

Macrophages IL-1 M11C (non-lectin components)

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The effect of Korean mistletoe extract M11C (non-lectin components) on IL-1 release and expression from macrophages

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Background: Korean mistletoe (*Viscum album*) extract has been found to possess immunostimulatory activity. In this study, Korean mistletoe extract, M11C (non-lectin components), was used to know whether this extract might activate mouse peritoneal macrophages to produce interleukin 1 (IL-1). **Methods:** Hemagglutination assay was carried out to examine whether M11C contained a lectin or not. To know the effect of M11C on the production of IL-1, the macrophages were treated by the M11C, and then collected the supernatant (M11C stimulated macrophages-conditioned media; MMCM). MMCM was analyzed for the IL-1 quantification and mRNA expression by means of ELISA and RT-PCR, respectively. **Results:** Maximum effective dose and time of M11C on IL-1 production from macrophages were 20 $\mu\text{g}/\text{M}\ell$ and 8 hours, respectively. This ELISA data was reconfirmed by immunoblotting assay, indicating that M11C is a good candidate for an immunomodulator. The dose and time dependent effects of M11C on the expression of IL-1 mRNA from macrophages was also shown in expression of mRNA detected by RT-PCR. Treatment dose and time for the maximum expression of IL-1 mRNA were 20 $\mu\text{g}/\text{M}\ell$ and 4 hours, respectively. Maximum gene expression of IL-1 was much earlier than maximum production of it. **Conclusion:** As results, Korean mistletoe extract, M11C, may be used for an immunomodulator. This will be able to make up for and solve the problems caused by existent immunoagent with many adverse effects through many other studies in future including one molecule extraction.

Key Words: Korean mistletoe, non-lectin components, M11C, macrophages, IL-1 release, IL-1 expression

(1-4). helper T cell, NK cell
 hematopoietic cell, fibroblast, vascular endothelial cell (15, 16).
 (1). 가
 1990 가
 (2-7). 가
 가
 macrophage IL-1
 macrophage IL-1 가
 가
 polysaccharides, oligo-saccharides, amines, alkaloids (8-12).
 T
 macrophages macrophages RPMI-1640 MEM, fetal bovine serum (FBS), L-glutamine acid, penicillin-streptomycin, trypsin-EDTA Gibco-BRL (Grand Island, NY, USA), culture plates, flasks, tube Falcon(Franklin Lakes, NJ, USA) IL-1 enzyme-linked immunosorbent assays (ELISA) kit immunoblotting Chemicon (Temecula, CA, USA) (RT-PCR) Takara (Otsu, Shiga, Japan)
 Chloroform, hexane, methanol, ethanol, isoprophanol Merck (Berlin, Germany)
 0.1% mouse
 (,)
 22±
 IL-1 , , macrophage 1 , 55± 5%, 150 Lux . Macro- phages Balb/c
 macrophage B lymphocyte, dendritic cell, fibroblast, keratinocyte, Langerhans cell, neutrophil, astrocyte, epithelial cell, endothelial cell (15, (12). M 11C
 16). IL-1 (17-19). IL-1 (82A,) 2

4 (,)
 30
 chloroform hexane
 (60µm 0.45µm; Millipore, Bedford, MA 01730, USA), polymyxin-B column (Biorad, Hercules, CA, USA) lipopolysaccharide (LPS) PBS (M11C). M11C LPS Limulus ES II kit (Wako, Osaka, Japan), 42 endotoxin units (EU)/ml (data not shown). 가 LPS 350 EU/ml (20), M11C 1/8 가 가 .
 3. (hemagglutination test) M11C (12, 21, 22). B PBS 3 2%가 PBS solution M11C negative control PBS, positive control Korean mistletoe lectin KML 가 96 well microtiter plate 1 M11C .
 4. Mouse macrophages Macrophage (12, 23). , RPMI-1640 24 well plate macrophages plating . macrophages 1 macrophages가 , Wright and Giemsa stain macrophages 98% (data not shown). M11C macrophage plate incomplete RPMI-1640 medium , serum free RPMI-1640 medium (+penicillin-streptomycin, +L-glutamine) 1 Ml . Macrophages M11C 가 , 37 medium -20

5. Sandwich ELISA

IL-1 ELISA kit (Chemicon, Temecula, CA, USA) . IL-1 standard samples 96 well (precoated with rat anti-mouse IL-1) 100 µl . primary antibody rabbit anti-mouse TNF- 25 µl 가 , 4 , wash buffer . 100 µl second antibody goat anti-rabbit conjugated alkaline phosphatase well 가 45 wash buffer , vacuum color reagent well , well stop solution 50 µl . 490 nm ELISA reader (Bio-Tek, Highland park, Vermont, USA) IL-1 (24).

6. Western blotting

Western blotting (12, 25). , MMCM sample buffer 16% separating gel 0.1% SDS-PAGE . gel nitrocellulose membrane (NC; Schleicher and Schuell, Verkaufsleiter, Germany) , NC blocking buffer (5% nonfat dry milk in TBS-T buffer) . TBS-T buffer , rabbit anti-murine IL-1 polyclonal primary antibody 가 hybridization incubator (Robbins, CA, USA; speed 10rpm) 4 12 . TBS-T buffer , 2 (goat anti-rabbit IgG horseradish peroxidase conjugated affinity purified antibody) 가 hybridization incubator 4 3 , TBS-T . NC ECL(oxidase substrate, Amersham, Cleveland, Ohio, USA) 5 . , 10W safety lamp (Kodak, Rochester, NY, USA)가 x-ray film(Agfa, Devaert, Belgium)) x-ray film developing,

Table 1. Primers used in RT-PCR

Target mRNA	Primer sequences	Product size (bp)
IL-1	sense: 5'-ATGGCAACTGTTCTGAACTCAAC-3' anti-sense: 5'-CAGGACAGGTATAGATTCTTTCTTT-3'	563
-actin	sense: 5'-GGAGAAGATCTGGCACCAACACC-3' anti-sense: 5'-CCTGCTTGCTGATCCACATCTGCTGG-3'	840

washing, fixing, washing IL-1 band

7. (reverse transcription - polymerase chain reaction)

(RT-PCR) kit

kit(Takara, Otsu, Shiga, Japan)

, Trisol(Gibco-BRL, Grand Island, NY, USA) total RNA

2 µg total RNA (total volume: 50 µl; 10 µl of 5x RAV-RTase buffer, 20 µl of 2.5 mM dNTP, 0.02 µM of IL-1 or -actin anti-sense primer, 10 units of RAV-RTase)

IL-1 -actin cDNA

cDNA (total volume: 50

µl; 5 µl of 10x Taq buffer, 4 µl of 2.5 mM dNTP, 1 µl of 10 pmoles/µl IL-1 or -actin sense or anti-sense primer, 8 units of Taq)

RT-PCR 1% agarose gel primer

Table 1

8.

group mean ± SD PC-SAS Excel

T- p 0.05

가

1. M11C

(non - lectin)

M11C가 agglutinin 가

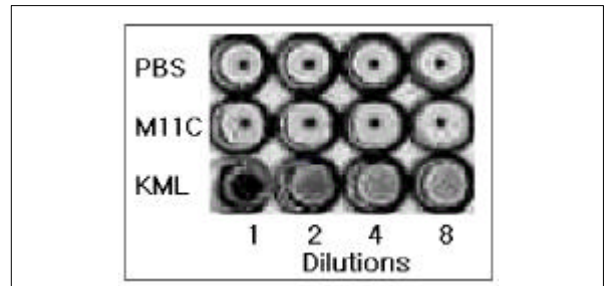


Fig. 1. The effect of Korean mistletoe crude extract (M11C) on agglutination of RBC. 1, 2, 4 and 8 express 1, 2, 4, 8 multiple dilutions, respectively. PBS, M11C, and KML represent negative control, Korean mistletoe crude extract, and Korean mistletoe lectin, respectively. The result is one of the four experiments. All other details are as described under "Materials and Methods".

(Fig. 1)

negative control PBS M11C

. Positive control

KML

8 µg/ml

(data not shown).

M11C agglutinin

2. M11C macrophages IL-1

M11C macrophage 8

M11C 0.02 µg/ml medium alone IL-1 (data not shown). ED50 2 µg/ml 가

가 IL-1 가 M11C

20 µg/ml 245 ± 6 pg/ml IL-1

IL-1 0.02

µg/ml

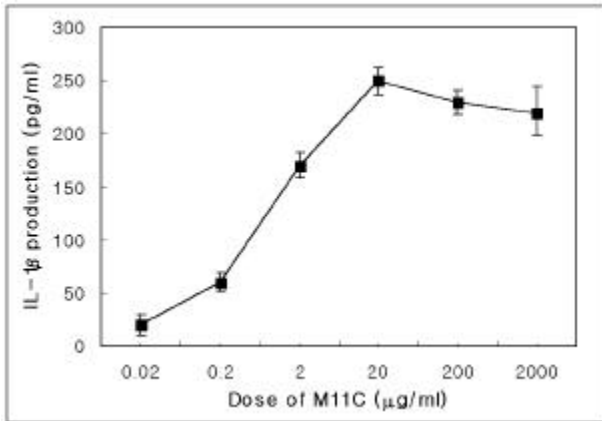


Fig. 2. The dose dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) release from macrophage. Bars represent the mean ± S.E.M for three experiments with triplicate. All other details are as described under "Materials and Methods".

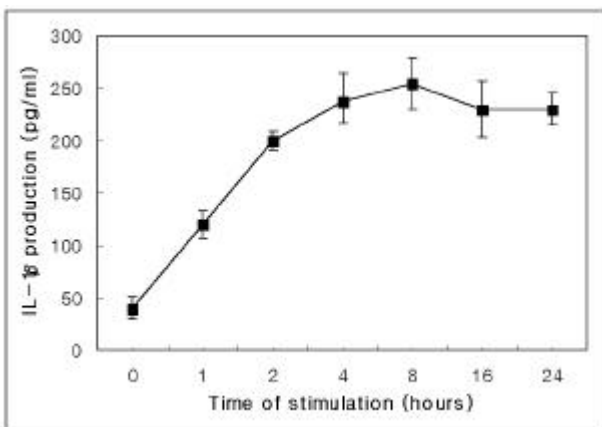


Fig. 3. The time dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) release from macrophage. Bars represent the mean ± S.E.M for three experiments with triplicate. All other details are as described under "Materials and Methods".

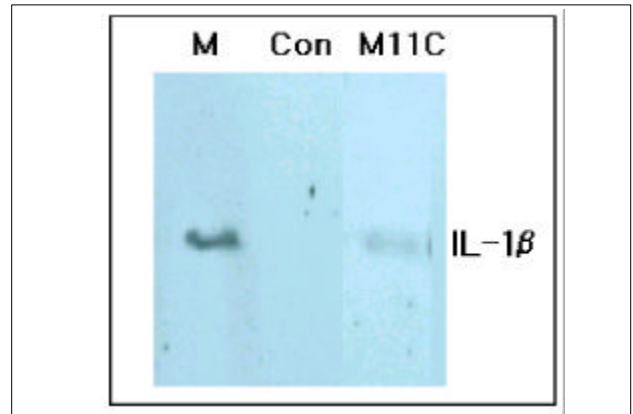


Fig. 4. Immunoblot analysis of conditioned media obtained from Korean mistletoe crude extract (M11C)-treated macrophage using polyclonal antibody of rabbit anti-mouse IL-1. M and Con represent marker and control (medium alone) for IL-1, respectively. The result is one of the five experiments. All other details are as described under "Materials and Methods".

(p<0.001). plateau (Fig. 2). IL-1 M11C 20 μg/ml macrophage TNF- (data not shown). 3. M11C macrophages IL-1

M11C macrophage 20 μg/ml 8 M11C TNF- 1/10 (data not shown; 12). ELISA immunoblotting M11C macrophages ELISA IL-1 가 (Fig. 4). 4. M11C macrophages IL-1 mRNA

PCR

, IL-1 mRNA

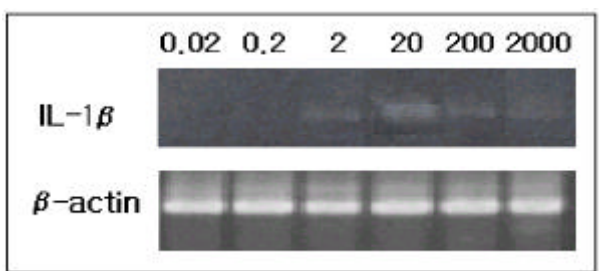


Fig. 5. The dose dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) mRNA expression from macrophage. Expression of mRNA was detected by RT-PCR. Equivalent quantities of mRNA were used since similar amounts of β -actin were expressed in each specimen. 0.02, 0.2, 2, 20, 200, and 2000 represent 0.02 $\mu\text{g/ml}$, 0.2 $\mu\text{g/ml}$, 2 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$, and 2000 $\mu\text{g/ml}$ of M11C dose, respectively, that were used for the stimulation of macrophage for 8 hours. The result is one of the five experiments. All other details are as described under "Materials and Methods".

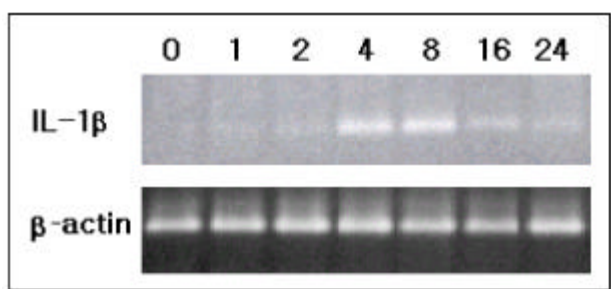


Fig. 6. The time dependent effect of Korean mistletoe crude extract (M11C) on interleukin-1 (IL-1) mRNA expression from macrophage. Expression of mRNA was detected by RT-PCR. Equivalent quantities of mRNA were used since similar amounts of β -actin were expressed in each specimen. 0, 1, 2, 4, 8, 16, and 24 represent 0 hour, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, and 24 hours of culture time, respectively, that were used for the stimulation of macrophage with M11C (20 $\mu\text{g/ml}$). The result is one of the five experiments. All other details are as described under "Materials and Methods".

M11C
 M11C macrophage M11C 0.02 $\mu\text{g/ml}$
 0.2 $\mu\text{g/ml}$ IL-1 mRNA
 (Fig. 5). ELISA
 M11C 0.2 $\mu\text{g/ml}$ 가 macrophage
 IL-1 (Fig. 2, 4).

sensitivity , PCR
 ELISA sensitivity 가
 M11C 2
 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$. M11C
 IL-1
 M11C 20 $\mu\text{g/ml}$
 IL-1 IL-1 mRNA
 가 (Fig. 2, 5).

5. M11C macrophages IL-1 mRNA
 IL-1 mRNA
 , M11C 20 $\mu\text{g/ml}$
 macrophage , 1 2
 가 4 8
 (Fig. 6).

가 M11C macrophage
 20 $\mu\text{g/ml}$
 4 8 (Fig. 5-6). 8
 IL-1 plateau
 , IL-1 mRNA
 (Fig. 3, 6).

가
 (1-7, 12). 가

가 macrophage IL-1
 KML
 M11C 가
 가 (Fig. 1).
 M11C (non-lectin com-
 ponents)
 가

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