

Effects of 3-Phenyl-Propenal on the Expression of Toll-Like Receptors and Downstream Signaling Components on Raw264.7 Murine Macrophages

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Abstract: 3-phenyl-propenal is one of the principle compounds isolated from Guizhi (Ramulus Cinnamomi), the principal drug in Guizhi-Tang (GZT), a famous traditional Chinese medical formula. The aim of the present study was to investigate the effects of 3-phenyl-propenal on the expression of toll-like receptor 3 (TLR3), TLR4 and the downstream signaling components on Raw264.7 murine macrophages. Raw264.7 cells were cultured in RPMI-1640 medium containing LPS (lipopolysaccharide) or poly (I:C) in the presence or absence of 3-phenyl-propenal. After 24-hour incubation, the medium was collected and the amount of TNF- α and IFN- β was measured by ELISA. mRNA expression of TLR3, TLR4, myeloid differentiation factor (MyD88), TRAF-6 (tumor necrosis factor receptor-associated), TRAM (toll-like receptor-associated molecule) and TRIF (TIR domain-containing adaptor inducing IFN- β) were analyzed by real-time PCR with SYBR green dye. Protein expression of TLR3 and TLR4 was analyzed by Western blotting and that of MyD88 and TRAF-6 was analyzed by immunofluorescence assay. The results indicate that LPS increased the expression of TLR4, MyD88, TRAF-6, TRAM and TRIF, but had no influence on TLR3, while poly (I:C) up-regulated the expression of TLR3, MyD88, TRAM and TRIF. 3-phenyl-propenal significantly decreased the expression of LPS-induced TLR4, MyD88, TRAF-6, while possessing no effect on LPS-induced TRAM and TRIF expression in Raw264.7 cells. When cells were stimulated by poly (I:C), 3-phenyl-propenal significantly decreased TLR3 and MyD88 expression. In conclusion, 3-phenyl-propenal blocked the over-expression of TLR3, TLR4, their downstream signaling components MyD88 and TRAF-6, which indicate that it had an antagonistic effect on TLR3 and TLR4.

Keywords: 3-Phenyl-Propenal; Macrophage; Guizhi (Ramulus Cinnamomi); TLR3; TLR4; Downstream Signaling Components.

Introduction

Guizhi-Tang (GZT), a famous traditional Chinese medical formula which contains 5 herbs: *Cinnamomum cassia* Presl., *Paeonia lactiflora* Pall., *Zingiber officinale* Rosc., *Ziziphus jujuba* Mill., and *Glycyrrhiza uralensis* Fisch.. In the theory of TCM, GZT expels pathogenic factors from muscles and skin, regulates Ying and Wei, which increases patients' ability to prevent invasion of external pathogenic factors. GZT has been extensively used clinically for pyrexia, influenza, sore throat and inflammation. Recent studies showed that it affected the cellular signal transduction in the hypothalamus via the regulation of the level of prostaglandin E₂ (PGE₂), cyclooxygenase-2 (COX-2) and other enzymes *in vitro* (Guo *et al.*, 2006; Sun *et al.*, 2004) and reduced the amount of inflammatory factors induced by LPS and poly (I:C) (Zhao *et al.*, 2006).

3-phenyl-propenal is one of the principle compounds in GZT. Some studies showed that it relieved fever and pain (Ma *et al.*, 2006), inhibited the *Bacillus tuberculosis* and influenza virus effectively (Wang, 1997), and regulated immunological function (Huang *et al.*, 2006). Another study also showed that it reduced the amount of COX-1, COX-2 and PGE₂, which would account for its fever-relieving effect (Guo *et al.*, 2005).

Toll-like receptors (TLRs) are essential recognition and signaling components in mammalian host defense. At least 13 TLRs have been cloned in rats, and 11 TLRs in human. Studies showed that macrophages, which can eliminate viral- or bacterial-infected cells, express TLR3 and TLR4 and undergo over-expression when stimulated by poly (I:C) or LPS.

TLR3 mediated the activation of the IFN- β promoter, and to a lesser extent, the NF- κ B promoter. Poly (I:C) is its specific ligand. When cells interacted with poly (I:C), two signaling pathways, MyD88-dependent and MyD88-independent, were associated with the activation of TLR3 (Alexopoulou *et al.*, 2001). Some studies showed that the adaptor proteins TRIF/TICAM-1 (Yamamoto *et al.*, 2002; Oshiumi. *et al.*, 2003) mediated the production of IFN- β through the MyD88-independent pathway. Over expression of TRIF not only induced the high expression of the IFN- β report gene, but also coprecipitated with endogenous IRF3, the latter being the main transcription factor to IFN- β production. In other words, TLR3 also induced cytokine production through the signaling pathway dependent on MyD88 (Doyle *et al.*, 2003).

LPS is one of the cell membrane components of gram-negative bacteria, and can up-regulate the level of TLR4 mRNA. TLR4 signaling activates the NF- κ B and MAPK promoters to produce cytokine such as TNF- α , mainly by MyD88-dependent pathway. The ability of TLR4 to induce IFN- β is attributed to a TRAM, TRIF-mediated MyD88-independent pathway (Gohda *et al.*, 2004).

The present study investigated the effect of 3-phenyl-propenal on the expression of TLR3, TLR4, their downstream signaling components (MyD88, TRAF-6, TRAM and TRIF) and related gene expression productions (IFN- β , TNF- α) in order to better understand the mechanism of its immunological regulation effect.

Materials and Methods

Reagents and Antibodies

3-phenyl-propenal was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (China). LPS and poly (I:C) were provided by Sigma (USA). ELISA kits of TNF- α and INF- β were obtained from R&B Company (USA). RNA extraction kit, RNAsafe, Oligo (dT) 15, dNTP and SYBR green were obtained from Tiangen Biotech Company (China). M-MLV Reverse Transcriptase was purchased from Promega (USA). Primers sequences were designed and synthesized by Invitrogen Inc (USA).

Cell Culture and Treatments

Raw264.7 cell strain was obtained from the Shanghai Cytology Biology Institute, Chinese Academy of Sciences. The cells were cultured in RPMI-1640 medium which was supplemented with 10% (v/v) heat-inactivated FCS, penicillin (100 $\mu\text{g/ml}$), streptomycin (100 $\mu\text{g/ml}$) and NaHCO_3 (2 mg/ml) in 5×10^5 cells/well.

For study on the mechanisms of 3-phenyl-propenal in immunological regulation effect, we chose IC_{50} of 3-phenyl-propenal as the experimental dosage. According to the previous study (data not show), 3-phenyl-propenal's IC_{50} (10 $\mu\text{g/ml}$) was used to investigate its effect on TNF- α and IFN- β which were activated by LPS and poly (I:C) respectively. Macrophages were divided into 5 groups: control group, LPS-induced model group, poly (I:C)-induced model group, 3-phenyl-propenal (LPS-induced) 10 $\mu\text{g/ml}$ group and 3-phenyl-propenal (poly (I:C)-induced) 10 $\mu\text{g/ml}$ group. The cells excepted control group were stimulated with LPS (5 $\mu\text{g/ml}$) or poly (I:C) (50 $\mu\text{g/ml}$), and treated with 3-phenyl-propenal simultaneously.

Assay of TNF- α and IFN- β

After 24-hour incubation, the medium was collected for measurement of TNF- α and IFN- β with ELISA kits, the procedures were followed as indicated in kit instructions. The absorbance was obtained at 410 nm and 450 nm by an enzyme immunoassay instrument (Bio-Rad Model 550, USA).

Real-Time PCR

Total RNA from different experimental conditions was obtained by Trizol method. The concentration of RNA was determined by an absorbance at 260 nm and RNA was reverse transcribed to cDNA using the Taqman[®] Reverse Transcription Reagents (Applied Biosystems). Reverse transcription was performed at 42°C for 30 min followed by inactivation at 94°C for 5 min. cDNA was analyzed immediately or stored at -20°C until

Table 1. Sequences of Primers Used for Real-Time PCR

Name	Oligo	Primers Sequences (5'-3')	Product Length (bp)	Product Region
TLR-3	Sense:	TCG GAT TCT TGG TTT CAA GG	299	2619–2917
	Anitsense:	CTT GCT GAA CTG CGT GAT GT		
TLR-4	Sense:	GGT GGC TGT GGA GAC AAA AT	286	411–696
	Anitsense:	AAT TCC CTG AAA GGC TTG GT		
MyD88	Sense:	ACT GGC CTG AGC AAC TAG GA	219	1091–1309
	Anitsense:	CGT GCC ACT ACC TGT AGC AA		
TRAF-6	Sense:	GCG CTG TGA AGT CTC TAC CC	226	1915–2140
	Anitsense:	GCT CGT GAC CTC ACT GAT GA		
TRIF	Sense:	CCA CGT CCT ACA CGG AAG AT	246	326–571
	Anitsense:	AAC AGC ATC TGC AGC TAC CA		
TRAM	Sense:	ATA AGT GCC CCC TTT CTT GG	243	26–268
	Anitsense:	CCT CGT CGG TGT CAT CTT CT		
GAPDH	Sense:	CTC ATG ACC ACA GTC CAT GC	201	572–753
	Anitsense:	CAC ATT GGG GGT AGG AAC AC		

use. The oligonucleotide primer sequences and predicted product lengths in real-time PCR were listed in Table 1 and GAPDH was used as the calibrator gene. Real-time PCR assay was carried out with IQ5-Type real-time PCR meter (Bio-Rad, USA). The experimental data were analyzed by using the ddCt method as described by the manufacturer. PCR mix was prepared according to the manufacturer's instructions and thermal cycler conditions were as follows: 1 × 5 min 94°C, 55 cycles denaturation (30 sec, 94°C), annealing (30 sec, 60°C) and extension (30 sec, 72°C). Relative expression of the TLR3, TLR4, MyD88, TRAF-6, TRIF and TRAM genes were calculated using the ddCt method (2^{-ddCt}).

Western Blotting Analysis

Raw264.7 cells were preincubated in culture flasks at a density of 1×10^6 cells per flask for 5 hours, then exposed to LPS or poly (I:C), and 3-phenyl-propenal (10 µg/ml) for 24 hours. After the appropriate treatment, the cells were harvested by centrifugation and washed with PBS, then lysed in lysis buffer (1% Triton X-100, 150 mM NaCl, 25 mM Tris (pH 7.8), 1 mM EDTA, 2 mM EGTA, 10 mM β-glycerophosphate, 1 mM sodium orthovanadate, 50 mM sodium fluoride, and a protease inhibitor mixture). The lysates were heated to 100°C for 5 min and centrifuged at 20,000 g for 5 min. The supernatant containing the cytosolic extract was loaded on a 10% SDS-PAGE and the proteins were transferred to a nitrocellulose membrane (Millipore, USA) in transfer buffer. After incubation of membranes in blocking buffer (5% non-fat milk in 100 mM Tris-HCl (pH 7.5), NaCl (145 mM), and 0.05% Tween 20; TBST), primary Abs, anti-TLR3 or anti-TLR4 antibodies, were added, and membranes were incubated at 4°C overnight with gentle rocking. Membranes, washed twice in TBST, were incubated with the appropriate HRP-conjugated secondary Ab (Zhongshan, China). Chemiluminescence was developed by adding a peroxidase/luminol-based substrate

(Zhongshan, China). Signals were detected by radiographic film and gray scale of every group was analyzed with ImageTool 3.00.

Immunofluorescence Assay

For the detection of expression of MyD88 and TRAF-6 proteins, Raw264.7 cells (2×10^4 /well) were cultured in sterile cover 48 well plates and treated with different condition as described above. Twenty-four hours after the treatment, cells were fixed with 4% formaldehyde in PBS for 20 min and washed with PBS for 5 min. The fixed cells were then permeabilized with 0.2% Triton X-100 in PBS for 25 min at RT, washed with PBS for 3 times, then treated with anti-MyD88 or TRAF-6 polyclonal antibody (Santa Cruz, USA) for 12 hours at 4°C and again washed with PBS for 3 times. Cells were then incubated in a 1:100 dilution of fluorescein-conjugated affinipure goat anti-rabbit IgG or TRITC-conjugated affinipure rabbit anti-goat IgG for 40 min at RT and washed with PBS for 3 times. Finally, the cells were sealed with glycerine. The expression of MyD88 and TRAF-6 of every group was evaluated with Qwin Standard V3.1.0 data processing system.

Statistical Analysis

The significance of differences between the groups was established using student's t-test. Differences were considered statistically significant at $p < 0.05$.

Results

Effects of 3-Phenyl-Propenal on TNF- α and IFN- β Induced by LPS or Poly (I:C)

To elucidate the effect of 3-phenyl-propenal, IC₅₀ (10 μ g/ml) of 3-phenyl-propenal was added when the cells were activated by LPS (5 μ g/ml) or poly (I:C) (50 μ g/ml), the amount of TNF- α and IFN- β was measured using the ELISA method. Data showed that both TNF- α and IFN- β were up-regulated by LPS or poly (I:C) ($p < 0.01$), while 3-phenyl-propenal down-regulated the expression of TNF- α and IFN- β markedly ($p < 0.01$) (Table 2).

Effect of 3-Phenyl-Propenal on the Expression of TLR3 and TLR4 Induced by Poly (I:C) or LPS

Real-time PCR was performed to analyze the expression of TLR3 mRNA and TLR4 mRNA. Data in Table 3 indicated that TLR3 mRNA and TLR4 mRNA expression were up-regulated by poly (I:C) and LPS stimulation ($p < 0.01$) respectively. These results are consistent with the previous finding that poly (I:C) is a specific ligand for TLR3, and LPS is a specific ligand for TLR4.

Table 2. The Influence of 3-Phenyl-Propenal on the Inflammatory Factors Released by Raw 264.7 Cells

	Dosage ($\mu\text{g/ml}$)	TNF- α (ng/ml)	IFN- β (ng/ml)
Control Group	—	1.056 \pm 0.282	110.052 \pm 29.556
LPS-Induced Group	—	2.134 \pm 0.181**	193.955 \pm 9.481**
LPS + 3-Phenyl-Propenal	10	1.448 \pm 0.268 ^{††}	146.605 \pm 15.724 ^{††}
Poly (I:C)-Induced Group	—	2.367 \pm 0.077**	193.427 \pm 18.827**
Poly (I:C) + 3-Phenyl-Propenal	10	1.487 \pm 0.277 ^{‡‡}	147.779 \pm 14.925 ^{‡‡}

Values are mean \pm SD from 9 samples per group. *Compared to the control group ($p < 0.05$); **compared to the control group ($p < 0.01$); [†]compared to LPS-induced group ($p < 0.05$); ^{††}compared to LPS-induced group ($p < 0.01$); [‡]compared to the poly (I:C)-induced group ($p < 0.05$); ^{‡‡}compared to the poly (I:C)-induced group ($p < 0.01$). The same as follows.

Table 3. The Influence of 3-Phenyl-Propenal on the Expression of TLR3 and TLR4 mRNA

	Dosage ($\mu\text{g/ml}$)	TLR3	TLR4
Control Group	—	1.03 \pm 0.26	1.01 \pm 0.13
LPS-Induced Group	—	0.94 \pm 0.22	2.26 \pm 0.31**
LPS + 3-Phenyl-Propenal	10	0.94 \pm 0.29	1.55 \pm 0.32 ^{††}
Poly (I:C)-Induced Group	—	2.37 \pm 0.37**	1.10 \pm 0.12
Poly (I:C) + 3-Phenyl-Propenal	10	1.28 \pm 0.32 ^{‡‡}	1.17 \pm 0.33

Table 4. The Influence of 3-Phenyl-Propenal on the Expression of MyD88 and TRAF-6 mRNA

	Dosage ($\mu\text{g/ml}$)	MyD88	TRAF-6
Control Group	—	1.01 \pm 0.19	1.01 \pm 0.18
LPS-Induced Group	—	2.46 \pm 0.44**	7.04 \pm 1.59**
LPS + 3-Phenyl-Propenal	10	1.55 \pm 0.30 ^{††}	4.11 \pm 1.00 ^{††}
Poly (I:C)-Induced Group	—	2.97 \pm 0.52**	1.07 \pm 0.17
Poly (I:C) + 3-Phenyl-Propenal	10	2.08 \pm 0.60 [‡]	0.95 \pm 0.14

3-phenyl-propenal down-regulated the expression of LPS-induced TLR4 mRNA and poly (I:C)-induced TLR3 mRNA ($p < 0.01$) significantly (Table 3).

Effects of 3-Phenyl-Propenal on MyD88 and TRAF-6 Induced by Poly (I:C) or LPS

Table 4 indicates that LPS significantly up-regulated the expression of MyD88 and TRAF-6 mRNA ($p < 0.01$) and poly (I:C) increased the expression of MyD88 ($p < 0.01$), but had no effect on the expression of TRAF-6 mRNA. 3-phenyl-propenal decreased the high expression of LPS-induced MyD88, TRAF-6 ($p < 0.01$). As to poly (I:C)-activated cells, 3-phenyl-propenal mainly inhibited the expression of MyD88 mRNA (Table 4).

Table 5. The Influence of 3-Phenyl-Propenal on the Expression of TRAM and TRIF mRNA

	Dosage ($\mu\text{g/ml}$)	TRAM	TRIF
Control Group	—	1.03 \pm 0.26	1.01 \pm 0.16
LPS-Induced Group	—	2.86 \pm 0.65**	3.54 \pm 0.41**
LPS + 3-Phenyl-Propenal	10	2.50 \pm 0.84	3.00 \pm 0.57
Poly (I:C)-Induced Group	—	2.84 \pm 0.51**	3.72 \pm 0.58**
Poly (I:C)+3-Phenyl-Propenal	10	2.52 \pm 0.75	3.49 \pm 0.61

Effect of 3-Phenyl-Propenal on TRAM and TRIF Induced by Poly (I:C) or LPS

Both LPS and poly (I:C) markedly up-regulated the expression of TRAM and TRIF ($p < 0.01$), but 3-phenyl-propenal had no influence on the expression of TRAM and TRIF mRNA activated by LPS or poly (I:C) (Table 5).

Effect of 3-Phenyl-Propenal on TLR3 and TLR4 Proteins Induced by Poly (I:C) or LPS

As we know that the expression of TLR3 and TLR4 mRNA reflected the gene transcription activity, but from mRNA to protein, it needs the process of translation and modification. the over expression of mRNA is not exactly equal to the increase of definite protein. Therefore, by using the Western blotting method, we analyzed the expression of TLR3 and TLR4 proteins when the cell were activated by LPS or poly (I:C) respectively and the influence of 3-phenyl-propenal onto TLR3 and TLR4 proteins.

The results (Table 6, Fig. 1) indicate that poly (I:C) induced the high expression of TLR3 protein ($p < 0.01$) and LPS induced the high expression of TLR4 protein ($p < 0.01$). 3-phenyl-propenal decreased the over expression of TLR3 induced by poly (I:C) ($p < 0.01$), and TLR4 induced by LPS ($p < 0.05$). This result is similar to that of mRNA expression.

Effect of 3-Phenyl-Propenal on MyD88 and TRAF-6 Proteins Induced by Poly (I:C) or LPS

As shown in Table 7, LPS induced the high expression of MyD88 and TRAF-6 proteins ($p < 0.01$, while poly (I:C) induced the high expression of MyD88 protein only ($p < 0.01$). 3-phenyl-propenal down-regulated the expression of MyD88 protein activated by LPS and poly (I:C) ($p < 0.01$), it also inhibited the TRAF-6 protein expression induced by LPS ($p < 0.05$).

Discussion

The ability of individual TLRs to discriminate different invading pathogens is an important determinant of the unique gene expression profiles activated by different microorganisms. Whereas the specificity of microbe detection begins with the ligand recognition features of one or more TLR, the discovery of a family of TIR domain-containing adaptor molecules,

Table 6. The Influence of 3-Phenyl-Propenal on the Expression of TLR3 Protein

	Dosage ($\mu\text{g/ml}$)	TLR3/GAPDH	TLR4/GAPDH
Control Group	—	1.115 ± 0.068	1.125 ± 0.066
LPS-Induced Group	—	1.138 ± 0.088	$1.391 \pm 0.129^{**}$
LPS + 3-Phenyl-Propenal	10	—	$1.199 \pm 0.064^{\dagger}$
Poly (I:C)-Induced Group	—	$1.889 \pm 0.150^{**}$	1.195 ± 0.116
Poly (I:C) + 3-Phenyl-Propenal	10	$1.579 \pm 0.215^{\ddagger}$	—

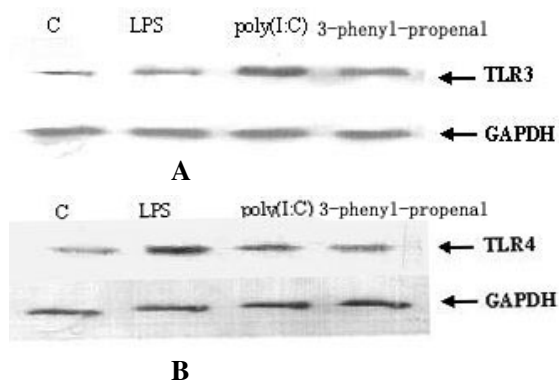


Figure 1. The influence of 3-phenyl-propenal on the expression of TLR3 and TLR4 proteins. Raw264.7 cells were preincubated in culture flasks at a density of 1×10^6 cells per flask for 5 hours. Cells were then exposed to LPS or poly (I:C), and 3-phenyl-propenal (10 $\mu\text{g/ml}$) for 24 h. Cells were harvested and lysed with lysis buffer, centrifugated and mixed with $2 \times$ SDS and run onto a 10% SDS-PAGE, then the protein was transferred onto PVDF membranes. The membranes were blocked with non-fat milk, incubated with primary and secondary Abs, developed with radiographic film. A: poly (I:C) induced the expression of TLR3 protein, but LPS did not. As for 3-phenyl-propenal, it significantly reduced the expression of poly (I:C)-induced TLR3 protein, B: poly (I:C) had no influence on the expression of TLR4 in Raw264.7 cells. On the contrary, LPS induced the high expression of TLR4 protein, and 3-phenyl-propenal inhibited the expression of LPS-induced TLR4 protein in Raw264.7 cells.

Table 7. The Influence of 3-Phenyl-Propenal on the Expression of MyD88 and TRAF-6 Protein

	Dosage ($\mu\text{g/ml}$)	MyD88/GAPDH	TRAF-6/GAPDH
Control Group	—	8.988 ± 0.896	16.580 ± 0.903
LPS-Induced Group	—	$20.670 \pm 1.560^{**}$	$20.605 \pm 1.874^{**}$
LPS + 3-Phenyl-Propenal	10	$11.198 \pm 1.194^{\dagger\dagger}$	$17.887 \pm 1.060^{\dagger}$
Poly (I:C)-Induced Group	—	$20.785 \pm 1.143^{**}$	17.203 ± 1.036
Poly (I:C) + 3-Phenyl-Propenal	10	$15.333 \pm 0.498^{\ddagger\ddagger}$	16.630 ± 1.305

including MyD88 (Muzio *et al.*, 1997), TRAF-6, TRIF (Varadaradjalou *et al.*, 2003; Muzio *et al.*, 1997) and TRAM, suggest that the outcome of induced pathogen recognition also depends on the TLR-restricted utilization of these molecules, alone and in combination, to drive a stimulus- specific response. The TLR3- and TLR4-restricted utilization of IRF-inducing adaptor molecules such as TRAM and TRIF induces not only the cytokines,

co-stimulatory molecules, and antimicrobial peptides that are induced by all TLRs, but also anti-viral type I interferon and specific chemokines including IP-10 and RANTES. Studies showed that while TLR3 and TLR4 activated the MyD88-independent pathway, the patterns of cytokines they induced were not equivalent (Re and Strominger, 2004).

TLR3 is mainly distributed on the placenta, pancreas, macrophages and monocytes. TLR3's special actor is double-strand RNA (dsRNA), when viruses infect cells, it can duplicate dsRNA in cells, dsRNA combines dsRNA-dependent protein kinase (PKR), induces the anti-virus action (Clemens and Lia, 1997).

TLR4 distributed on the macrophages, B/T lymphocyte and spleen, liver, lung, placenta, etc. mainly mediates the signal transduction of LPS, causing nuclear translation of NF- κ B activation of immunogene, and release co-stimulate molecules, which play a part in the inflammatory reaction regulations (Vogel, 1998). LPS is one of the components to the Gram-negative bacteria ectal membrane.

Both TLR3 and TLR4 have MyD88-independent signal transduction through TRIF, down-regulating the expression of IFN- β inducing gene and activation of NF- κ B subsequently.

The protein, MyD88 has a molecular weight of 3.5×10^4 kDa (Takeuchi *et al.*, 2000). When its TIR domain interact with the cytomere of IL-1R/TLRs, MyD88 can collect and connect with IRAK (IL-1R associated kinase); (Burns *et al.*, 2000), mediating the signal transduction accordingly.

Alexopoulou *et al.* (2001) found that MyD88, essential for dsRNA to mediate macrophage to produce cytokines, induces the expression of IL-1, TNF- α , IL-6 and IL-12, and participate in the non-specificity anti-virus action. Through MyD88-independent signal pathway, dsRNA also induces the expression of co-stimulatory molecules such as CD80, CD86 and cytokines such as IFN- β , IP-10, participate in the DC cell differentiation and maturing, and antiviral immunity reactions (Varadaradjalou *et al.*, 2003; Muzio *et al.*, 1997). However, overexpression of TLRs and the downstream signaling components would promote the inflammatory and destructive process to tissues (Sacre *et al.*, 2007).

It was interesting to note that the expression of TLR4, MyD88 and TRAF-6 mRNA were increased by twofold, twofold and sevenfold respectively when the cells were activated by LPS. The inhibition ratio of 3-phenyl-propenal to these signal transduction components mRNA expression were nearby to 50% in our experiment. The fact implied that the effect of 3-phenyl-propenal on the production of inflammatory cytokines such as TNF- α and IFN- β in LPS-activated Raw 264.7 would mainly be through TLR4 and its MyD88-dependent signaling transduction pathway.

When the cells were activated by poly (I:C), 3-phenyl-propenal could inhibit the expression of TLR3, MyD88 mRNA and TNF- α , IFN- β production. The inhibition ratios were 81.2%, 36.4%, 67.2% and 54.8% respectively. No effect was found on the expression of TRAF-6, TRAM and TRIF. This indicates that 3-phenyl-propenal maybe also inhibit TLR3 and its MyD88-dependent signaling transduction pathway.

From the results of this experiment, the conclusion could be drawn that 3-phenyl-propenal could inhibit the over expression of TLR3 or TLR4 and their MyD88- dependent signaling transduction pathway components in Raw264.7 cells activated by poly (I:C)

and LPS separately. Therefore, 3-phenyl-propenal decreased the production of IFN- β and TNF- α , down-regulated the over-expression of TLR3, TLR4 and some signal components in their downstream to avoid more damage to host tissue, that would be the immunological base for the effect of 3-phenyl-propenal.

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