, and isoeugenodilol) were determined by the changes of viability of RAW 264.7 cells using the MTT assay. As shown in Fig. 1, the cell viability was not affected by most of them except in extremely high concentrations of coglyceryl-isoeugenol (100 μM) or isoeugenodilol (50 and 100 μM).

Effects on NO production, and expression of iNOS protein, COX-2 protein and iNOS mRNA in RAW 264.7 cells

To compare effects of these derivatives on LPS-induced NO production in RAW 264.7 cells, culture media were harvested and nitrite level was measured. We first found that eugenolol and glyceryl-isoeugenol had more profound inhibitory effects on LPS-induced NO production than others, and exerted their effects in a concentration-dependent manner (Fig. 2). We further determined their effects on iNOS and COX-2 protein expression, and found that eugenolol and glyceryl-isoeugenol strongly inhibited their expression dose-dependently (Fig. 3). Moreover, RT-PCR analysis showed the effects of eugenolol and glyceryl-isoeugenol on the expression of iNOS mRNA were in parallel with their effects on iNOS protein expression (data not shown). However, all these derivatives had no effect on the expression of COX-2 mRNA (data not shown).

Effects on LPS-induced NF-κB activation in RAW 264.7 cells

Given the fact that NF-κB activation is critically required for the expression of iNOS and COX-2 by LPS, we used gel shift analysis to determine whether these derivatives changed NF-κB DNA binding activity. Pretreatment with eugenolol, isoeugenolol or glyceryl-isoeugenol (all in the concnetrations of 10 μM) for 1 h significantly attenuated LPS-induced NF-κB binding (Fig. 4). Since p65 was the major component of NF-κB activated by LPS in macrophages, we further determined the nuclear translocation of p65. As shown in Fig. 5, pretreatment with eugenolol (10 and 50 μM), isoeugenolol (50 μM) or glyceryl-isoeugenol (10 and 50 μM) for 1 h significantly decreased LPS-induced NF-κB protein expression in the nucleus. PARP, a constitutive nuclear protein, was used as control to confirm that there was no

contamination during extraction of each fraction.

Translocation of NF-κB to the nucleus is preceded by proteolytic degradation of IκBα subunit. To investigate whether these derivatives could directly affect IκBα, the level of IκBα protein was examined in RAW 264.7 cells incubated with or without these derivatives. We found that LPS reduced the IκBα level at 30 min, and both eugenolol and glyceryl-isoeugenol significantly preserved the level of IκBα protein (Fig. 6). Since phosphorylation of IκBα precedes degradation of IκBα, we further determined effects of these derivatives on IκBα phosphorylation and found that both eugenolol and glyceryl-isoeugenol inhibited LPS-inducible IκBα phosphorylation (Fig. 6). Taken together, we demonstrated that eugenolol and glyceryl-isoeugenol inhibited nuclear NF-κB binding through prevention of IκBα phosphorylation and subsequent nuclear translocation of p65 protein.

Effects phosphorylation of ERK, JNK, p38 MAP Kinase , and Akt in RAW 264.7 cells

MAPKs cascades have been implicated in the regulation of iNOS expression at the transcriptional level in LPS-stimulated macrophages. To investigate whether the inhibition of iNOS by these derivatives is mediated through modulation of MAPKs pathway, we further examined effects on phosphorylation of ERK, JNK, and p38 MAPK. We found that LPS induced phosphorylation of MAPKs, and these events were inhibited by pretreatment with glyceryl-isoeugenol (Fig. 7). In addition, eugenolol attenuated the LPS-induced phosphorylation of ERK in a concentration-dependent manner, but not affected phosphorylation of JNK and p38 MAPK. However, high concentration of eugenolol (50 μM) also significantly reduced the phosphorylation of p38 MAPK (Fig. 7).

Effects of HIF-1α expression in RAW 264.7 cells

Recent evidence suggest that pro-angiogenic transcription factor hypoxia-inducible factor (HIF)-1α has a role in the upregualtion of proinflammatory cytokines and these effects are mediated through MAPK- and PI3K-mediated pathways (Jung et al., 2003; Liu et al., 2008; Scortegagna et al., 2008). We finally investigated the role of HIF-1α activation in our study and found that eugenolol and glyceryl-isoeugenol inhibited the LPS induced HIF-1α activation in a concentration-dependent manner (Fig. 8).

Discussion

In this study, we demonstrated that eugenolol and glyceryl-isoeugenol potently inhibited the LPS-induced production of NO, TNF-α and IL-1β, with parallel suppressive effects on expression of iNOS protein and iNOS mRNA, suggesting that the inhibition of NO production by these two derivatives are mediated by down-regulation of iNOS expression. In addition, our findings suggest that these anti-inflammatory effects are mediated by blocking MAPKs and Akt/IκBα signaling pathways with associated deactivation of NF-κB, HIF-1α and AP-1. Thus, the present study provides for the first time the molecular evidence for the anti-inflammatory properties of eugenolol and glyceryl-isoeugenol.

Transcriptional factors NF-κB and AP-1 are both important regulators of LPS-mediated iNOS expression in macrophages with their binding sites identified on the murine iNOS promoter (Lee et al., 2003). In particular, NF-κB can regulate expressions of other proinflammatory enzymes and cytokines, including COX-2, TNF-α and IL-6 (Tsoyi et al., 2008). We therefore investigated the effects on transcriptional factors and found that eugenolol and glyceryl-isoeugenol inhibited DNA binding activity of NF-κB and AP-1. Since in the cytoplasm, NF-κB is bound to tightly by its inhibitory subunit, IκB, we further determined the effects of eugenolol and glyceryl-isoeugenol on the nuclear translocation of activated NF-κB, and the degradation and phosphorylation of IκB-α. Our findings indicate that mechanisms underlying inactivation of NF-κB by eugenolol and glyceryl-isoeugenol in

LPS-treated macrophages are associated with suppression of phosphorylation of IκB-α and nuclear translocations of the p65 subunit of NF-κB. Given that fact that Akt is an important upstream signaling pathway for activation of NF-κB, our study further showed that eugenolol and glyceryl-isoeugenol significantly decreased the phosphorylation of Akt, supporting the notion that that inactivation of Akt/IκB/NF-κB pathway mediates the anti-inflammatory actions of these two pharmacologic agents.

Hypoxia inducible factor 1 (HIF-1) is a heterodimeric protein composed of an HIF-1α subunit and a constitutively expressed HIF-1β subunit, and recent studies have indicated that HIF-1α plays an essential role in the regulation of inflammation. Indeed, it has been demonstrated that LPS increased HIF-1α mRNA expression by activating upstream ERK1/2 (Frede et al., 2006). Recently, it has been established that HIF-1 can induce NF-κB activation via phosphorylating IκB and enhancing nuclear translocation of p65 (Liu et al., 2010). In line with previous studies, we observed that LPS induced HIF-1α expression, and importantly, we found that HIF-1α expression was inhibited by eugenolol and glyceryl-isoeugenol (Fig. 11). However, whether these suppressive effects on HIF-1 contributed to the inhibition of IκB/NF-κB pathway were not clarified in the present study and may warrant further investigations.

In summary, we suggest that eugenolol and glyceryl-isoeugenol may suppress LPS-mediated iNOS expression and pro-inflammatory cytokines by blocking MAPKs and Akt signaling pathways with the deactivation of NF-κB, HIF-1α and AP-1 (Fig. 12). Our findings provide the first molecular basis for the anti-inflammatory properties of eugenolol and glyceryl-isoeugenol, and imply their potential therapeutic roles in the management of inflammatory diseases.

References

Li W, Tsubouchi R, Qiao S, Haneda M, Murakami K, Yoshino M. Inhibitory action of eugenol compounds on the production of nitric oxide in RAW264.7 macrophages. Biomed Res 2006;27:69-74.

Ko FN, Liao CH, Kuo YH, Lin YL. Antioxidant properties of demethyldiisoeugenol. Biochim Biophys Acta 1995;1258:145-52.

Ito M, Murakami K, Yoshino M. Antioxidant action of eugenol compounds: role of metal ion in the inhibition of lipid peroxidation. Food Chem Toxicol 2005;43:461-6.

Choi CY, Park KR, Lee JH, Jeon YJ, Liu KH, Oh S, Kim DE, Yea SS. Isoeugenol suppression of inducible nitric oxide synthase expression is mediated by down-regulation of NF-kappaB, ERK1/2, and p38 kinase. Eur J Pharmacol 2007;576:151-9.

Murakami Y, Shoji M, Hirata A, Tanaka S, Yokoe I, Fujisawa S. Dehydrodiisoeugenol, an isoeugenol dimer, inhibits lipopolysaccharide-stimulated nuclear factor kappa B activation and cyclooxygenase-2 expression in macrophages. Arch Biochem Biophys 2005;434:326-32.

Atsumi T, Murakami Y, Shibuya K, Tonosaki K, Fujisawa S. Induction of cytotoxicity and apoptosis and inhibition of cyclooxygenase-2 gene expression, by curcumin and its analog, alpha-diisoeugenol. Anticancer Res 2005; 25:4029-36.

Lin CH, Kuo YH, Lin YL, Teng CM. Inhibition of platelet thromboxane formation and phosphoinositides breakdown by diisoeugenol. J Pharm Pharmacol 1994;46:54-7.

Chen SJ, Huang YC, Wu BN, Chen IJ. Eugenolol: a eugenol-derived beta-adrenoceptor blocker with partial beta2-agonist and calcium mobilization inhibition associated vasorelaxant activities. Drug Develop Res 1997;40: 239-50.

Lin YT, Wu BN, Horng CF, Huang YC, Hong SJ, Lo YC, Cheng CJ, Chen IJ.

Isoeugenolol: a selective beta1-adrenergic antagonist with tracheal and vascular smooth muscle relaxant properties. Jpn J Pharmacol 1999;80:127-36.

Yeh JL, Yang TH, Liang JC, Huang YC, Lo YC, Wu JR, Lin YT, Chen IJ. Isoeugenodilol: a vasorelaxant alpha/beta-adrenoceptor blocker with antioxidant activity. Drug Develop Res 2000;51:29-42.

Lee AK, Sung SH, Kim YC, Kim SG. Inhibition of lipopolysaccharide-inducible nitric oxide synthase, TNF-alpha and COX-2 expression by sauchinone effects on I-kappaBalpha phosphorylation, C/EBP and AP-1 activation. Br J Pharmacol 2003;139:11-20.

Tsoyi K, Kim HJ, Shin JS, Kim DH, Cho HJ, Lee SS, Ahn SK, Yun-Choi HS, Lee JH, Seo HG, Chang KC. HO-1 and JAK-2/STAT-1 signals are involved in preferential inhibition of iNOS over COX-2 gene expression by newly synthesized tetrahydroisoquinoline alkaloid, CKD712, in cells activated with lipopolysacchride. Cell Signal 2008;20:1839-47.

Frede S, Stockmann C, Freitag P, Fandrey J. Bacterial lipopolysaccharide induces HIF-1 activation in human monocytes via p44/42 MAPK and NF-kappaB. Biochem J 2006;396:517-27.

Liu YN, Pan SL, Peng CY, Huang DY, Guh JH, Chen CC, Shen CC, Teng CM. Moscatilin repressed lipopolysaccharide-induced HIF-1alpha accumulation and NF-kappaB activation in murine RAW264.7 cells. Shock 2010;33:70-5.

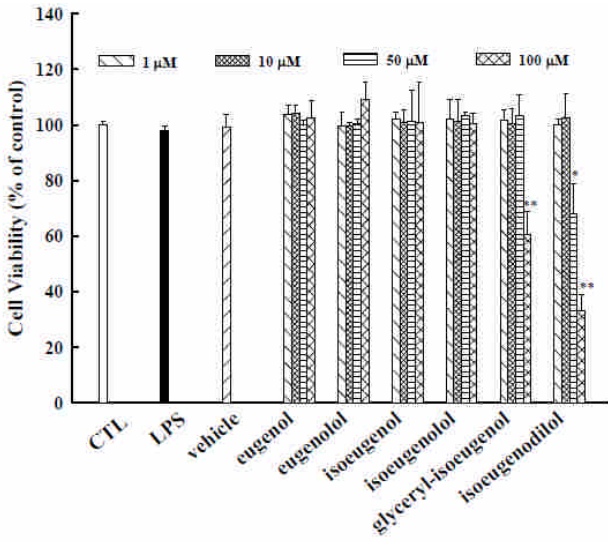


Figure 1. Cytotoxic effects of eugenol and isoeugenol derivatives in RAW 264.7 cells. Cell viability was performed by MTT assay. Cells were pretreated for 30 min with these derivatives followed by incubation with LPS (100 ng/ml) for 24 h. Each value represents the mean ± S.E.M. of three independent experiments, with triplicate determinations in each experiment. $^{**}p < 0.01$ compared with the control (CTL).

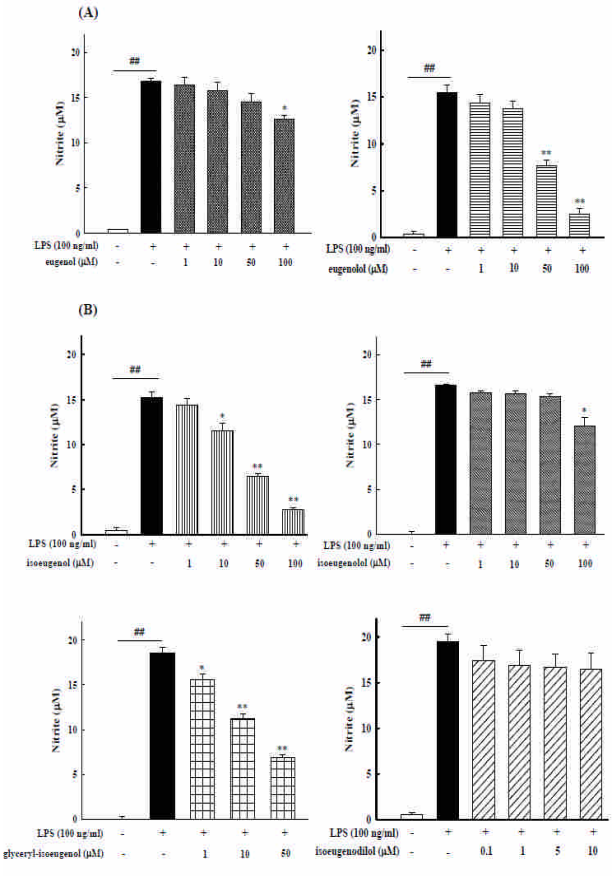


Figure 2. Effects on LPS-induced nitrite

production. Cells were pretreated with each derivative for 30 min followed by incubation with LPS (100 ng/ml) for 24 h. Each value represents the mean ± S.E.M. of three independent experiments, with triplicate determinations in each experiment. $^{\#\#}$ $p < 0.01$ compared with the control ; $^{*}p < 0.05$, ** $p < 0.01$ compared with LPS.

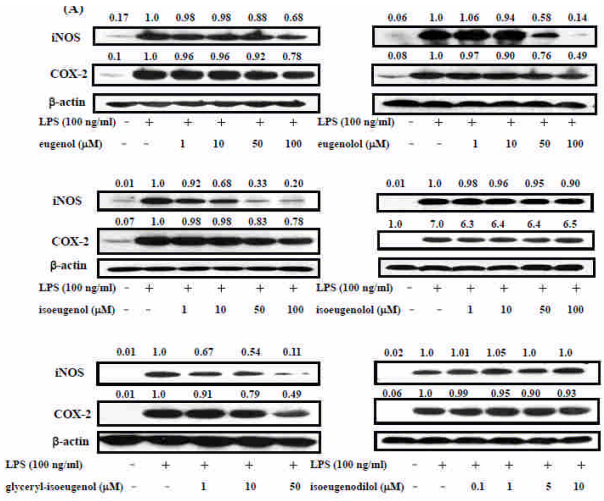


Figure3. Effects on the protein level of iNOS and COX-2 induced by LPS. These experiments were repeated for three times with similar results obtained.

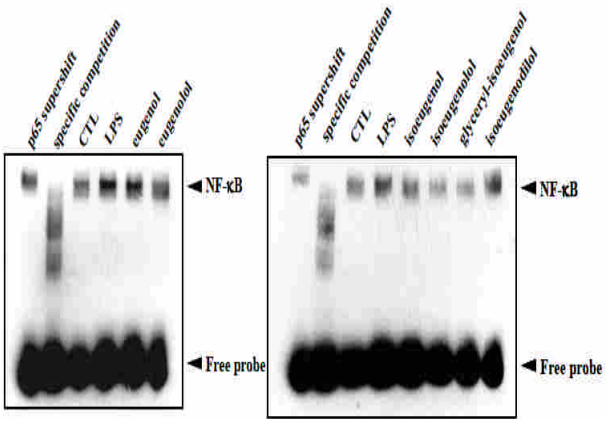


Figure 4. Effects on NF-κB DNA binding activity in LPS-induced RAW 264.7 cells. The results are representative of three different experiments.

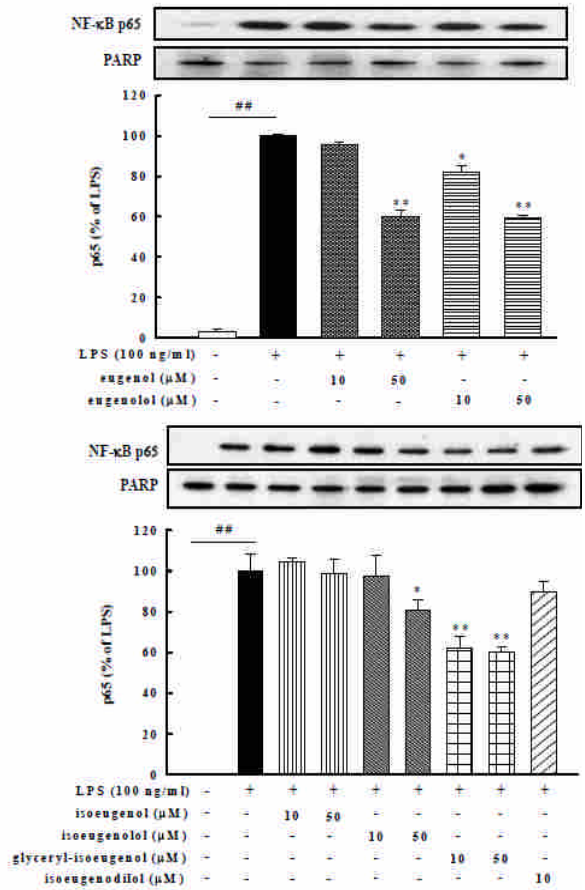
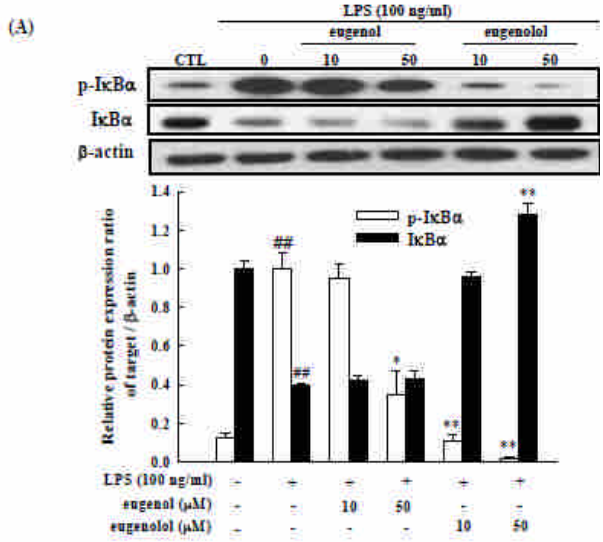


Figure 5. Effects on the inhibition of nuclear translocation of NF-κB. Cells were pretreated with each derivative for 30 min followed by incubation with LPS (100 ng/ml) for 30 min. Densitometry analyses are presented as the relative ratio of p65 protein to PARP protein. Each value represents the mean ± S.E.M. of three independent experiments, with triplicate determinations in each experiment. $^{\#\#}$ $p < 0.01$ compared with the control ; $^{*}p < 0.05$, ** $p < 0.01$ compared with LPS. ANOVA followed by Dunnett's test.



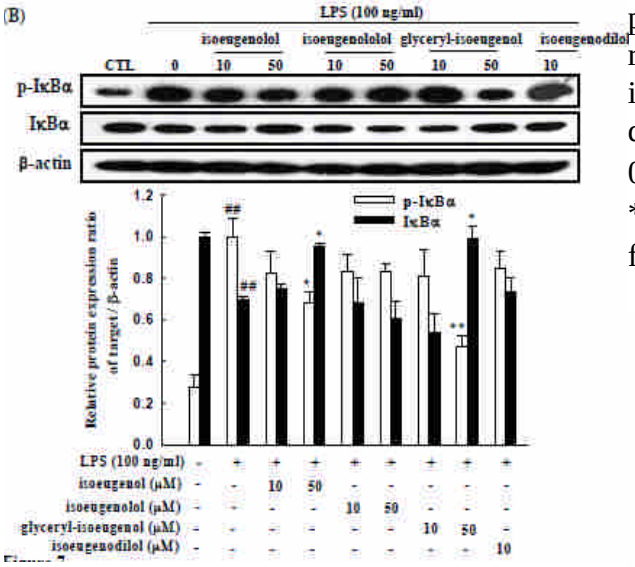


Figure 6. Effects on LPS-inducedphosphorylationand degration of IκBα. Each value represents the mean ± S.E.M. of three independent experiments, with triplicate determinations in each experiment. $^{\#\#}$ $p<0.01$ compared with the control ; $^{*}p<0.05$, ** $p<0.01$ compared with LPS. ANOVA followed by Dunnett's test.

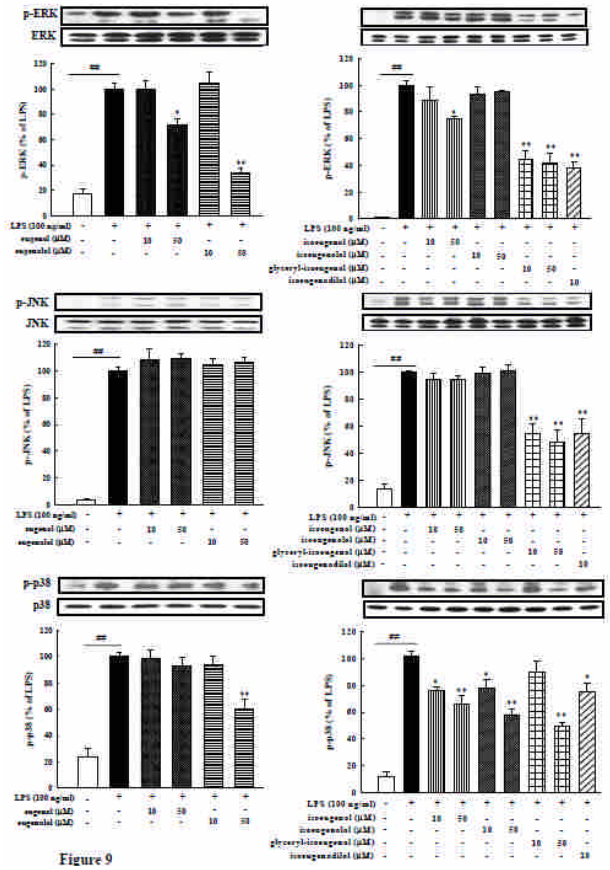


Figure 7. The effects on LPS-induced phosphorylation of MAPKs. Each value represents the mean ± S.E.M. of three independent experiments, with triplicate determinations in each experiment. $^{\#\#}$ $p<0.01$ compared with the control ; $^{*}p<0.05$, ** $p<0.01$ compared with LPS. ANOVA followed by Dunnett's test.

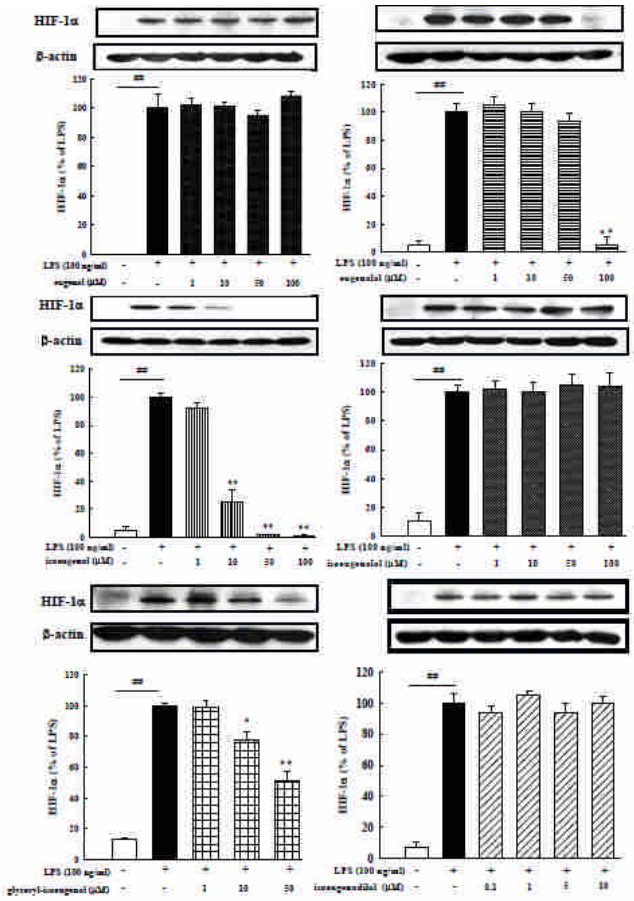


Figure 8. The inhibitory effects on LPS-induced HIF-1α expression. Each value represents the mean ± S.E.M. of three independent experiments, with triplicate determinations in each experiment. $^{\#\#}$ $p<0.01$ compared with the control ; $^{*}p<0.05$, ** $p<0.01$ compared with LPS. ANOVA followed by Dunnett's test.

國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：99 年 7 月 28 日

計畫編號	NSC 98－2320－B－041－001		
計畫名稱	丁香醇和異丁香醇衍生物之體外細胞模式及體內內毒素模式之抗發炎活性評估與訊息傳遞路徑之探討		
出國人員姓名	劉淑芬	服務機構及職稱	嘉南藥理科技大學藥學系助理教授
會議時間	99 年 7 月 19 日至 99 年 7 月 23 日	會議地點	西班牙巴塞隆納
會議名稱	(中文)第十二屆國際毒理學會 (英文) The XII International Congress of Toxicology		
發表論文題目	(中文) 以脂多醣體刺激之巨噬細胞模式探討丁香醇與異丁香醇衍生物之抗發炎活性及機轉 (英文) Anti-inflammatory evaluation and mechanisms of eugenol and isoeugenol derivatives in lipopolysaccharide-stimulated macrophages.		

一、參加會議經過

此次參加第十二屆國際毒理學會是經由學會網站公告後得知，由於此會議在西班牙巴塞隆納舉行因此可以趁機和歐洲的毒理學者相互討論，由於本人首次以脂多醣體刺激巨噬細胞進行抗發炎的新藥開發相關研究，因此報名與會學習這個領域的新知。本校一同前往的有施美份 副教授，搭乘新加坡航空公司班機從桃園出發飛往新加坡再轉機到西班牙巴塞隆納。

此次會期自 7 月 19 日 ~ 23 日在巴塞隆納著名的 Fira de Barcelona 舉行。 7 月 19 日上午 9：00 開始辦理報到後分別有 4 場持續教育課程，下午 6：00 舉行開幕隨後於晚上 8：00 舉辦歡迎晚宴與會學者歡聚一堂。主辦單位除了早、午場的學術報告外，特別於中午午餐時段安排了壁報展示，提供與會學者進行壁報討論，提供餐點讓學者在輕鬆愉悅的心情下進行學術交流。本次大會安排了 3 天 Poster Session 的時段，由於本人研究的屬性是關於藥物開發領域，因為被歸類 Drugs Development。在這 5 天我和許多國際學者相互討論交流研究心得，感到獲益匪淺。

二、與會心得

本次大會中有 2 場 Special Lectures，主題分別為 ”The diet in colon cancer development” 與 ”New insights into acetaminophen toxicity”最令人印象深刻。其中，在 The role of flat dysplastic aberrant crypt foci induced in the colon by food contaminants 的研究中指出，最近有一項發現對腺瘤性息肉與大腸癌之間的關係有了進一步的了解。息肉之前的病變可成為微小腺瘤的異常腺管灶(aberrant crypt focus, ACF)，初期的 ACF 是從腸腺側發出的內襯上皮細胞的囊狀凸起，然後擴大並最終成為獨立的廣口腺管，因其在腸腔的開口較正常腺管大許多，所以，可通過腸鏡下的染色或病理切片診斷。微小腺瘤有不典型增生，

其抑癌基因缺失和原癌基因激活的發生率與息肉狀腺瘤相似，由於 ACF 在動物致癌模型中可轉化為腺瘤或癌，因此，ACF 成為診斷結腸癌的一個重要 biomarker，其中又以 flat dysplastic ACF 在結腸癌檢體中表現更是顯著，報告中並指出表皮生長因子受體 (EGFR)的活化會促進 flat dysplastic ACF 與惡性結腸腫瘤的進展。

另外，在 "New insights into acetaminophen toxicity" 的主題中，Dr. Jack A Hinson (Division of Toxicology, Univ Arkansas, USA) 發表了題目為 new insights into mechanisms of acetaminophen toxicity 的研究，研究中指出高劑量的 acetaminophen 會引起致命性的肝小葉壞死，主要的機制包括: (1) 細胞色素 P450 代謝，代謝物的一種反應耗盡 GSH 和蛋白質共價結合; (2) 虧損增加 GSH 的形成與活性氧和氮屬在肝細胞發生壞死物種的變化;（3）增加氧化壓力 (oxidative stress)，並改變鈣恆定和啟動信號轉導的反應，導致粒線體通透性轉變;（4）線粒體通透性轉變產生了過多的氧化壓力，使粒線體膜電位減少，並喪失粒線體合成 ATP 的能力;及（5）因 ATP 的減少而導致肝壞死。而炎症介質似乎與這些事件有關，例如細胞激素和趨化因子已被證明可藉由改變氧化壓力來減少 acetaminophen 的毒性的。另外，現有的數據也支持細胞激素、趨化因子及生長因子在肝細胞啟動再生重建肝結構和功能中扮演重要的角色。

在會場也見到許多位台灣學者參加本次國際會議，並且和日本及歐美學者相互交流認識。

三、考察參觀活動(無是項活動者略):無

四、建議

參加這次的學會時與一些其他國家的相關研究領域學者進行討論，趕到獲益匪淺，一些改進的建議對自己的研究有所幫助，很感謝國科會經費補助，希望將來能有機會能再參與類似的國際會議，也希望國科會能繼續補助研究學著參與國際會議。

五、攜回資料名稱及內容

大會議程手冊與摘要書本。

論文摘要

XII International Congress of Toxicology. 19-23 July 2010 Barcelona, Spain. (ABSTRACT ADDENDA)

P302-076

ANTI-INFLAMMATORY EVALUATION AND MECHANISMS OF EUGENOL AND ISOEUGENOL DERIVATIVES IN LIPOPOLYSACCHARIDE-STIMULATED MACROPHAGES

S. F. Liou [(1)], Y. S. Hong [(2)], C. L. Chen [(1)], Y. W. Liu [(2)], J. L. Yeh [(2)].

[(1)] Chia-Nan University of Pharmacy and Science, Tainan, Taiwan; [(2)] College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

During the past decade, various eugenol and isoeugenol derivatives such as eugenolol, isoeugenolol, isoeugenodilol and glyceryl-isoeugenol have been synthesized and investigated in our laboratory. Several studies have demonstrated isoeugenol and eugenol exert anti-inflammatory actions. However, the anti-inflammatory action of these derivatives is still not determined. Therefore we evaluate the anti-inflammatory effects of these derivatives and study the mechanisms. In this study, we demonstrated that eugenol derivative, eugenolol, exhibited stronger inhibition effects on cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor-alpha (TNF-alpha), interleukin -1beta (IL-1beta) as well as NO production than those of eugenol in lipopolysaccharide (LPS)-induced RAW264.7 macrophages. We also found that eugenolol reduced LPS-induced nuclear translocation of nuclear factor-kappaB (NF-kappaB) subunit p65 and the DNA binding activity of NF-kappaB more potently than eugenol by blocking phosphorylation of Akt and inhibitor kappaB (IkappaB)alpha as well as the subsequent degradation of Ikappa B alpha. We also revealed that eugenolol repress the phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) but not c-Jun NH2-terminal kinase (JNK) and p-38. Besides, the anti-inflammatory effects of isoeugenol derivatives are also investigated, and we discovered that glyceryl-isoeugenol is the strongest COX-2, iNOS and TNF-alpha inhibitor of these derivatives by preventing the Akt and Ikappa B alpha phosphorylation and the following nuclear translocation of p65. Moreover, glyceryl-isoeugenol can repress all three MAPKs. Aside from this, we also studied other transcription factor such as activator protein-1(AP-1), cAMP-response element-binding protein (CREB), hypoxia-induced factor (HIF), and found that eugenolol and glyceryl-isoeugenol inhibited the LPS-induced HIF-1alpha expression and AP-1 binding activity. Taken together, eugenolol and glyceryl-isoeugenol can suppress iNOS and COX-2 expression by blocking MAPKs-mediated pathways with the attendant activation of AP-1, HIF-1alpha and CREB, and also by preventing the phosphorylation of Akt and Ikappa B alpha and the subsequent p65 nuclear translocation as well as the NF-kappaB binding activity.

Anti-Inflammatory Evaluation and Mechanisms of Eugenol and Isoeugenol Derivatives in Lipopolysaccharide-Stimulated Macrophages

Shu-Fen Liou[1*], Yu-Shu Hong[2], Chiu-Lan Chen[1], Yu-Wen Liu[2], Jwu-Lai Yeh[2]

[1]Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Tainan;
[2]Department and Graduate Institute of Pharmacology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

INTRODUCTION

During the past decades, various eugenol and isoeugenol derivatives such as eugenolol, isoeugenolol, isoeugenodilol, and glyceryl-isoeugenol have been synthesized and investigated in our laboratory. Our previous studies have demonstrated that the agents possess vasodilatory, antioxidant, tracheal relaxant β-adrenoceptor blocking properties, or α-adrenoceptor blockade. Several studies have demonstrated isoeugenol and eugenol exert anti-inflammatory actions. However, the anti-inflammatory action of these derivatives is still not determined. In this study, we demonstrated that eugenol derivative, eugenolol, exhibited stronger inhibition effects on cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) as well as NO production than those of eugenol in lipopolysaccharide (LPS)-induced RAW264.7 macrophages. We also found that eugenolol reduced LPS-induced binding activity of NF-κB more potently than eugenol by blocking phosphorylation of Akt and inhibitor κB (IκB)α as well as the subsequent degradation of IκBα. We also revealed that eugenolol repress the extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 but not c-Jun NH_2-terminal kinase (JNK). Besides, the anti-inflammatory effects of isoeugenol derivatives are also investigated, and we discovered that glyceryl- isoeugenol is the strongest COX-2 and iNOS inhibitor of these derivatives by preventing the Akt and IκBα phosphorylation. Moreover, glyceryl-isoeugenol can repress all three MAPKs. Aside from this, we also studied other transcription factor such as activator protein-1 (AP-1), hypoxia-induced factor (HIF) and found that eugenolol and glyceryl-isoeugenol inhibited the LPS-induced HIF-1α expression and AP-1 binding activity. Taken together, eugenolol and glyceryl-isoeugenol can suppress iNOS and COX-2 expression by blocking MAPKs-mediated pathways with the attendant activation of AP-1 and HIF-1α, and also by preventing the phosphorylation of Akt - IκBα as well as the NF-κB and AP-1 binding activities.

MATERIALS AND METHODS

1. **Cell culture**
   RAW264.7 murine macrophage cell line
2. **Cell viability**
   MTT assay
3. **Nitrite quantification**
   Griess reagent assay
4. **Western blotting**
   iNOS, COX-2, p-Akt, p-IκBα, MAPKs, HIF-1α
5. **Electrophoretic mobility shift assay (EMSA)**
   NF-κB, AP-1

RESULTS

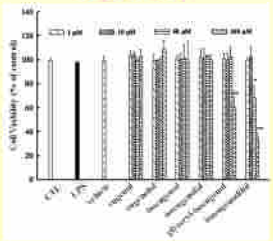


Fig 1. Cytotoxic effects of eugenol and isoeugenol derivatives in RAW264.7 cells. The values are expressed as means ± standard error of triplicate tests. (significance compared with control, *$p<0.05$, ** $p<0.01$)

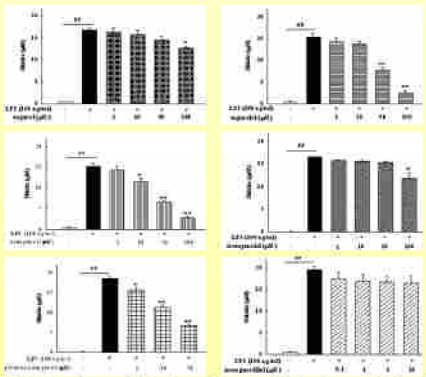


Fig 2. Effects of eugenol derivatives and isoeugenol derivatives on LPS-induced nitrite production in RAW264.7 cells. The values are expressed as means ± standard error of triplicate tests. (significance compared with the control, ## $p<0.01$; significance compared with LPS, *$p<0.05$, ** $p<0.01$)

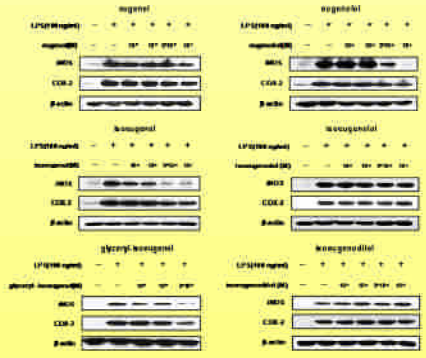


Fig 3. Effects of eugenol and isoeugenol derivatives on LPS-induced iNOS and COX-2 expression in RAW264.7 cells.

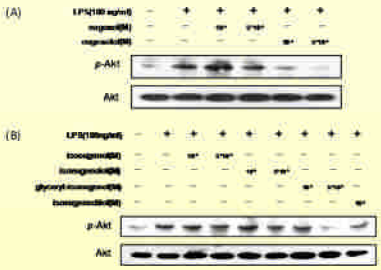


Fig 4. Effects of (A) eugenol and (B) isoeugenol derivatives on LPS-induced phosphorylation of Akt in RAW264.7 cells.

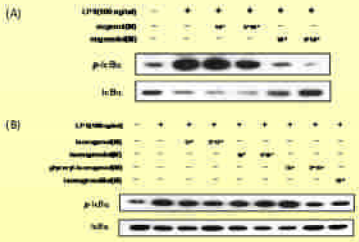


Fig 5. Effects of (A) eugenol and (B) isoeugenol derivatives on LPS-induced phosphorylation and degradation of IκBα in RAW264.7 cells.

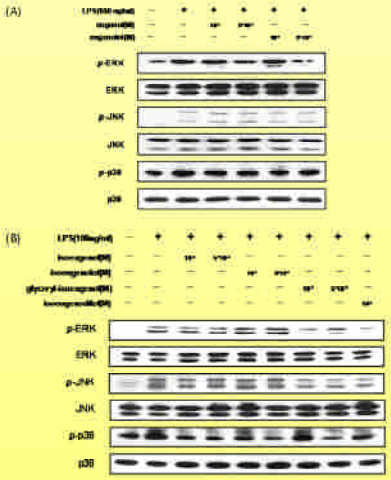


Fig 6. Effects of (A) eugenol and (B) isoeugenol derivatives on LPS-induced phosphorylation of ERK, JNK and p38 in RAW264.7 cells.

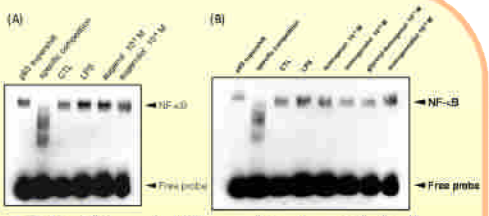


Fig 7. Effects of (A) eugenol and (B) isoeugenol derivatives on the binding of transcription factor NF-κB in LPS-induced RAW264.7 cells.

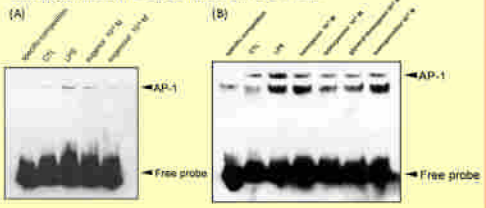


Fig 8. Effects of (A) eugenol and (B) isoeugenol derivatives on the binding of transcription factor AP-1 in LPS-induced RAW264.7 cells.

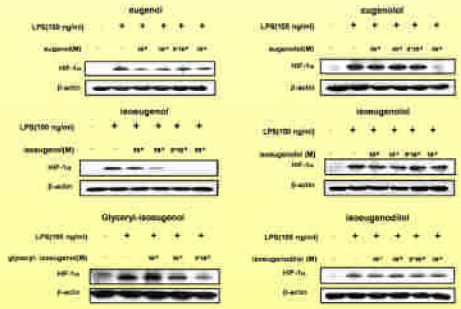
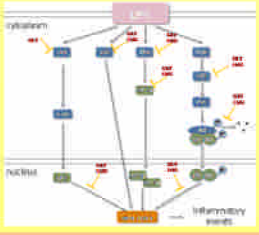


Fig 9. Effects of eugenol and isoeugenol derivatives on LPS-induced transcription factor HIF-1α expression in RAW264.7 cells.

CONCLUSION

In this study, we revealed that eugenolol and glyceryl-isoeugenol, which are the derivatives of eugenol and isoeugenol, respectively, can inhibit LPS-stimulated iNOS, COX-2 and NO production though down-regulating the Akt/IKK/IκBα pathway and the following NF-κB binding activity. Besides, they also reduced the MAPKs phosphorylation, which is considered to be involved in the LPS-induced iNOS and COX-2 expression. Furthermore, they can repress LPS-induced transcription factor AP-1 binding activity and HIF-1α expression.



附件四

國科會補助專題研究計畫項下出席國際學術會議心得報告

日期：99年7月28日

計畫編號	NSC 98－2320－B－041－001		
計畫名稱	丁香醇和異丁香醇衍生物之體外細胞模式及體內內毒素模式之抗發炎活性評估與訊息傳遞路徑之探討		
出國人員姓名	劉淑芬	服務機構及職稱	嘉南藥理科技大學藥學系助理教授
會議時間	99年7月19日至 99年7月23日	會議地點	西班牙巴塞隆納
會議名稱	(中文)第十二屆國際毒理學會 (英文) The XII International Congress of Toxicology		
發表論文題目	(中文) 以脂多醣體刺激之巨噬細胞模式探討丁香醇與異丁香醇衍生物之抗發炎活性及機轉 (英文) Anti-inflammatory evaluation and mechanisms of eugenol and isoeugenol derivatives in lipopolysaccharide-stimulated macrophages.		

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此次會期自7月19日 ~ 23日在巴塞隆納著名的 Fira de Barcelona 舉行。 7月19日上午9：00開始辦理報到後分別有4場持續教育課程，下午6：00舉行開幕隨後於晚上8：00舉辦歡迎晚宴與會學者歡聚一堂。主辦單位除了早、午場的學術報告外，特別於中午午餐時段安排了壁報展示，提供與會學者進行壁報討論，提供餐點讓學者在輕鬆愉悅的心情下進行學術交流。本次大會安排了 3 天 Poster Session 的時段，由於本人研究的屬性是關於藥物開發領域，因為被歸類 Drugs Development。在這5天我和許多國際學者相互討論交流研究心得，感到獲益匪淺。

二、與會心得

本次大會中有2場 Special Lectures，主題分別為 "The diet in colon cancer development" 與 "New insights into acetaminophen toxicity"最令人印象深刻。其中，在 The role of flat dysplastic aberrant crypt foci induced in the colon by food contaminants 的研究中指出，最近有一項發現對腺瘤性息肉與大腸癌之間的關係有了進一步的了解。息肉之前的病變可成為微小腺瘤的異常腺管灶(aberrant crypt focus, ACF)，初期的 ACF 是從腸腺側發出的內襯上皮細胞的囊狀凸起，然後擴大並最終成為獨立的廣口腺管，因其在腸腔的開口較正常腺管大許多，所以，可通過腸鏡下的染色或病理切片診斷。微小腺瘤有不典型增生，其抑癌基因缺失和原癌基因激活的發生率與息肉狀腺瘤相似，由於 ACF 在動物致癌模型中可轉化為腺瘤或癌，因此，ACF 成為診斷結腸癌的一個重要 biomarker，其中又以 flat dysplastic ACF 在結腸癌檢體中表現更是顯著，報告中並指出表皮生長因子受體 (EGFR)的活化會促進 flat dysplastic ACF 與惡性結腸腫瘤的進展。

另外，在 "New insights into acetaminophen toxicity" 的主題中，Dr. Jack A Hinson (Division of Toxicology, Univ Arkansas, USA) 發表了題目為 new insights into mechanisms of acetaminophen toxicity 的研究，研究中指出高劑量的 acetaminophen 會引起致命性的肝小葉壞死，主要的機制包括: (1) 細胞色素 P450 代謝，代謝物的一種反應耗盡 GSH 和蛋白質共價結合; (2) 虧損增加 GSH 的形成與活性氧

和氮屬在肝細胞發生壞死物種的變化;（3）增加氧化壓力 (oxidative stress)，並改變鈣恆定和啟動信號轉導的反應，導致粒線體通透性轉變;（4）線粒體通透性轉變產生了過多的氧化壓力，使粒線體膜電位減少，並喪失粒線體合成 ATP 的能力;及（5）因 ATP 的減少而導致肝壞死。而炎症介質似乎與這些事件有關，例如細胞激素和趨化因子已被證明可藉由改變氧化壓力來減少 acetaminophen 的毒性的。另外，現有的數據也支持細胞激素、趨化因子及生長因子在肝細胞啟動再生重建肝結構和功能中扮演重要的角色。

在會場也見到許多位台灣學者參加本次國際會議，並且和日本及歐美學者相互交流認識。

三、考察參觀活動(無是項活動者略):無

四、建議

參加這次的學會時與一些其他國家的相關研究領域學者進行討論，趕到獲益匪淺，一些改進的建議對自己的研究有所幫助，很感謝國科會經費補助，希望將來能有機會能再參與類似的國際會議，也希望國科會能繼續補助研究學著參與國際會議。

五、攜回資料名稱及內容

大會議程手冊與摘要書本。

六、其他

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：劉淑芬	計畫編號：98-2320-B-041-001-
計畫名稱：丁香醇和異丁香醇衍生物之體外細胞模式及體內內毒素模式之抗發炎活性評估與訊息傳遞路徑之探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	1	1	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 ■未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

發炎反應在體內算是一種保護作用，主要在於控制感染和促進組織的修復；然而過度發炎或是失去控制機轉，往往會造成組織的損害及疾病的發生，甚至危及生命。例如：類風濕關節炎、一些腸道性發炎疾病、粥狀動脈硬化症、癌症等。也因此，近年來抗發炎的藥物或食物逐漸引起重視，希望藉由減少不當的發炎反應以延緩慢性疾病的生成。本研究中的四種衍生物分別是以丁香醇（eugenol）與異丁香醇（isoeugenol）為主體，結合上具有抗氧化與交感神經阻斷作用的官能基，經由實驗的進行可證實衍生物比 eugenol 與 isoeugenol 在更低的濃度下即能有效抑制對內毒素所引起的發炎媒介物，如 iNOS/NO 的製造和釋放、減少發炎性細胞激素，例如：IL-1β、TNF-α的釋放，更可探知是否會影響轉錄因子活化與基因表現之機轉，包括 IκBα的水解、MAPKs 的磷酸化及 4 種轉錄因子與 DNA 的結合能力；以這些分子作為篩選的工具，期望建立一套系統以利於將來進行類似藥物之篩選，不僅在基礎研究上有所貢獻，並可作為相關藥物開發的參考及依據。我們也期望結構修飾後的衍生物在進一步活體實驗中也具有顯著的抗發炎效果來改善內毒素血症引起的症狀。