Inflammatory properties of ribosome-inactivating protein momorcharin derived from bitter melon: abridged secondary publication

YJ Chen, ZL Yu, AKW Tse *

KEY MESSAGES

1. The inflammatory responses induced by alpha-momorcharin (α-MMC) derived from *Momordica charantia* (bitter melon) were studied.
2. α-MMC induced cytokines release in human monocytic THP-1 cells.
3. α-MMC-induced inflammatory gene expression were mediated by activation of IKK-NF-κB and JNK pathways.
4. α-MMC induced inflammatory responses in vivo.

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YJ Chen, ZL Yu, AKW Tse

School of Chinese Medicine, Hong Kong Baptist University

* Principal applicant and corresponding author: anfernee@hkbu.edu.hk

Introduction

Ribosome-inactivating proteins (RIPs) are a family of highly potent protein toxins that inhibit protein synthesis by inactivating the ribosomes or by modification of factors involved in translation.[1] The biological activity of RIPs is not completely clarified and, sometimes, independent of the inhibition of protein synthesis. One important biological activity of RIPs is production of cytokines, which may be released by macrophages and inflammatory reactions.[2] The mechanism of cytokines induction by RIPs is not fully understood. A significant role played by RIPS in inflammation is the activation of kinases (JNK, p38, and MAPK) and key inflammatory regulating transcription factors (NF-κB, AP-1).

RIPs are almost ubiquitous among plants and are distributed in different plant tissues (seed, leaf, sarcocarp, bark).[2] RIPs may undergo degradation under high cooking temperature. RIPs can be found in edible plant materials and eaten raw by humans or animals, including plant tissues (such as *Allium coepa, Spinacia oleracea, Apium graveolens, Daucus carota, Cucurbita moschata*) and plant organs (such as the seeds of *Lycopersicon esculentum*). The ground powder of the seeds of *Momordica charantia* (bitter melon) contains RIPs such as α- and β-momorcharin (MMC)[3] and is commonly used to lower blood pressure, cholesterol level, and blood glucose. Immune-related adverse effects of the seeds of *Momordica charantia* (bitter melon) have been reported,[3] but no large-scale studies have been undertaken to establish its safety and potential adverse effects when taken as a nutritional supplement. We proposed a preclinical study to determine the inflammatory responses induced by recombinant α-MMC using cell culture and animal models. We aimed to define the underlying molecular mechanisms of how α-MMC induces cytokines production.

Methods

This study was conducted from March 2014 to May 2016. Recombinant α-MMC was generated in *Escherichia coli* Rosetta (DE3) pLysS strain and purified by nickel–nitrilotriacetic acid affinity chromatography. Recombinant α-MMC solutions were passed twice through Zeba spin desalting columns and high capacity endotoxin removal spin columns to remove imidazole and endotoxin residues, respectively. Endotoxin contamination was kept below the detection limit of LAL chromogenic endotoxin quantitation kit (Thermo Scientific). The concentration and purity of recombinant α-MMC protein were analysed by SDS-PAGE.

The cytokines concentrations in cell culture supernatants were measured using the human ELISA Kit (eBioscience), and the protein concentrations of corresponding cell extracts were measured using the Bradford method. The cytokines concentrations in mouse serum and duodenum extracts were measured using the mouse ELISA Kit (eBioscience).

Total RNA was extracted using the TRIzol reagent (Invitrogen), and reverse transcription was conducted. cDNA products were analysed using the Human Inflammatory Response & Autoimmunity RT² Profiler PCR Array following the manufacturer's

instruction. Alternatively, real-time PCR on cDNA was carried out in an Applied Biosystems ViiA 7 real-time PCR machine using SYBR Green assays.

Male mice were intragastrically administered with either 6 mg α-MMC/kg/day and saline or saline alone. At the end of treatment, retro-orbital blood samplings under anaesthesia were performed, and sera were collected by centrifugation. Duodenum tissues were dissected and homogenised in ice-cold saline using an ultra-Turrax T-25 homogeniser and then sonicated.

Results

Ribosome-inactivating protein and related proteins can induce activation of IKK-NF-κB pathway.[4] After a 60-minute treatment, α-MMC caused a rapid degradation of IκBα, followed by a slow but dramatic restoration of the IκBα level at 120 minutes (Fig 1a). Moreover, α-MMC induced p65 levels in the nucleus. Treatment of cells with specific IKKβ inhibitors (TPCA-1, SC-514, or BMS-345541) suppressed the α-MMC-induced IL-1β protein expression (Fig 1b). An IKKβ inhibitor TPCA-1 inhibited α-MMC-induced p65 nuclear translocation (Fig 1c), cytokine secretion (Fig 1d), and cytokine mRNA levels (Fig 1e). Taken together, these results suggest that activation of IKK-NF-κB pathway is indispensable for α-MMC inflammatory action.

A significant role in the inflammatory responses of RIPs is played by the activation of MAPK kinases pathway (including JNK, p38 and ERK).[5] We detected elevated levels of JNK, but not p38 or ERK, in THP-1 cells treated with α-MMC (Fig 2a). To test whether cytokine expression in response to α-MMC is dependent on JNK activation, we treated THP-1 cells with SP600125 for 30 minutes before the addition of α-MMC. Treatment of JNK inhibitor SP600125 inhibited α-MMC-induced IL-1β protein expression (Fig 2a), cytokine secretion (Fig 2c), and cytokine mRNA levels (Fig 2d). Overall, these results indicate that activation of JNK pathway is essential for α-MMC-induced inflammatory responses.

To determine whether intragastric administration of α-MMC in mice leads to elevated levels of cytokines in serum and duodenum, four groups of mice treated with vehicle control, TPCA-1 alone, α-MMC alone, or TPCA-1 with α-MMC were exsanguinated at the end of treatment. The serum and duodenum levels of IL-1β, TNF-α, and MCP-1 protein were up-regulated upon α-MMC treatment, and these effects were inhibited by co-administration of IKKβ inhibitor TPCA-1 (Fig 3). These data suggest that the α-MMC induces inflammatory responses in vivo.

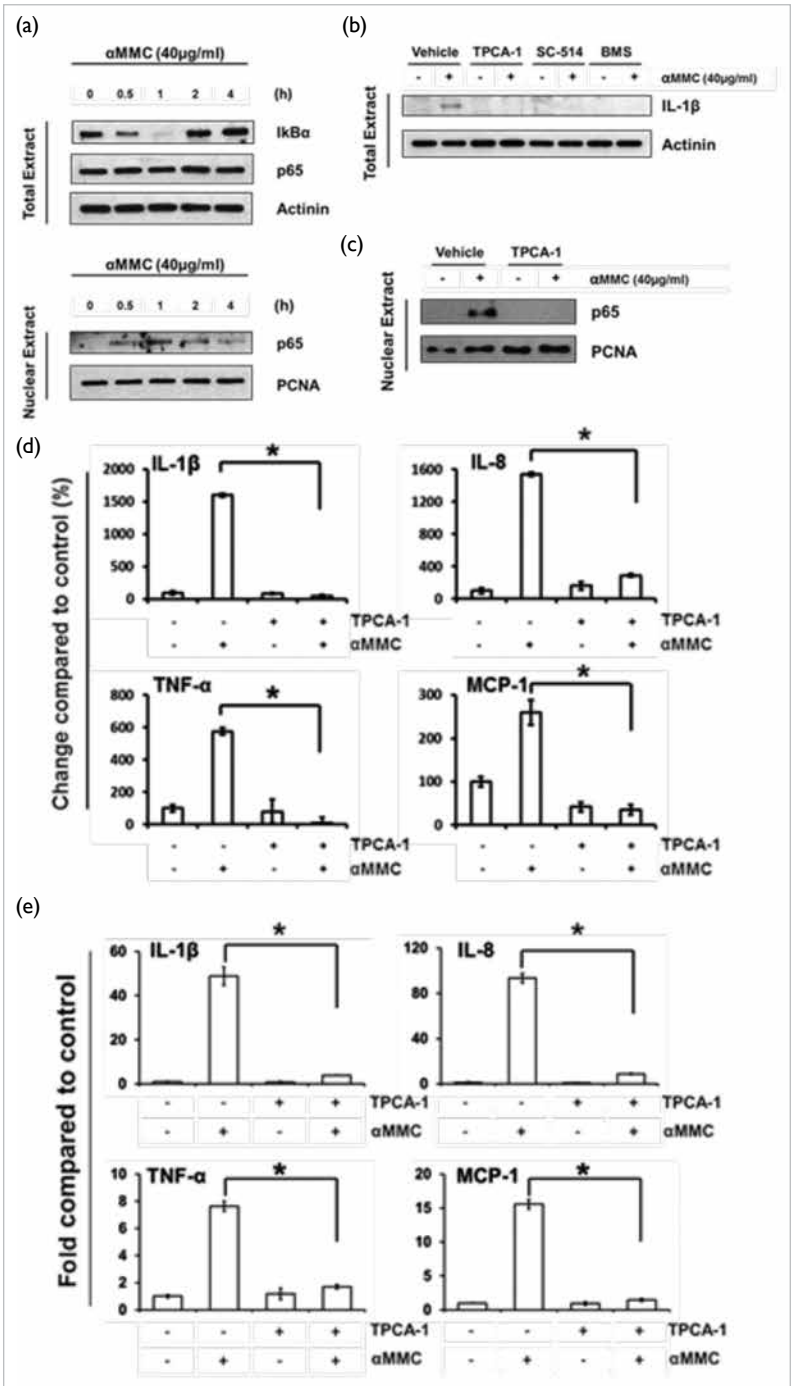

FIG 1. NF-κB activation is essential for α-MMC-induced inflammatory responses in THP-1 cells. (a) THP-1 cells are treated with α-MMC, and the level of IκBα and p65 proteins in the whole cell, and lysates and p65 protein in the nuclei are assayed by western blotting. (b) THP-1 cells are pretreated with TPCA-1 (5 μM), SC-514 (50 μM), BMS-345541 (2.5 μM) for 30 minutes and then further treated with or without α-MMC (40 μg/mL) for 24 hours. Whole-cell extracts are analysed for protein expression levels of IL-1β by western blotting. (c) THP-1 cells are exposed to vehicle or α-MMC (40 μg/mL) for 24 hours after pretreatments with or without 5 μM TPCA-1 for 30 minutes. Western blot analysis of nuclei p65 is shown. (d & e) THP-1 cell cultures are pretreated with TPCA-1 (5 μM) for 30 minutes, followed by the addition of α-MMC (40 μg/mL) and incubation for 24 hours. (d) The secretion levels of indicated pro-inflammatory cytokines in the cell culture supernatant are examined using corresponding ELISA kits. (e) The mRNA level of the four pro-inflammatory cytokines is analysed by real-time PCR.

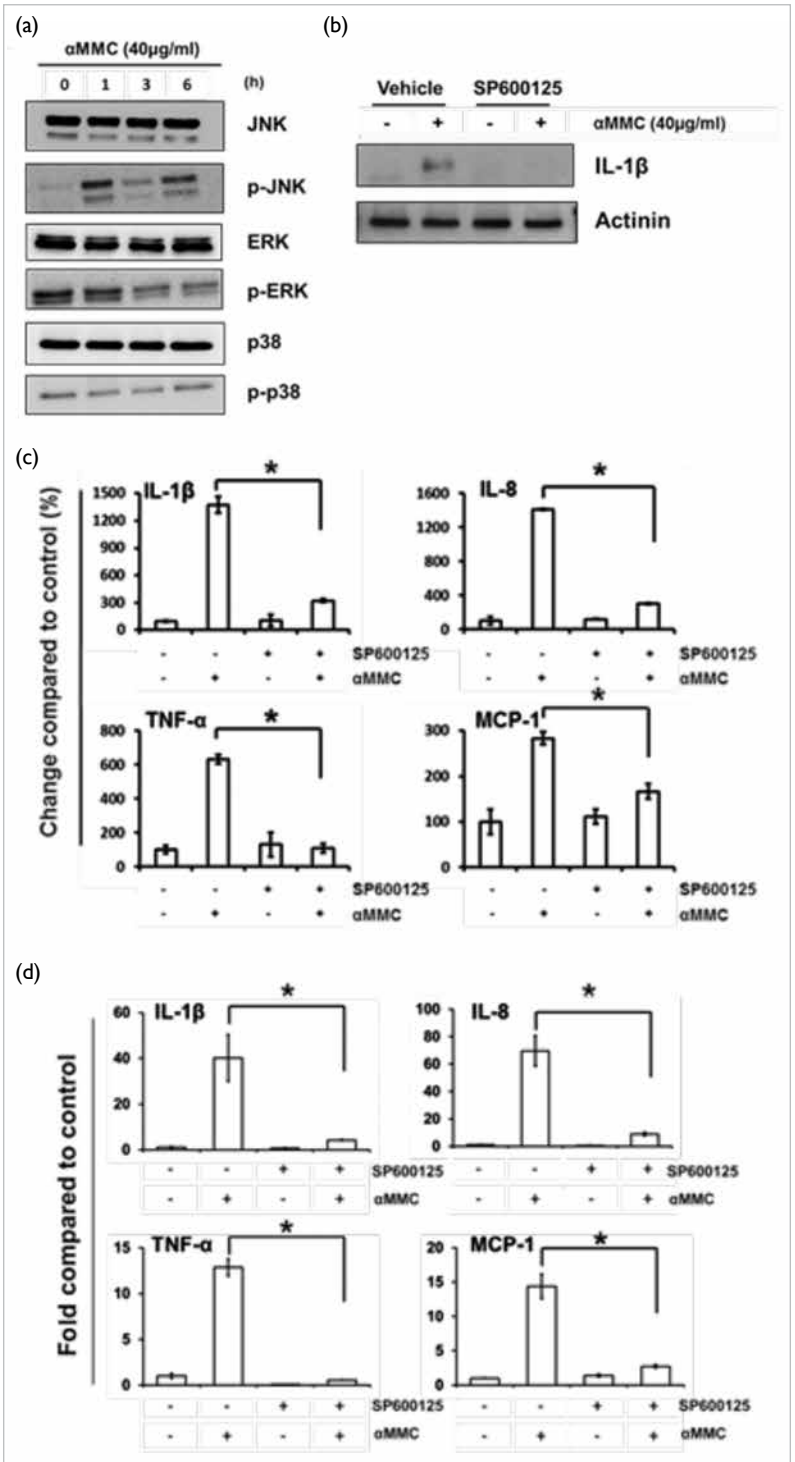

FIG 2. JNK activation is essential for α-MMC induced inflammatory responses in THP-1 cells. (a) THP-1 cells are treated with α-MMC and the whole-cell extracts are subjected to western blot analysis using JNK, ERK, and p38 antibodies and their respective phospho-specific antibodies. (b) THP-1 cells are pretreated with 15 μM SP600125 for 30 minutes, followed by the addition of α-MMC (40 μg/mL) and incubation for 24 hours. Whole-cell extracts are analysed for protein expression levels of IL-1β by western blotting. (c & d) THP-1 cells are exposed to vehicle or α-MMC (40 μg/mL) for 24 hours after pretreatments with or without 15 μM SP600125 for 30 minutes. (c) Levels of IL-1β, IL-8, TNFα, and MCP-1 in the culture medium of THP-1 cells are detected by ELISA. (d) The mRNA level of the indicated cytokines is analysed by real-time PCR.

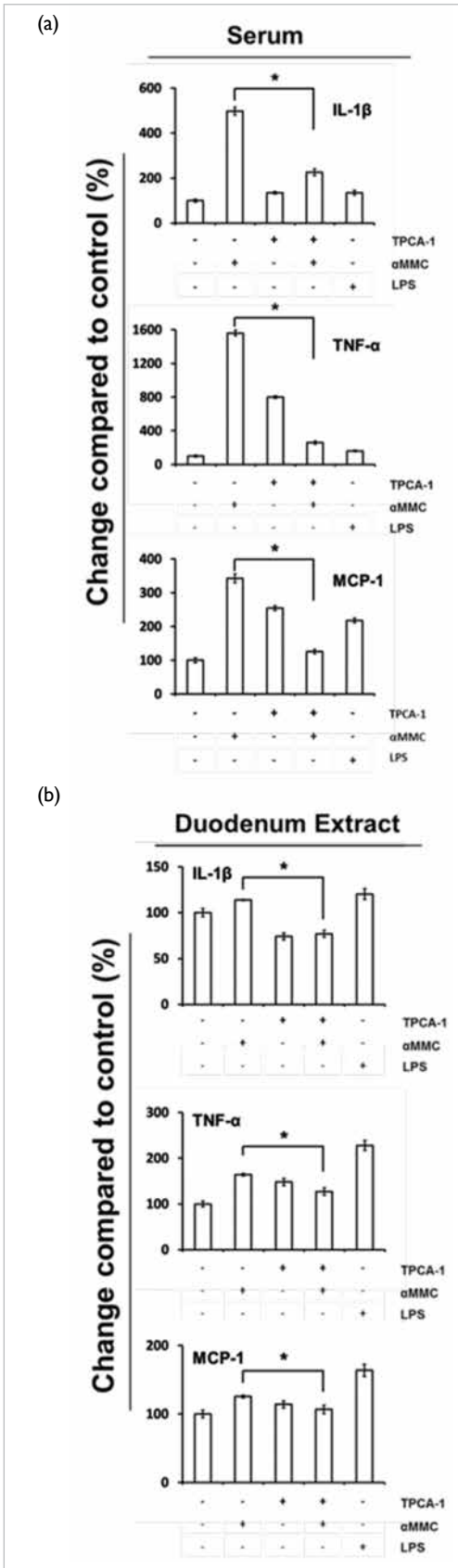

FIG 3. Inflammatory gene expression in mouse after administration of α-MMC and α-MMC plus TPCA-1. Mice are treated with α-MMC (6 mg/kg), TPCA-1 (15 mg/kg), or their combination for 3 consecutive days. LPS (10 mg/kg) is used as positive control. Levels of IL-1β, IL-8, TNFα, and MCP-1 in the (a) sera and (b) duodenum extracts are detected by ELISA.

Discussion

Several RIPs have been reported to induce secretion of cytokines. We report that α-MMC, a ribosome-inactivating protein alpha-momorcharin derived from *Momordica charantia* (bitter melon), induces the release of cytokines including IL-1β, IL-8, TNF-α, MCP-1 in human peripheral-blood mononuclear cells. IL-1β, TNF-a, and MCP-1 were detected in the plasma and duodenum of α-MMC-treated mice. There are a number of reported immune-related adverse effects of the seeds of *Momordica charantia* (bitter melon).[3] Our results suggest that these cytokines produced by α-MMC may contribute to the pathogenesis and other effects after α-MMC poisoning.

The results can enhance our understanding of the function of RIPs and their possible involvement in inflammatory-related diseases. Importantly, these results support the establishment of guidance for the safe use of RIP-containing edible plant materials. Moreover, characterisating new RIPs or homogeneous recombinant RIPs may play important roles in cancer treatment, immunotherapy, and treatment of viral diseases.

Taken together, the present study demonstrates that α-MMC induces inflammatory responses via activation of IKK-NF-κB and JNK MAPK activities in a mouse model and in vitro monocytes.

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Disclosure

The results of this research have been previously published in:

1. Chen YJ, Zhu JQ, Fu XQ, et al. Ribosome-inactivating protein α-momorcharin derived from edible plant Momordica charantia induces inflammatory responses by activating the NF-kappaB and JNK pathways. Toxins 2019;11:694.

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