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INHIBITORY EFFECT OF EMODIN ON RAW 264.7 ACTIVATED WITH DOUBLE STRANDED RNA ANALOGUE POLY I:C

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Abstract

Background: Emodin (3-methyl-1, 6, 8-trihydroxyanthraquinone) is a compound which can be found in Polygoni Multiflori Radix (PMR). PMR is the root of *Polygonum multiflorum*. PMR is used to treat dizziness, spermatorrhea, sores, and scrofula as well as chronic malaria traditionally in China and Korea. The anti-tumor property of emodin was already reported. However, anti-viral activity of emodin on macrophages are not fully reported.
Materials and Methods: Effects of emodin on RAW 264.7 mouse macrophages induced by polyinosinic-polycytidylic acid (poly I:C), a synthetic analog of double-stranded RNA, were evaluated.
Results: Emodin restored the cell viability in poly I:C-induced RAW 264.7 at concentrations of up to 50 μM. Emodin significantly inhibited the production of nitric oxide, IL-1α, IL-1β, IL-6, GM-CSF, G-CSF, M-CSF, MCP-1, MIP-1α, MIP-1β, MIP-2, RANTES, and IP-10 as well as calcium release and mRNA expression of signal transducer and activated transcription 1 (STAT1) in poly I:C-induced RAW 264.7 ($P < 0.05$).
Conclusion: This study shows the inhibitory effect of emodin on poly I:C-induced RAW 264.7 via calcium-STAT pathway.

Keywords: Emodin; dsRNA; Inflammation; Macrophages; Cytokine; Calcium; STAT1

Introduction

To date, pandemic viral diseases such as Ebola hemorrhagic fever and Zika virus infection is still being undissolved. Both Ebola virus and Zika virus belong to the single-stranded RNA virus. In 2015, people of South Korea suffered from Middle East respiratory syndrome caused by the Middle East respiratory syndrome coronavirus (a novel positive-sense, single-stranded RNA virus of the genus Betacoronavirus). Recently, Zika virus disease in South America was reported to be dangerous for public health. Thus, it deserves to contemplate how to effectively deal with new pandemic infectious diseases such as Ebola hemorrhagic fever, Middle East respiratory syndrome, severe acute respiratory syndrome, Zika virus infection, and avian influenza.

Cytokine is one of important elements in immunity and inflammation; cytokines are known to be small proteins; as a cell signalling molecule, cytokine plays a role in autocrine, paracrine, and endocrine mechanism of immunomodulation; cytokine are produced not only immune cells but also endothelial cells and fibroblasts; cytokines are classified as interleukins, lymphokines, interferons, tumor necrosis factors, colony stimulating factors, and chemokines; cytokines bind cell-surface receptors, which triggers intracellular signalling and host responses to infection, trauma, cancer, immune reaction, and other inflammatory phenomena; the concentration of circulating cytokine can increase up to 1,000-fold in the case of infection or trauma; the dysregulation of cytokines might become pathological in inflammation and be linked to various diseases ranging from neuroinflammatory diseases to cancer.

Immunity and inflammation play an important role in protecting human body from pathogenic infections including viral infection. Infections with virus sometimes provoke unregulated hyper-inflammation such as cytokine storm and the uncontrolled inflammatory reaction caused by viral infection could be harmful to human life (Hu et al., 2014). ‘Cytokine storm’ is one of major characteristics caused by the pandemic viral infection and leads to multiple

organ dysfunction syndrome (Sordillo and Helson, 2015). Thus, cytokine storm is becoming one of important topics concerned with caring incurable pandemic viral diseases.

Emodin (3-methyl-1, 6, 8-trihydroxyanthraquinone) is a compound which can be found in Polygoni Multiflori Radix (PMR). PMR is the root of *Polygonum multiflorum*. PMR is used to treat dizziness, spermatorrhea, constipation, sores, scrofula, prematurely gray hair, goiter, and neck lumps as well as chronic malaria traditionally in Korea and China (Thiruvengadam et al., 2014; Cho et al., 2010; Avula et al., 2007). According to the pharmacological theory of Korean Medicine, PMR is known to taste bitter, sweet, and acerbic; PMR has a warm character and is nontoxic; the pharmacological activity of PMR is mainly done through Liver Meridian, Heart Meridian, and Kidney Meridian; the efficacy of PMR is complementing Liver function, strengthening Kidney function, nourishing Blood-part, removing Wind-pathogen, benefiting Essence-part; some herbal formulae including PMR are used as the medicinal drug; for example, the herbal composition of PMR, Panax Ginseng Radix, Angelica Gigas Radix, Anemarrhenae Asphodeloidis Radix, and Amydae Carapax could be used for chronic intermittent fever and chills similar to chronic malaria; the herbal composition of PMR, Sophora flavescens Radix, Dictamnus dasycarpus Radicis Cortex, and Schizonepeta tenuifolia Herba can be used to treat itching; the herbal composition of PMR, Rehmannia glutinosa Radix Preparata, and Cornus officinalis fructus can be used to treat strengthen liver and kidney function (Chen et al., 2016)

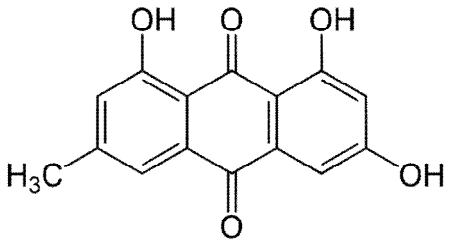

Figure 1: Structural formula of anthraquinone Emodin.

Many RNA viruses make cytosolic double-stranded RNA (dsRNA) during the replication process in the infected cell. Polyinosinic-polycytidylic acid (poly I:C), a synthetic analog of dsRNA, is regarded as a viral mimic toll-like receptor 3 stimulant (Lee et al., 2011) and poly I:C is used to stimulate macrophages in *in vitro* assay for evaluating anti-inflammatory activity on viral infections. It is well known that dsRNA could trigger macrophage activation. Molecular patterns of invasive pathogens might be recognized by toll-like receptors on cell membrane or Nod-like receptors inside cytoplasm of immune cells, which activates an inflammatory cascade and results in the massive expression of inflammatory mediators such as nitric oxide and cytokine.

Although Choi et al. have reported the anti-inflammatory activity of emodin in lipopolysaccharide (LPS)-induced RAW 264.7 (Choi et al., 2013), effects of emodin on virus-induced macrophages are not fully reported. In this study, we investigated effects of emodin on poly I:C-induced RAW 264.7 by *in vitro* assay. Data represents that emodin inhibits productions of NO, cytokines, chemokines, and growth factors in poly I:C-induced RAW 264.7 via calcium –STAT pathway.

Materials and Methods

Materials

DMEM, FBS, penicillin, streptomycin, PBS, and other tissue culture reagents were purchased from Gibco BRL (Grand Island, NY, USA). Emodin, poly I:C, indomethacin, Griess reagent, and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The multiplex bead-based cytokine assay kits used for the determination of cytokine concentration were purchased from Millipore (Billerica, MA, USA). The Fluo-4 calcium assay kit was purchased from Molecular Probes (Eugene, OR, USA). QuantiGene Plex 2.0 Reagent System for direct quantification of multiple RNA targets was purchased from Panomics (Redwood City, CA, USA).

Cell viability

RAW 264.7 were obtained from the Korea Cell Line Bank (Seoul, Korea). RAW 264.7 cells were cultured and cell viability was evaluated with MTT assay according to the previous study (Lee et al., 2011) with a microplate reader (Bio-Rad, Hercules, CA, USA).

NO concentration

NO concentration in culture medium was determined by the Griess reaction (Lee et al., 2011) according to the previous study (Lee et al., 2011) with a microplate reader (Bio-Rad).

Multiplex cytokine assay

This assay was performed with multiplex cytokine assay kits and Bio-Plex 200 suspension array system (Bio-Rad) as described previously (Lee et al., 2011). The following cytokine productions were analyzed: interleukin (IL)-1α, IL-1β, IL-6, IL-10, granulocyte macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-2, CXCL10 (IP-10), monocyte chemotactic activating factor (MCP)-1, RANTES, leukemia inhibitory factor (LIF; IL-6 class cytokine), lipopolysaccharide-induced CXC chemokine (LIX; CXCL5), and tumor necrosis factor -α (TNF-α).

Intracellular calcium level

Intracellular calcium level was determined using Fluo-4 assay according to the previous study (Lee et al., 2011) with a spectrofluorometer (Dynex, West Sussex, UK) with excitation and emission filters of 485 nm and 535 nm, respectively.

STAT1 mRNA expression

The mRNA expression of STAT1 (GenBank: NM_009283) was evaluated with the bead-based QuantiGene Plex assay according to the manufacturer's protocol. The relative mRNA level of each sample for STAT1 was normalized to that of GAPDH (GenBank: NM_001001303).

Statistical analysis

The data represent the mean ± SD of three independent experiments. Significant differences were examined using one-way analysis of variance test followed by Tukey's multiple comparison test with SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). In all cases, a P value < 0.05 was considered significant.

Results

Effects of emodin on cell viability

In this study, emodin up to a concentration of 50 μM restored the cell viability in poly I:C-induced RAW 264.7. The cell viability in poly I:C-induced RAW 264.7 incubated with emodin at concentrations of 5, 10, 25, and 50 μM for 24 h were 118.6 ± 20.12%, 122.05 ± 14.24%, 114.72 ± 12.99%, and 116.48 ± 10.1% of the control value, respectively. With this result, emodin concentrations of up to 50 μM were chosen for subsequent experiments (Figure 2A).

Effects of emodin on NO production

Data represented that emodin significantly inhibits excessive production of NO in poly I:C-induced RAW 264.7 (Figure 2B). The NO production in poly I:C-induced RAW 264.7 incubated with emodin at concentrations of 5, 10, 25, and 50 μM for 24 h were 85.29 ± 4.93%, 83.55 ± 5.18%, 81.59 ± 4.64%, and 79.30 ± 5.6% of the control value, respectively.

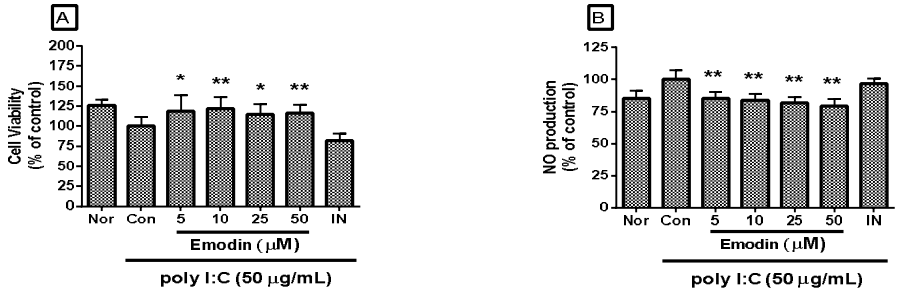

Figure 2: Effects of emodin on cell viability (A) and NO production (B) in poly I:C-induced RAW 264.7. Normal group (Nor) was treated with media only. Control group (Con) was treated with poly I:C (50 μg/mL) alone. IN means indomethacin (0.5 μM). Values are the mean ± SD of three independent experiments. * $P < 0.05$ vs. Con; ** $P < 0.01$.

Effects of emodin on cytokine production

Emodin significantly inhibited cytokine's productions in poly I:C-induced RAW 264.7 (Figure 3; Figure 4). In details, IL-1α productions in RAW 264.7 incubated with media only, poly I:C only, emodin (10 μM) plus poly I:C, emodin (25 μM) plus poly I:C, emodin (50 μM) plus poly I:C, and indomethacin plus poly I:C for 24 h were 214.43 ± 45.69 pg/mL, 4531.5 ± 1888.25 pg/mL, 1312.5 ± 128.4 pg/mL ($P < 0.05$ vs. poly I:C only), 550.25 ± 86.58 pg/mL ($P < 0.01$ vs. poly I:C only), 261.88 ± 27.23 pg/mL ($P < 0.01$ vs. poly I:C only), and 248.5 ± 41.24 pg/mL, respectively; IL-1β productions were 74.0 ± 10.46 pg/mL, 288.88 ± 89.69 pg/mL, 136.5 ± 6.81 pg/mL ($P < 0.05$ vs. poly I:C only), 85.75 ± 2.25 pg/mL ($P < 0.01$ vs. poly I:C only), 71.0 ± 4.02 pg/mL ($P < 0.01$ vs. poly I:C only), and 61.88 ± 1.18 pg/mL; IL-6 productions were 144.93 ± 27.17 pg/mL, 23243.5 ± 4977.11 pg/mL, 9966.0 ± 1524.36 pg/mL ($P < 0.01$ vs. poly I:C only), 6680.13 ± 828.28 pg/mL ($P < 0.01$ vs. poly I:C only), 925.0 ± 248.76 pg/mL ($P < 0.001$ vs. poly I:C only), and 241.75 ± 58.62 pg/mL; GM-CSF productions were 132.14 ± 24.01 pg/mL, 3371.0 ± 1369.23 pg/mL, 1586.88 ± 101.71 pg/mL ($P < 0.05$ vs. poly I:C only), 1078.88 ± 115.1 pg/mL ($P < 0.05$ vs. poly I:C only), 211.38 ± 9.18 pg/mL ($P < 0.01$ vs. poly I:C only), and 107.0 ± 7.26 pg/mL; G-CSF productions were 532.08 ± 244.9 pg/mL, 30629.88 ± 135.87 pg/mL, 29767.63 ± 177.41 pg/mL ($P < 0.001$ vs. poly I:C only), 30030.5 ± 257.09 pg/mL ($P < 0.01$ vs. poly I:C only), 29766.63 ± 440.0 pg/mL ($P < 0.05$ vs. poly I:C only), and 18905.13 ± 2537.64 pg/mL; M-CSF productions were 120.14 ± 14.72 pg/mL, 159.13 ± 19.01 pg/mL, 116.38 ± 2.29 pg/mL ($P < 0.01$ vs. poly I:C only), 130.13 ± 9.54 pg/mL ($P < 0.05$ vs. poly I:C only), 115.63 ± 3.82 pg/mL ($P < 0.01$ vs. poly I:C only), and 95.38 ± 3.97 pg/mL; TNF-α productions were 1096.88 ± 289.84 pg/mL, 23294.75 ± 1810.21 pg/mL, 23111.75 ± 473.83 pg/mL ($P > 0.05$ vs. poly I:C only), 22388.67 ± 1747.93 pg/mL ($P > 0.05$ vs. poly I:C only), 21271.63 ± 1867.28 pg/mL ($P > 0.05$ vs. poly I:C only), and 22405.25 ± 672.02 pg/mL; IL-10 productions were 88.36 ± 13.29 pg/mL, 253.13 ± 99.84 pg/mL, 142.13 ± 13.86 pg/mL ($P > 0.05$ vs. poly I:C only), 205.13 ± 63.88 pg/mL ($P > 0.05$ vs. poly I:C only), 168.38 ± 48.18 pg/mL ($P > 0.05$ vs. poly I:C only), and 76.13 ± 7.64 pg/mL; MCP-1 productions were 136.14 ± 28.53 pg/mL, 22243.75 ± 4701.86 pg/mL, 11139.38 ± 3717.66 pg/mL ($P < 0.05$ vs. poly I:C only), 2352.25 ± 784.77 pg/mL ($P < 0.001$ vs. poly I:C only), 250.0 ± 55.87 pg/mL ($P < 0.001$ vs. poly I:C only), and 101.25 ± 7.09 pg/mL; MIP-1α productions were 28051.64 ± 1506.35 pg/mL, 31009.88 ± 230.92 pg/mL, 30027.5 ± 191.56 pg/mL ($P < 0.01$ vs. poly I:C only), 30284.0 ± 308.35 pg/mL ($P < 0.01$ vs. poly I:C only), 30220.5 ± 226.22 pg/mL ($P < 0.01$ vs. poly I:C only), and 29296.63 ± 207.43 pg/mL; MIP-1β productions were 27142.86 ± 1588.67 pg/mL, 30357.0 ± 176.58 pg/mL, 29383.0 ± 278.2 pg/mL ($P < 0.01$ vs. poly I:C only), 29511.75 ± 172.42 pg/mL ($P < 0.001$ vs. poly I:C only), 29435.63 ± 461.03 pg/mL ($P < 0.05$ vs. poly I:C only), and 27760.88 ± 284.25 pg/mL; MIP-2 productions were 276.64 ± 190.26 pg/mL, 30573.63 ± 128.84 pg/mL, 29575.13 ± 161.94 pg/mL ($P < 0.001$ vs. poly I:C only), 29880.75 ± 176.97 pg/mL ($P < 0.01$ vs. poly I:C only), 29589.5 ± 367.64 pg/mL ($P < 0.01$ vs. poly I:C only), and 28463.13 ± 235.65 pg/mL; RANTES productions were 396.14 ± 123.14 pg/mL, 28046.0 ± 714.64 pg/mL, 26238.0 ± 299.63 pg/mL ($P < 0.01$ vs. poly I:C only), 26401.17 ± 403.62 pg/mL ($P < 0.05$ vs. poly I:C only), 25690.25 ± 731.67 pg/mL ($P < 0.01$ vs. poly I:C only), and 23804.75 ± 241.02 pg/mL; IP-10 productions were 6462.64 ± 2226.76 pg/mL, 29242.0 ± 258.89 pg/mL, 25910.88 ± 700.89 pg/mL ($P < 0.001$ vs. poly I:C only), 26720.88 ± 651.6 pg/mL ($P < 0.001$ vs. poly I:C only), 25845.75 ± 759.08 pg/mL ($P < 0.001$ vs. poly I:C only), and 24266.13 ± 496.65 pg/mL; LIF productions were 154.07 ± 29.67 pg/mL, 24987.13 ± 754.81 pg/mL, 22950.75 ± 907.25 pg/mL ($P < 0.05$ vs. poly I:C only), 24999.38 ± 487.17 pg/mL ($P > 0.05$ vs. poly I:C only), 24440.38 ± 362.56 pg/mL ($P > 0.05$ vs. poly I:C only), and 3283.5 ± 249.74 pg/mL; LIX productions were 994.79 ± 92.36 pg/mL, 24741.5 ± 1632.49 pg/mL, 23491.0 ± 1907.13 pg/mL ($P > 0.05$ vs. poly I:C only), 23338.75 ± 1485.07 pg/mL ($P > 0.05$ vs. poly I:C only), 24446.38 ± 320.22 pg/mL ($P > 0.05$ vs. poly I:C only), and 10588.88 ± 3139.32 pg/mL.

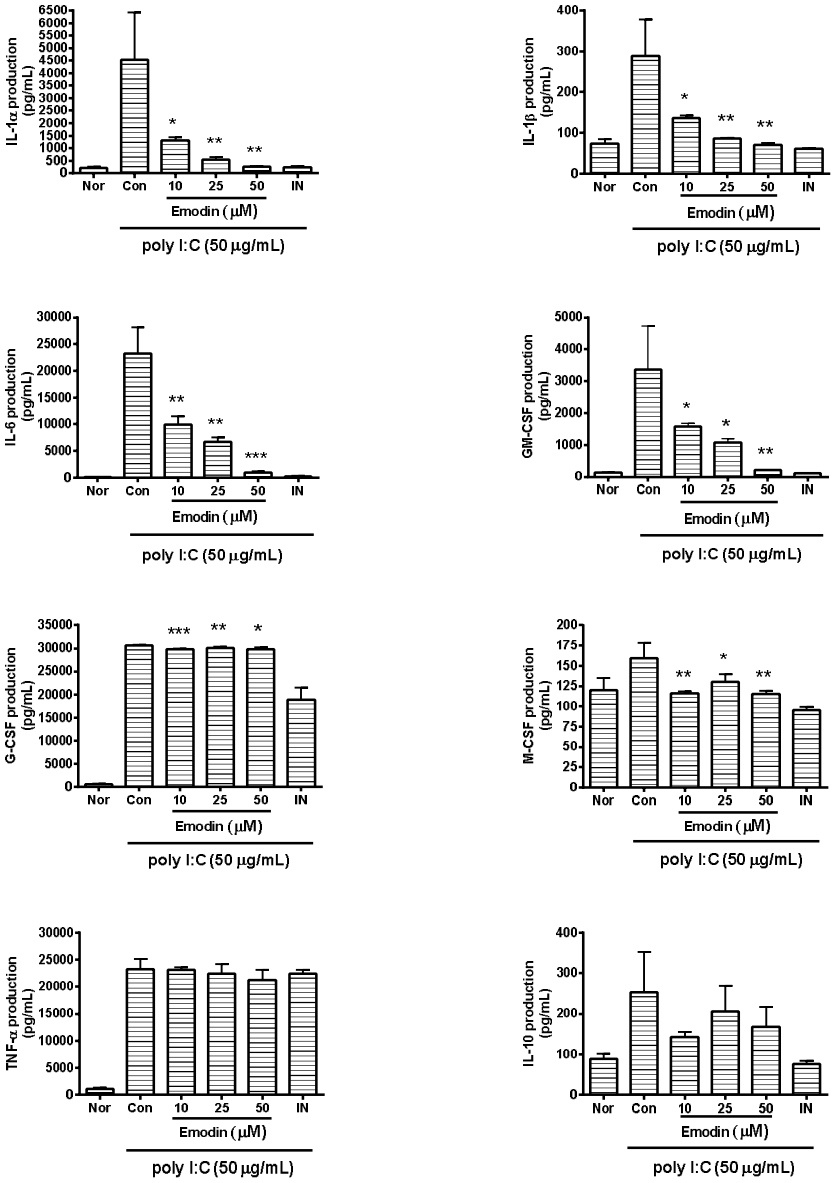

Figure 3: Effects of emodin on production of cytokines and growth factors) in poly I:C-induced RAW 264.7. Normal group (Nor) was treated with media only. Control group (Con) was treated with poly I:C (50 μg/mL) alone. IN means indomethacin (0.5 μM). Values are the mean ± SD of three independent experiments. * $P < 0.05$ vs. Con; ** $P < 0.01$; *** $P < 0.001$.

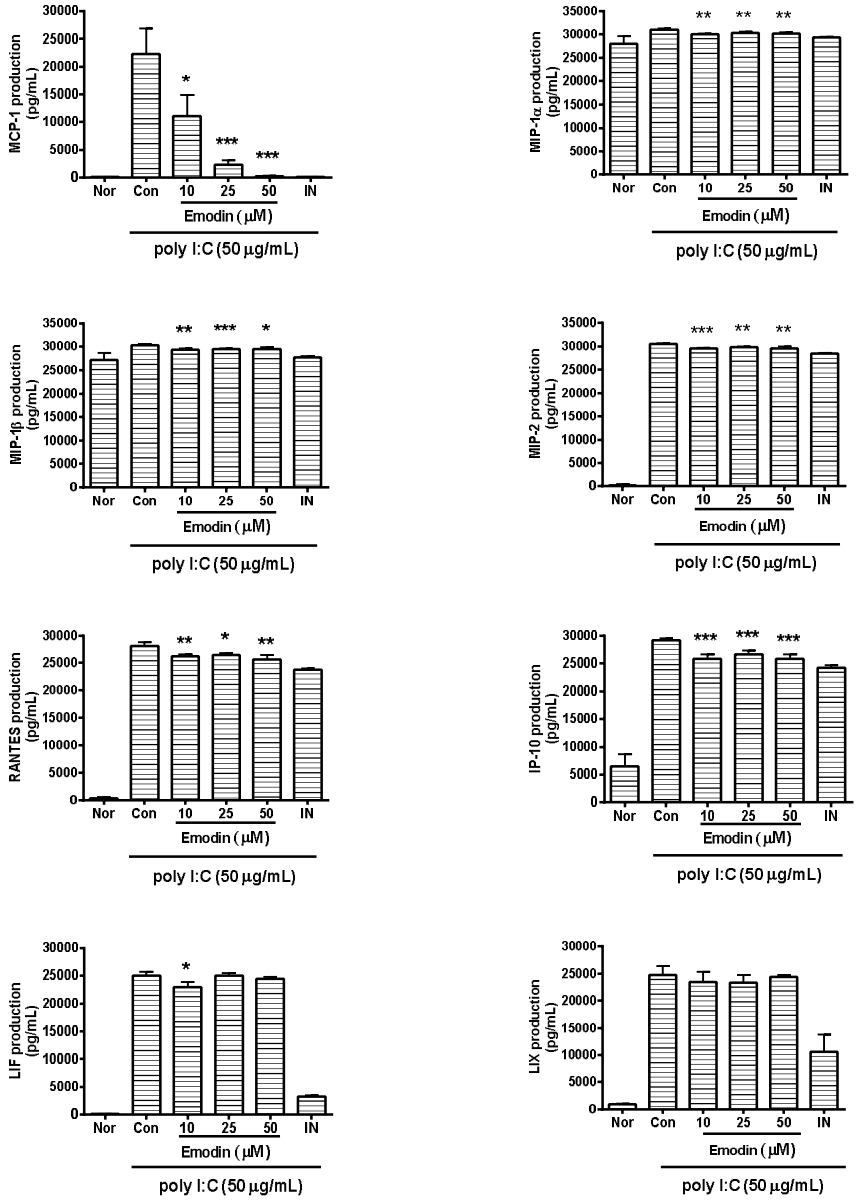

Figure 4: Effects of emodin on production of chemotactic cytokines in poly I:C-induced RAW 264.7. Normal group (Nor) was treated with media only. Control group (Con) was treated with poly I:C (50 μg/mL) alone. IN means indomethacin (0.5 μM). Values are the mean ± SD of three independent experiments. * $P < 0.05$ vs. Con; ** $P < 0.01$; *** $P < 0.001$.

Effects of emodin on intracellular calcium release

In the present study, emodin significantly inhibited the calcium release in poly I:C-induced RAW 264.7 (Figure 5). The calcium release in poly I:C-induced RAW 264.7 incubated with emodin at concentrations of 5, 10, 25, and 50 μM for 24 h were 90.9 ± 6.21%, 92.3 ± 5.79%, 94.2 ± 4.3%, and 91.41 ± 5.27% of the control value, respectively.

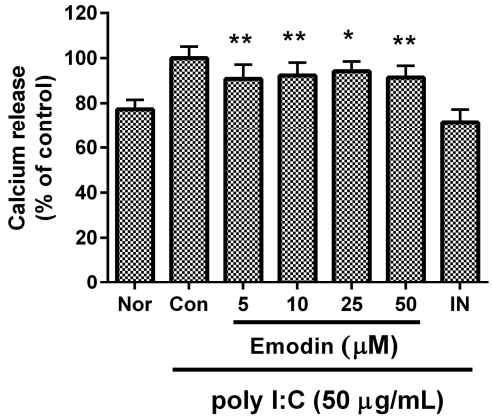

Figure 5: Effects of emodin on calcium release in poly I:C-induced RAW 264.7. Normal group (Nor) was treated with media only. Control group (Con) was treated with poly I:C (50 μg/mL) alone. IN means indomethacin (0.5 μM). Values are the mean ± SD of three independent experiments. * $P < 0.05$ vs. Con; ** $P < 0.01$.

Effects of emodin on mRNA expression of STAT1

The mRNA expression of STAT1 in poly I:C-induced RAW 264.7 incubated with emodin at concentrations of 10, 25, and 50 μM for 24 h were 36.14 ± 2.56%, 20.17 ± 2.87%, and 16.97 ± 1.56% of the control value, respectively (Figure 6). Specifically, emodin significantly decreased STAT1 mRNA expression of poly I:C-activated mouse macrophages in a dose-dependent manner.

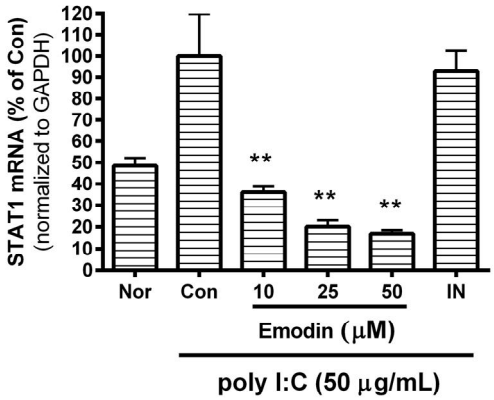

Figure 6: Effects of emodin on STAT1 mRNA expression in poly I:C-induced RAW 264.7. STAT1 mRNA was normalized to the housekeeping gene GAPDH mRNA. Normal group (Nor) was treated with media only. Control group (Con) was treated with poly I:C (50 μg/mL) alone. IN means indomethacin (0.5 μM). Values are the mean ± SD of three independent experiments. ** $P < 0.01$ vs. Con.

Discussion

The newly occurring viral disease has become an important issue for the world public health more and more. For example, Ebola hemorrhagic fever, Middle East respiratory syndrome, severe acute respiratory syndrome, Zika virus infection, and avian influenza are still hazardous to the health of specific regional people, which might attain the world level. Till now, effective and safe drugs for these pandemic diseases have not been reported. It is difficult and expensive to develop a suitable vaccine for a new viral disease in time. Therefore, nontoxic and immuno-modulatory herbal materials attract researcher's attention in these days. The property of herbal medicines noticed by researchers is not the removal of pathogenic virus in human body but modulating immune reaction against viral infection because the antiviral agent sometimes shows a side effect. For example, Yoon et al. reported the anti-inflammatory activity of Scutellaria baicalensis radix water extract (Yoon et al., 2009) and Yuk et al. reported the anti-inflammatory activity of Epimedium brevicornum herba water extract (Yuk et al., 2010). In the addition, Kim et al. have reported that the water

extract of Liriope Platyphylla tuber represents anti-inflammation efficacy through *in vitro* assay using LPS-triggered mouse macrophages in the recent study (Kim et al., 2016).

PMR is traditionally used to treat various inflammatory diseases in Korea and China. In Korean Medicine, the herbal composition of PMR, Lycium chinense Fructus, Psoraleae Corylifoliae Fructus, and Cuscuta chinensis Semen can be used to treat premature graying of the hair and premature aging.

Important ingredients of PMR are oxymethylanthraquinone, emodin, sargencuneside, chrysophanol, rhein, physcion, and chrysophanol anthrone, lecithin, questin, polygonimitin B, polygonimitin C as well as starch and fatty oils (Cho et al., 2010; Avula et al., 2007; Park et al., 2016). Thiruvengadam et al. have reported that emodin is an active component of PMR (Thiruvengadam et al., 2014). Other name of emodin is 3-methyl-1, 6, 8-trihydroxyanthraquinone. Various studies for bioactive potentials of emodin have been reported: Hwang et al. reported anti-tumor, antibacterial, vasorelaxant, and diuretic effects of emodin (Hwang et al., 2013). Wei et al. reported the anti-tumor effect of emodin on human tongue squamous cancer and gastric cancer (Wei et al., 2013). In the addition, Cha et al. reported that emodin induces androgen receptor degradation (Cha et al., 2005). Lim et al. reported the inhibitory effect of emodin on phenylephrine-induced vasoconstriction (Lim et al., 2014). Interestingly, Li et al. reported emodin inhibited the nuclear translocation of NF-κB in LPS-induced RAW 264.7 (Li et al., 2005). Xiao et al. also reported emodin potently inhibits LPS-induced pulmonary inflammation and cytokine production in mice (Xiao et al., 2014). However, the inhibitory effect of emodin on viral inflammation in human body is not fully reported. Thus, we investigated effects of emodin on poly I:C-induced RAW 264.7 by *in vitro* assay in this study.

Immunity is regarded to be a necessary function for human life but, at the same time, it is thought to be a double-edged sword biologically. For example, the strong inflammatory reaction with immune activity in human body is a natural reaction against threats from anything which is infectious or foreign but the hyper-inflammation can be inversely a severe attack to human life itself. In the case of viral infection, such a double-sided character of immune activity is also embarrassing researchers of the bio-science. Immune reactions include inflammatory mediators. Among inflammatory mediators, cytokine is regarded to be one of very important elements in immunity and inflammation; cytokines are known to be small proteins (~20 kDa); as a cell signalling molecule, cytokine plays a role in autocrine, paracrine, and endocrine mechanism of immunomodulation; cytokine are produced not only immune cells (macrophages, monocytes, neutrophils, lymphocytes, mast cells etc.) but also endothelial cells and fibroblasts; cytokines are classified as interleukins, lymphokines, interferons, tumor necrosis factors, colony stimulating factors (G-CSF, GM-CSF, M-CSF etc.), and chemokines (IP-10, MCP-1, MIP-1α, MIP-1β, MIP-2, RANTES etc.); cytokines bind cell-surface receptors, which triggers intracellular signalling and host responses to infection, trauma, cancer, immune reaction, and other inflammatory phenomena; as in G-CSF and GM-CSF, some cytokines can be classified as growth factors; the concentration of circulating cytokine can increase up to 1,000-fold in the case of infection or trauma; the dysregulation of cytokines might become pathological in inflammation.

Infection caused by pathogens (such as bacteria, virus, fungus etc.) is always dangerous to human life. Inflammation with immune activity plays an important role in protecting human body from pathogenic infections including the viral infection. However, the viral infection sometimes provokes the unregulated hyper-inflammation such as 'cytokine storm (hypercytokinemia)' (Sordillo and Helson, 2015). And the uncontrolled inflammatory reaction against viral infection could be harmful to human life (Hu et al., 2014; Pribul et al., 2008). Up to recently, studies on anti-virus vaccines and anti-viral drugs have been emphasized, but the present data shows that the modulation on cytokine productions caused by viral infectious diseases also deserves to be inspected carefully. Interestingly, Hutchinson et al. have reported that chemokines such as (MCP-1, MIPs, RANTES, IP-10, etc.) are increased in Ebola virus-infected primates (Hutchinson et al., 2001).

Poly I:C, which is an analog of viral dsRNA and regarded as a viral mimic toll-like receptor 3 stimulant, induces macrophages to produce various inflammatory mediators, which might be related with dsRNA-relative macrophage activation (Maggi et al., 2000). It is well known that dsRNA is produced during viral replication (Maggi et al., 2000). Additionally, Ioi et al have reported that rotavirus, a kind of dsRNA virus, causes cytokine storm followed by Reye syndrome (Ioi et al., 2006). In this study, emodin inhibited the excess production of NO and cytokines in poly I:C-induced RAW 264.7. Data means that more study to evaluate the pathway of emodin's activity is required for a new antiviral therapy.

Meanwhile, the infection-induced excess production of NO leads to ER stress response (unfolded protein response), which increases the concentration of intracellular calcium and activates STAT pathway (Gotoh et al., 2004; Timmins et al., 2009; Tabas et al., 2009; Wang et al., 2012). As such, ER stress response is an important pathway of virus-induced inflammation. Because the experimental data represented emodin downregulated NO production and calcium release in RAW 264.7 as well as STAT1 mRNA expression, the inhibitory effect of emodin on cytokine production in poly I:C-induced macrophages might be achieved via calcium-STAT pathway. But whether emodin regulates pyroptosis or apoptosis in poly I:C-induced macrophages is unclear in this study.

Because results of *in vitro* assay do not guarantee *in vivo* efficacy, it is insufficient to say that emodin can relieve cytokine storm in pandemic viral diseases. But this study deserves to be notified in order to develop a new material for modulation of cytokine production from virus-induced macrophages. Again, further study needs to elucidate the exact intracellular pathway about the inhibitory effect of emodin and how to regulate macrophages on

inflammation caused by viral infection.

Finally, emodin exerts the inhibitory effect on productions of NO, IL-1α, IL-1β, IL-6, GM-CSF, G-CSF, M-CSF, IP-10, MCP-1, MIP-1α, MIP-1β, MIP-2, and RANTES in poly I:C-induced macrophages via calcium-STAT pathway.

Conclusion

Emodin has anti-inflammatory property related with its inhibition of NO, cytokines, growth factors, and chemokines in poly I:C-induced macrophages via calcium-STAT pathway.

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Conflict of Interest

The authors declare no conflict of interest.

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