Animal Cells and Systems

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tacs20

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Published online: 30 Jun 2015.

To cite this article: Seok-Won Jang, Su-Geun Lim, Dong-Sub Lee, Kyoungho Suk & Won-Ha Lee (2015) Fermented bitter gourd extract differentially regulates lipopolysaccharide-induced cytokine gene expression through nuclear factor-κB and interferon regulatory factor-1, Animal Cells and Systems, 19:3, 194-200, DOI: 10.1080/19768354.2015.1042405

To link to this article: http://dx.doi.org/10.1080/19768354.2015.1042405

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Fermented bitter gourd extract differentially regulates lipopolysaccharide-induced cytokine gene expression through nuclear factor-κB and interferon regulatory factor-1

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(Received 27 February 2015; accepted 12 April 2015)

Bitter gourd is the fruit of a tropical vine in Asia, Africa, and South America where it is commonly used in traditional medicine. Our study tested the effects of a fermented extract of the bitter gourd on the inflammatory activities of the human monocytic leukemia cell line, THP-1. Treatment with the extract resulted in the suppression of phagocytic as well as lipopolysaccharide (LPS)-induced adhesion activity. Interestingly, the LPS-induced expression of matrix metalloproteinase-9 (MMP-9) and tumor necrosis factor-α (TNF-α) was suppressed by the extract while the expression of Interleukin-8 (IL-8) was upregulated. The extract inhibited the LPS-induced activation of p38 mitogen-activated protein kinase (MAPKs) and nuclear factor-κB (NF-κB), both of which are well known to be required for the expression of MMP-9 and TNF-α. In contrast, the expression of interferon regulatory factor (IRF) 1, a transcription factor involved in the expression of IL-8, but not TNF-α, was enhanced by the extract. Suppression of IRF-1 expression resulted in the elimination of the extract’s interleukin-8 (IL-8) enhancing effect. These results indicate that the fermented bitter gourd extract has general anti-inflammatory effects, with a differential effect on the expression of cytokines through modulation of NF-κB and IRF-1 activities.

Keywords: bitter gourd; macrophages; cytokine; signaling; LPS

Introduction

Inflammation, an important part of innate immunity, is directly involved in the pathogenesis of various chronic inflammatory diseases. It is also associated with cancer development and progression (Kidane et al. 2014; Wang et al. 2014) and metabolic syndromes that accompany lifestyle-related diseases such as diabetes, hypertension, and obesity (van den Oever et al. 2010; Mraz & Haluzik 2014). This indicates that the regulation of inflammation is pivotal in controlling these diseases.

Bitter gourd or bitter melon (Momordica charantia), a widely cultivated and commonly consumed vegetable in Asia, Africa, and South America, has been used in traditional medicines for the treatment of various infections, cancer, and diabetes (Leung et al. 2009; Nerurkar & Ray 2010; Fang & Ng 2011; Ooi et al. 2012). Investigations of its anti-inflammatory activity have been focused on its inhibitory effects on the activation-induced expression of pro-inflammatory cytokines, as well as its therapeutic effects in various animal models including sepsis, obesity, and alcoholic fatty liver. The butanol-soluble fraction of bitter gourd placenta extract suppressed LPS-induced tumor necrosis factor-α (TNF-α) production in RAW 264.7 cells (Kobori et al. 2008). Proponibacterium acne-induced expression of pro-inflammatory cytokines (such as TNF-α, IL-8, and IL-1β), and matrix metalloproteinase-9 (MMP-9) was suppressed by bitter gourd extract both in vivo and in vitro (Hsu et al. 2012). Bitter gourd extract also exhibited an anti-inflammatory effect in an LPS-induced sepsis model. Oral administration of the extract resulted in suppression of pro-inflammatory mediators (IL-1, IL-6, TNF-α, iNOS, and COX-2) with a concomitant increase in the anti-inflammatory cytokine IL-10 (Chao et al. 2014). Furthermore, bitter gourd was demonstrated to attenuate oxidative stress and the production of pro-inflammatory cytokines from the liver in a mouse model of chronic alcohol-induced liver injury (Lu et al. 2014).

Fermentation of a biologically active extract is commonly practiced to completely release functional substances and to produce metabolites with new functions (Gao et al. 2010; Bhat et al. 2014; Smith et al. 2014). In this study, fermented bitter gourd extract was used to treat the human macrophage-like cell line THP-1, and its effect was tested on cell adhesion, phagocytosis, and LPS-induced expression of pro-inflammatory mediators. Treatment with the extract resulted in the suppression of cell adhesion and phagocytosis. Interestingly, the LPS-induced expression of TNF-α was suppressed by the extract, while the expression of IL-8 was enhanced. The molecular mechanism responsible for this differential effect on cytokine expression was subsequently analyzed.
Materials and methods
Materials and Methods are provided in a supplementary file.

Results

Treatment with fermented bitter gourd extract suppressed LPS-induced inflammatory activation of macrophage-like cell line THP-1

Before the extract was tested for possible anti-inflammatory effects, THP-1 cells were incubated with various doses of the extract to determine cell viability (Figure 1A). Treatment of cells with concentrations higher than 10 μg/ml resulted in a significant decrease in cell viability. On the bases of these results, the extract was used at concentrations less than 3 μg/ml in subsequent analyses. The extract was then tested for its effect on the LPS-induced expression of MMP-9 (Figure 1B). LPS treatment resulted in the induction of MMP-9 expression, which was dose dependently suppressed by the extract. The suppressive effect was evident at a concentration as low as 0.3 μg/ml with a statistical significance.

In order to test the effect of the extract on cell adhesion, cells were pretreated with the extract and stimulated with or without LPS, and then cultured on fibronectin-coated culture vessels (Figure 2A). Treatment with the extract slightly reduced adhesion but was not statistically significant. Treatment of THP-1 cells with LPS resulted in a significant increase which was suppressed by additional treatment with the extract (Figure 2A).

The effect of the extract on phagocytic activity was then tested. When THP-1 cells were incubated with opsonized zymosan for 3 h, about 68 ± 3% of cells phagocytozed the zymosan particle. In the presence of the extract, the phagocytic activity was slightly suppressed with statistical significance (Figure 2B).

Treatment with fermented bitter gourd extract suppressed LPS-induced expression of TNF-α while enhancing the expression of IL-8

The effect of the extract was then tested on the LPS-induced expression of inflammatory cytokines. The expression of TNF-α was suppressed by treatment with the extract.
in a dose-dependent manner (Figure 3A). Unexpectedly, the LPS-induced expression of IL-8 was enhanced by the treatment (Figure 3B). This effect was detected only in the presence of LPS, and treatment with the extract alone did not induce the expression of IL-8 (Figure 3B, third lane).

In order to test whether this differential regulation occurs at the transcriptional level, RT-PCR analysis was performed on the THP-1 cells after treatment. Combined treatment with LPS and the extract resulted in the reduction of TNF-α mRNA in comparison to the LPS-treated samples (Figure 4A). IL-8 mRNA levels were increased 2 h after LPS stimulation and they increased again at 5 h. In cells that have been pretreated with the extract, the pattern of IL-8 expression was similar but the overall levels were upregulated (Figure 4B).

**Fermented bitter gourd extract enhanced LPS-induced expression of IL-8 through activation of interferon regulatory factor (IRF) 1**

Since the treatment with bitter gourd extract resulted in the enhancement of IL-8 expression, it is likely that the extract may have enhanced IL-8-specific transcription activator(s) that overcome the reduction of NF-κB activity. In order to identify the responsible factor, the activation status of various transcription factors including IRF-1 was tested. The expression levels of IRF-1 were enhanced by the extract at both the mRNA (Figure 7A) and protein levels (Figure 7B).

In order to confirm the role of IRF-1 in bitter gourd-mediated enhancement of IL-8 expression, IRF-1 expression was downregulated by siRNA (Figure 7C). The cells transfected with IRF-1-specific siRNA responded to LPS normally, but the enhancing effect of the extract disappeared. Furthermore, the IL-8 expression levels were suppressed by the extract to a level much lower than that of the LPS-treated samples (Figure 7D). These data indicate that IRF-1 is responsible for the IL-8 enhancing effect of the extract, and the increase in IRF-1 was masking the phosphorylation of one of its p65 subunit. When the phosphorylation status of NF-κB p65 subunit was tested, the extract had reduced LPS-induced phosphorylation levels of the subunit (Figure 5B).

In order to make sure that the extract affects NF-κB function, the activation status of NF-κB was then tested using the luciferase reporter gene under a promoter with NF-κB binding sites. The reporter construct was transfected into the HEK293 cell line with an expression construct for the constitutively active form of TLR4 (CD4-TLR4 fusion protein which has extracellular domain of CD4 and transmembrane and intracellular domain of TLR4) (Medzhitov et al. 1997). As shown in Figure 6, the activation of NF-κB was detected by transfection of CD4-TLR4 and was suppressed by treatment with the extract in a dose-dependent manner.

![Figure 3](image_url)

**Figure 3.** Treatment with the fermented bitter gourd extract suppressed LPS-induced expression of TNF-α while enhancing IL-8. THP-1 cells were pretreated with indicated concentrations of the extract for 30 min and then stimulated with 1 μg/ml of LPS for 24 h. The culture supernatants were then collected to measure TNF-α (A) and IL-8 (B) concentrations using ELISA (n = 3, *p < 0.05, **p < 0.01, ***p < 0.001 when compared with LPS-treated samples).
decrease in NF-κB activity with regard to the expression of IL-8. In the absence of IRF-1, the IL-8 expression pattern became similar to that of TNF-α.

Discussion

Current data indicate that fermented bitter gourd extract can suppress the inflammatory process in THP-1 cells and that targets of its anti-inflammatory action include the cell’s phagocytic activity and LPS-induced increase in cell adhesion and the expression of TNF-α and MMP-9. The anti-inflammatory effect of bitter gourd reported so far has only been related to the expression of inflammatory cytokines. As far as we know, this is the first case to report that the bitter gourd have inhibitory effect on cell adhesion and phagocytosis.

In numerous other studies, fermentation was employed in order to enhance the extract’s biologic effect or produce new metabolites with unexpected functions. In the case of ginseng extract, fermentation resulted in the enhancement of its anti-inflammatory function (Kawazoe et al. 2008; Jung et al. 2012; Park et al. 2013) and modification of ginsenoside content (Bae et al. 2011). Phenolic compounds have been produced by fermentation of red wine extract (Sanchez-Patan et al. 2012). In an experiment using a plant extract, two novel oligosaccharides were isolated (Kawazoe et al. 2008). Current data indicate that the bitter gourd extract retained its anti-inflammatory effect after fermentation and even appears to be enhanced. Previous studies which employed bitter gourd extract used the direct extract without fermentation, and the effective concentration ranges were 25–50 μg/ml in one study (Kobori et al. 2008) and 100–1000 μg/ml in another (Hsu et al. 2012). Since the fermented extract used in this study demonstrated its anti-inflammatory effect at the concentration range of 0.3–3 μg/ml, the fermentation process may have generated potent anti-inflammatory agents through metabolism of its contents. It is also possible that the fermentation process liberated active compounds from the complex polymer. Isolation of this active compound is expected to lead to the development of more effective anti-inflammatory agents.

Current data indicate that the extract downregulated expression of TNF-α through the suppression of LPS-induced activation of p38 MAPK and NF-κB. However, it is surprising that the expression of IL-8, which is also known to be regulated by NF-κB, was not suppressed by the extract. Analysis of IRF-1 activity indicated that the extract upregulated a transcription factor, IRF-1 which...
overcame the reduction of NF-κB activity and enhanced IL-8 expression. IRF-1 is a regulator of IL-8 expression but not for TNF-α or MMP-9. Since bitter gourd extract usually has a suppressive function on the LPS-induced expression of inflammatory cytokines, it is highly likely that a new compound generated during fermentation mediated this enhancing effect. It is unlikely that the extract was contaminated with a yeast cell wall component such as zymosan, since treatment with the extract alone does not induce the expression of TNF-α (Figure 3A, third lane) or IL-8 (Figure 3B, third lane).

As a multifunctional cytokine, IL-8 is involved in the pathogenesis of various diseases. In the synovial tissues of RA patients, IL-8 is the major T-cell chemoattractant (Nishiura et al. 1996). In atherogenesis, IL-8 induces the migration and proliferation of T-lymphocytes, macrophages, endothelial cells, and smooth muscle cells (Boisvert et al. 1998; Shin et al. 2002; Boisvert 2004). In human coronary atherosclerosis, IL-8 contributes to plaque formation as a potent angiogenic factor (Koch et al. 1992; Simonini et al. 2000). Identification of the compound responsible for the IL-8 enhancing effect in the fermented bitter gourd extract and investigation of the underlying mechanism may lead to the development of therapeutic agents that may control the diseases associated with abnormal regulation of IL-8 expression.

On the basis of current observations, it can be concluded that the fermented bitter gourd extract exhibit a differential effect on the LPS-induced expression of TNF-α and IL-8. This differential effect was mediated by NF-κB, which was suppressed by the extract, and IRF-1, which was activated. Currently, components of the extract that are responsible for the suppression of NF-κB or activation of IRF-1 are not known and will be the subject of further research. In addition, the mechanism that is responsible for the transcriptional activation of IRF-1 by the fermented bitter gourd extract needs to be investigated in the future.
Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A4A01005809 and 2014R1A1A2053381).

References


