Arch Pharm Res Vol 25, No 6, 954-963, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Immuno Activation of Lectin-Conjugated Praecoxin A on IL-6, IL-12 Expression

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(Received October 8, 2002)

Lectin-conjugated praecoxin A is a compound, which is combined Wheat Germ Agglutinin (WGA) Lectin with praecoxin A and also known to have an anti-tumor activity. In our lab, in order to investigate its immune reaction other than the anti-tumor activity ever known, we examined cytokines such as IL-6 and IL-12 through their mRNA expressions, which are generally secreted by macrophage both *in vivo* and *in vitro*. To analyze, we used RT-PCR for total RNAs of macrophages. As a result, we obtained that both *in vitro* and *in vivo*, lectin-conjugated praecoxin A showed an interesting increase on IL-6 and IL-12 even though it may be little hard to say the conjugated form is absolutely more effective than that of lectin or praecoxin A alone for immune response activities. Those results suggest that the conjugated form may give an additional opportunity in a future therapeutic use over its immuno activation properties.

Key words: Lectin-conjugated praecoxin A, Praecoxin A, IL-6, IL-12, Macrophage

INTRODUCTION

Generally in the body, there is a natural immune response reacting with target cells by a direct activation even in a certain state of non-experience to foreign antigens when exposed. The involved cells in these immune responses are polymorphonuclear lymphocyte, macrophage and NK cell. These cells are generally characterized by various functions with no relates to target cells to be attacked. Particularly, macrophage plays a role in various immune reactions and acts as an antigen presenting cell (APC) when exposed to antigen in process of a specific immune reaction. Furthermore, it plays an assistant role in the immune reaction of lymphocytes by producing various substances including cytokine (Adams *et al.*, 1984; Roitt *et al.*, 1989).

Macrophage is developed from stem cells in the midst of proliferation of the blood cells in bone marrow and moved into different body sites, where lastly located in matured form. During this period, certain cells may go to peritoneal area (Van, 1982). Monoblast producing myelomonocytic stem cells have great self-renewal capacity in cells (Goud *et al.*, 1975). In the next maturation stages of bone marrow, promonocyte is transformed into monocytes and maintained in circulatory system of the body. Monocyte then moves into other organs even though there is no local inflammatory, but monocytes located in tissues are not returned to its origin. In other words, monocytes are adequately assimilated in their tissues with a proper shape and function and become a site specific macrophage. These changes of monocyte are occurred by a tissue specific stimulation. For example, monocyte is developed to tissue specific cells, such as kuffer cell in liver, synovial macrophage in cartilage site, peritoneal macrophage in peritoneal cavity and microglia in brain. Moreover, monocytes also become alveolar macrophage in lungs, which pass epithelial cells.

Properties of macrophage include a specific response to stimulants, such as lipopolysaccharide (LPS) or IFN-γ, which is very attractive both in physiological and pharmacological way. Such responses of macrophage are known to produce approximately 100 substances ranging from 32 MW (superoxide anion) up to 440,000 (fibronectin) as well as cytokines, and to secrete cytolytic protease (CP) and tumor necrosis factor (TNF), a factor to degrade tumor cells (Ezekowitz *et al.*, 1984; Sarih *et al.*, 1992).

In murine, cytokines released from macrophage include IL-1, IL-6, IL-8, IL-12, TNF, GM-CSF. Cytokine is a protein of small MW (<8-25 kDa) and consists of single chains. Certain cytokine is reactive to specific cell forms, and also

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cytokines control important biological reactions, such as cell growth, cell activation, inflammation, immunity, tissue recover, fibrosis and morphology. In specific, IL-6 is known as B cell generator or intercellular stimulator and is produced in T cell, macrophage, B cell, fibroblast and endothelial cell. Although IL-6 usually acts in all cells, they induce antibody-forming cell when reacted with B cell. Moreover, it is considered to be an important growth factor for multiple myeloma and malignancy of plasma cell. In contrast, IL-12 is produced by both macrophage and B cell and induces a response of Th1 type and production of IFN-γ when activated by macrophage and NK cell (Brodsky *et al.*, 1994; Chantry *et al.*, 1991; Clark *et al.*, 1994; Parker, 1993; Romagnani, 1994; Ullman *et al.*, 1989; Abbas *et al.*, 1991; Akira *et al.*, 1993; Arai *et al.*, 1990; Balkwill *et al.*, 1989; Hirano *et al.*, 1990).

For the study, we used "lectin-conjugated praecoxin A", a conjugated form of praecoxin A (tannin; $C_{41}H_{28}O_{27}$ M.W. =952.08, Fig. 1.) with wheat germ agglutinin(WGA), a tumor cell specific (Kim *et al.*, 2001; Ogawara *et al.*, 1987; Ogawara, *et al.*, 1985). Basically, this lectin-conjugated praecoxin A was aimed for an antitumor missile agent, which is selectively effective on tumor cells (Lee *et al.*, 1992; Chang *et al.*, 1995).

Ellagitannin is one of hydrolytic tannins and includes hexahydroxydiphenoyl (HHDP) or HHDP particle in molecule. Ellagitannins have been isolated more than that of gallotannins, and these ellagitannins are differentiated from other tannins in that those can be isolated as relatively stable substances. In light of this, such properties of ellagitannin provide us with possibilities of physiological and pharmacological activities (Nishioka, 1983).

Based on the backgrounds of above, we tried to study on the role of macrophage when exposed to lectin-conjugated praecoxin A. This study was targeted to prove additive effects other than an anti-tumor effect by lectin-conjugated praecoxin A, particularly in immune reaction and mechanical activity of macrophage.

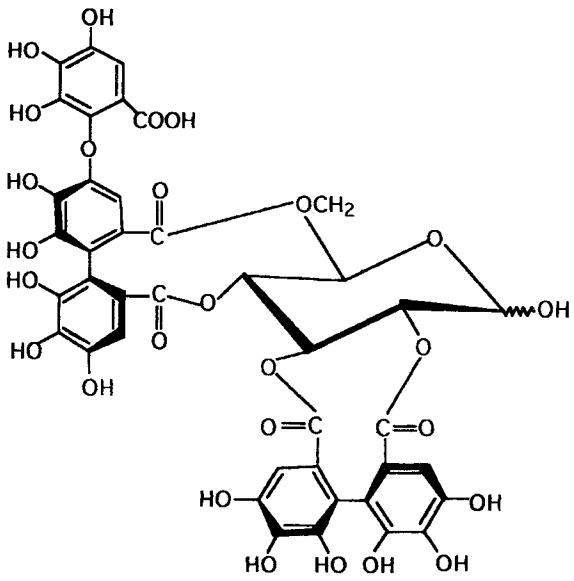

Fig. 1. The chemical structure of praecoxin A

METHODS AND MATERIALS

Test animal

The study was performed by using 5 week-old ICR mice with one week of adaptation period (total 6 week old ICR mice).

Test drug

Praecoxin A (a purified ellagitannin; M.W. = 952.08), Lectin (Wheat Germ Agglutinin, WGA), Lectin-conjugated Praecoxin A. were taken by sterilizing through 0.22 μm filter after the complete dissolution in RPMI and PBS (pH 7.2).

Reagent

LPS; *E. coli* serotype 0111:B4 (Sigma Chem. Co., U.S.A.), ethyl ether (Tedia Company, Inc. U.S.A.), FBS (Gibco BRL., U.S.A.), penicillin-streptomycin (Gibco BRL., U.S.A.), trypsin-EDTA (Gibco BRL., U.S.A.), total RNA extraction kit (Pharmacia Biotech Co., U.S.A.), DEPC (Sigma Chem. Co., U.S.A.), β-mercaptoethanol (Sigma Chem. Co., U.S.A.), ethanol (Merck Co., Germany), RT-PCR kit (Bioneer Co., U.S.A.), trizma base (Sigma Chem. Co., U.S.A.), EDTA (Sigma Chem. Co., U.S.A.), bromphenol blue (Sigma Chem. Co., U.S.A.), glycerol (Pharmaco-Chemical Industry Co., Ltd., Japan), acetic acid (Pharmaco-Chemical Industry Co., Ltd., Japan), ethidium bromide (Sigma Chem. Co., U.S.A.), agarose (Sigma Chem. Co., U.S.A.), RPMI 1640 (Sigma Chem. Co., U.S.A.) and trypan blue (Sigma Chem. Co., U.S.A.), 1Kb DNA ladder (Promega Co., U.S.A.) were used. In addition, other buffers and reagents used in the study were formulated in lab with the first or super graded reagents.

Tools and equipments

Clean bench (VISION Scientific Co., Korea), CO2-incubator (SANYO Electric Co., Japan), centrifuge (VISION Scientific Co., Korea), microcentrifuge (VISION Scientific Co., Korea), deep freezer (SANYO Electric Co., Japan), inverted microscope (OLYMPUS Optical Co., Japan), electrophoresis (VISION Scientific Co., Korea), vortex mixer (Scientific Industries INC., U.S.A.), thermal cycler (Barnstead Thermolyne Corporation., U.S.A.), electronic balance (A&D Co., Japan), UV-VIS spectrophotometer (Shimadzu Corporation., Japan), polaroid type 667 film (Polaroid Co., United Kingdom), pH meter (Mettler toledo, Switzerland), autoclave (Vision sci. co., Korea) were used,

and all other tools and equipments used during the study were autoclaved at 121°C, 1.1 kg/cm^2 for 20 minutes.

Peritoneal macrophage separation

Murine macrophage was separated as described previously (Coligan *et al.*, 1996; Joo *et al.*, 2002). 6 week-old (20~25 g, male) ICR mice were killed by cervical dislocation and peeled off the abdomen skins, and then serum free RPMI 1640 medium 6 and air 3 ml were injected into peritoneal and withdrawn 2-3 minutes after massage. After repeating those procedures up to 3 times, we took most the peritoneal cells from mouse. The cells were centrifuged for 10 minutes at 100 g after diluting an appropriate medium. Precipitated cells were suspended in RPMI 1640 medium and took plate-adhered macrophages 2 hrs after the culture in CO_2-incubator, 37°C, 5% CO_2, 95 % air.

Macrophage culture

Macrophages were cultured at RPMI 1640 medium in incubator at 37°C, 5% CO_2, and used 3-4 days after the culture periods for *in vitro* test.

***In vitro* Test**

Macrophages were taken from 6 week-old ICR mouse and placed 5×10^6 cells on to 75 cm^2 flask and cultured for 3~4 days. Lectin-conjugated praecoxin A, lectin, praecoxin A, LPS were used by dissolving in media. To investigate the efficacy in each drug with macrophage, we followed protocol as described previously (Coligan *et al.*, 1996).

After replacing media, lectin-conjugated praecoxin A 1, 10, 100 μg/ml (1.27, 12.7, 127 uM), lectin 10 μg/ml (12.7 uM), praecoxin A 10 μg/ml (12.7 uM) were added and cultured for 4, 8, 12, 24. Trypsin was added for collecting cells. Taken cells were stored at -70°C until RNA extraction. Negative controls were cultured in media and positive controls were cultured after adding LPS 1 μg/ml (12.7 μM) with the same method of above.

***In vivo* Test**

By designing four 6 week-old, ICR mice, as a group, the study was carried out as described previously (Coligan *et al.*, 1996). Lectin-conjugated praecoxin A, lectin, praecoxin A, LPS were melted at PBS (pH 7.2) and taken for the study.

For test groups, lectin-conjugated praecoxin A 1, 5, 10 mg/kg, lectin 5 mg/kg, praecoxin A 5 mg/kg were injected into mouse peritoneal, and macrophages were taken 4, 8, 12, 24 hours after above procedures. For negative control, PBS buffer 0.1 ml was added and as a positive control, LPS 1 mg/kg was added. Test method was performed with the same protocol as above, and the amount of each drug was adjusted to 0.1 ml and stored at -70°C until RNA extraction.

RNA extraction

5×10^6 cells of macrophage were taken after 4, 8, 12, 24 h and followed by RNA extraction. Total RNA extraction was carried out in accordance with instruction of Pharmacia Biotech. After deicing cells, LiCl solution 350, β-mercaptoethanol 3 μl, extraction buffer 150 μl, CsTFA 500 μl were added and lysed by vortexing. To wash the obtained total RNA, extraction buffer 75 μl, LiCl solution 175 μl, CsTFA 250 μl were added, and then 70% of ethanol was added. After the vortex, ethanol was eliminated after 5 minutes of centrifugation and they were dried for 15 minutes at room temperature. 0.1% DEPC-treated water was added to total RNA and placed them on ice for 15~30 minutes. We then vortexed and keep them for 10 minutes at 65°C. and stored at -20°C.

RNA confirmation (RNA loading)

To confirm the obtained RNAs from above, 2 μl of total RNA was taken and 3 μl of RNA loading buffer, 7 μl of DEPC water were mixed and then loaded on 1% agarose gel including ethidium bromide. Until bromphenol blue was moved up to 2/3 length of the gel, current 70~80A was maintained for 20~30 minutes, and then confirmed bands at 260 nm.

RNA quantification (OD measurement)

The concentration of obtained RNA was measured through spectrophotometer at 260 nm. After calculating total RNAs described below, we quantified 1 μg of total RNA for use of Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

$$[\text{RNA}] = \text{OD260 nm} \times D \times 40\ \mu\text{g/ml}\ (D = \text{final dilution factor})$$

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

1 μg of total RNA reverse transcription was used and 20 U of Moloney Murine Leukemia Virus reverse transcriptase, 10 U of RNasin, 1 U of thermostable DNA polymerase, and 20 μl RT-PCR kit including dNTP were used. Protocol of Bioneer was referred for RT-PCR. RNA 1 μg, oligo(dT) 18 25p mole, primer 50p mol, RNase-free water were filled up to 20 μl, and the response of reverse transcription was performed. RNA denaturation was processed for 10 minutes at 57°C by heating, and cDNA was synthesized for 60 minutes at 42°C. We then inactivated reverse transcriptase for 5 minutes at 94 by heating and placed them on ice (4°C). PCR amplification was responded for 0.5 min. at 94°C, for 1.5 min. at 55°C, 1 min. at 68°C and amplified for 34 cycles. 3 μl of PCR product was loaded on 1.8% agarose gel, and IL-6, 12 primers that used in PCR

were as below.

IL-6	5' primer	TGGAGTCACAGAAGGAGTGGCTAAG
	3' primer	TCTGACCACAGTGAGGAATGTCCAC
IL-12	5' primer	CTCACATCTGCTGCTCCACAA
	3' primer	CTCCTTCATCTTTTCTTTCTT

PCR quantification

The density obtained from cDNA band was measured through UVIDocMW (UVI tech, ver. 99.03) computer program. In brief, we took a photo of each PCR band, scanned and input the information in computer for the final measurement.

RESULTS

In vitro

In the study, we found that IL-6 mRNA and IL-12 mRNA were released when exposed to study drugs by macrophage in a timely manner. However, when adding LPS 1 μg/ml as positive control, both were increased at 4 to 24 hour compared to baseline. In specific, lectin-conjugated praecoxin A 1 μg/ml increased IL-6 mRNA at 24 hour compared to baseline, and little more increase than that was found in LPS. In contrast, at 4, 8, 12 hour, it was slightly increased compared to baseline signal but increased more weakly compared to LPS. In lectin-conjugated praecoxin A 10 μg/ml, IL-6 mRNA was increased at 4, 8, 24 hour compared to baseline but was also increased more weakly compared to LPS. Moreover, in lectin-conjugated praecoxin A 100 μg/ml, IL-6 mRNA and IL-12 mRNA were increased at 4 to 24 hour and similarly increased in LPS. In lectin 10 μg/ml, IL-6 mRNA and IL-12 were slightly increased at 4 to 24 hour but weakly increased compared to LPS. In praecoxin A 10 μg/ml, IL-6 mRNA was slightly increased at 8, 12, 24 hour compared to baseline but more weakly increased than that of LPS. Similar results were observed in IL-12 mRNA. When comparing lectin-conjugated praecoxin A 10 μg/ml, lectin 10 μg/ml and praecoxin A 10 μg/ml, IL-6 mRNA expression revealed little weaker effect in lectin-conjugated praecoxin A than that of praecoxin A and lectin (Fig. 2). However, no big differences were found in IL-12 mRNA expression (Fig. 3).

In vivo

As described *in vitro* test, we found that IL-6 mRNA and IL-12 mRNA were released at untreated baseline even though it was a small amount. When adding LPS 1 μg/ml as positive control, IL-6 mRNA was increased at 4 to 24 hour, and IL-12 mRNA was increased at 8 to 24 hour. In lectin-conjugated praecoxin A 1 mg/kg, IL-6 mRNA was slightly increased at 24 hour compared to baseline but increased more weakly than that was found in LPS. In addition, no changes were found in IL-12 mRNA compared to baseline signal. In lectin-conjugated praecoxin A 5 mg/kg, IL-6 mRNA was increased at 12, 24 hour compared to LPS. Similar results were observed in IL-12 mRNA. Moreover, in lectin-conjugated praecoxin A 10 mg/kg, IL-6 mRNA was increased very similarly to LPS, whereas IL-12 mRNA increased at 12 to 24 hour similarly to LPS and increased more weakly than that was found in LPS. In lectin 5 mg/kg and Praecoxin A 5 mg/kg, IL-6 mRNA and IL-12 mRNA were increased at 12 to 24 hour though such increase was weaker than that of LPS. In lectin-conjugated praecoxin A 5 mg/kg, lectin 5 mg/kg and praecoxin A 5 mg/kg, no big differences were found in IL-6 mRNA and IL-12 mRNA expression (Fig. 4, Fig. 5).

CONCLUSION

In general, there are IL-1 and IL-6, pro-inflammatory cytokines and IL-12, IL-15, T cell growth and differentiation factors. As described in introduction part, cytokines released from macrophage include IL-1, IL-6, IL-8, IL-12, TNF and GM-CSF. Of many cytokines released from macrophage, we focused on pro-inflammatory cytokine and T cell growth and differentiation such as IL-6 and IL-12.

In vitro, IL-6 and 12 mRNA resulted from the treatment of lectin-conjugated praecoxin A, lectin, praecoxin A with macrophage were measured by means of times and doses. As a result, IL-6 mRNA was increased at 24 hour In lectin-conjugated praecoxin A 1 μg/ml, at 4, 8, 24 hour in lectin-conjugated praecoxin A 10 μg/ml and at 24 hour both in lectin 10 μg/ml, praecoxin 10 μg/ml, respectively. However, those all were weakly increased compared to LPS.

Similarly, in lectin-conjugated praecoxin A 100 μg/ml, IL-6 mRNA was increased at 24 hour though it was a bit weaker than LPS. IL-12 mRNA was increased at 4, 8, 12 hour in lectin-conjugated praecoxin A 1 μg/ml, at 24 hour in lectin-conjugated praecoxin A 10 μg/ml and at 8, 12, 24 hour both in lectin 10 μg/ml and praecoxin 10 μg/ml though they were a bit weaker than LPS. In lectin-conjugated praecoxin A 100 μg/ml, IL-12 mRNA was increased at 24 hour and the increase was almost same with LPS. Therefore, *in vitro,* lectin-conjugated praecoxin A is thought to increase IL-6, 12 mRNA expressions in accordance with time periods and doses in normal murine macrophage. This represents that *in vitro,* lectin-conjugated praecoxin A can act as an activating agent of macrophage. In addition, lectin or praecoxin A itself may also have a possibility to activate macrophage in a certain degree, *in vitro*.

In vivo, IL-6, 12 mRNA were measured by times and doses from macrophage after adding lectin-conjugated praecoxin A, lectin and praecoxin A into mice. In this result, IL-6 mRNA was increased at 24 hour in lectin-conjugated

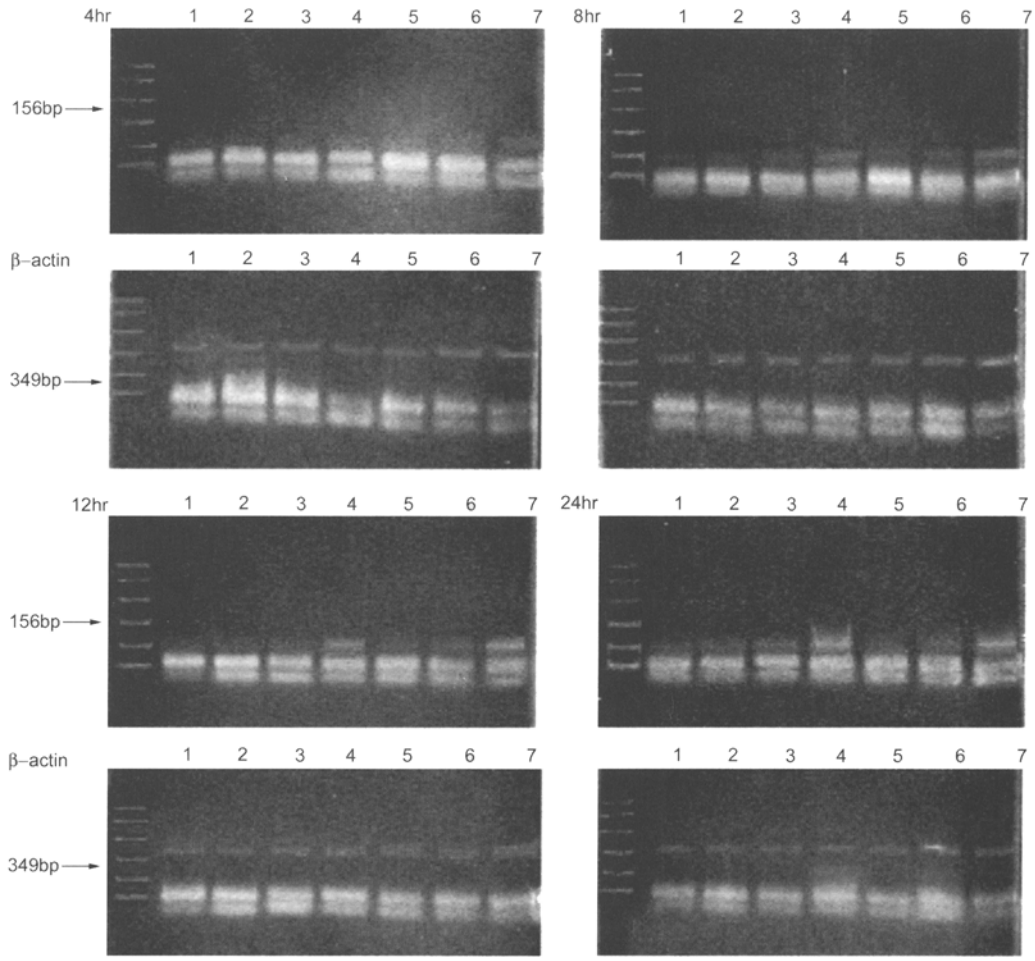

Fig. 2-1. The effect of lectin-conjugated praecoxin A inducing IL-6 mRNA in murine macrophage *in vitro*. Total RNA extracted from murine macrophage 4, 8, 12, 24 hr after applications of 1, 10, 100 μg/ml of lectin-conjugated praecoxin A, 10 μg/ml of lectin, 10/ of praecoxin A, was analyzed by RT-PCR for the identification and the induction of IL-6 mRNA and β-actin mRNA. 1 μg/ml of LPS was used as a positive control. 1 = Baseline signal, 2 = Lectin-conjugated praecoxin A 1 μg/ml, 3 = Lectin-conjugated praecoxin A 10 μg/ml, 4 = Lectin-conjugated praecoxin A 100 μg/ml, 5 = Lectin 10 μg/ml, 6 = Praecoxin A 10 μg/ml, 7 = LPS 1 μg/ml.

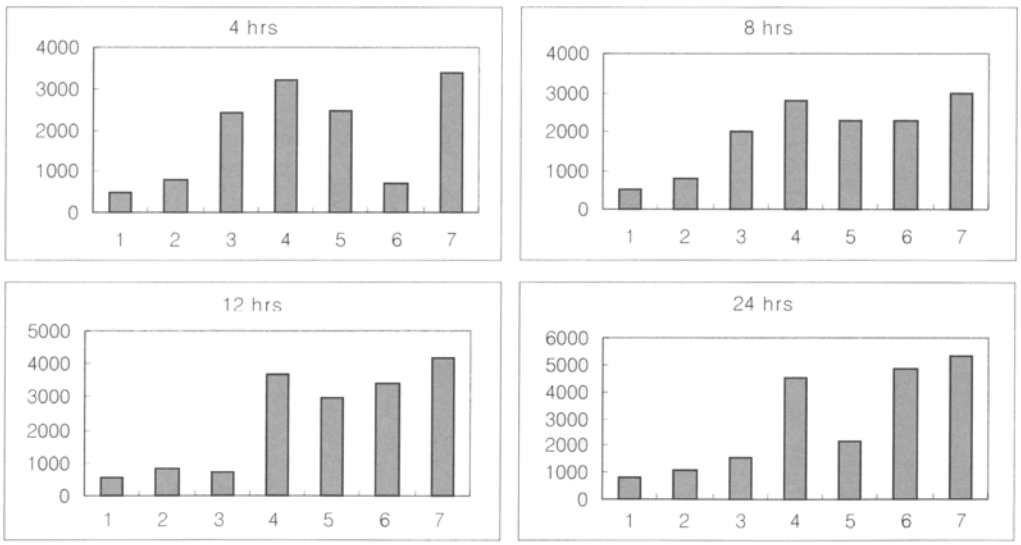

Fig. 2-2. The effect of lectin-conjugated praecoxin A inducing IL-6 mRNA in murine macrophage *in vitro*. (density comparison).

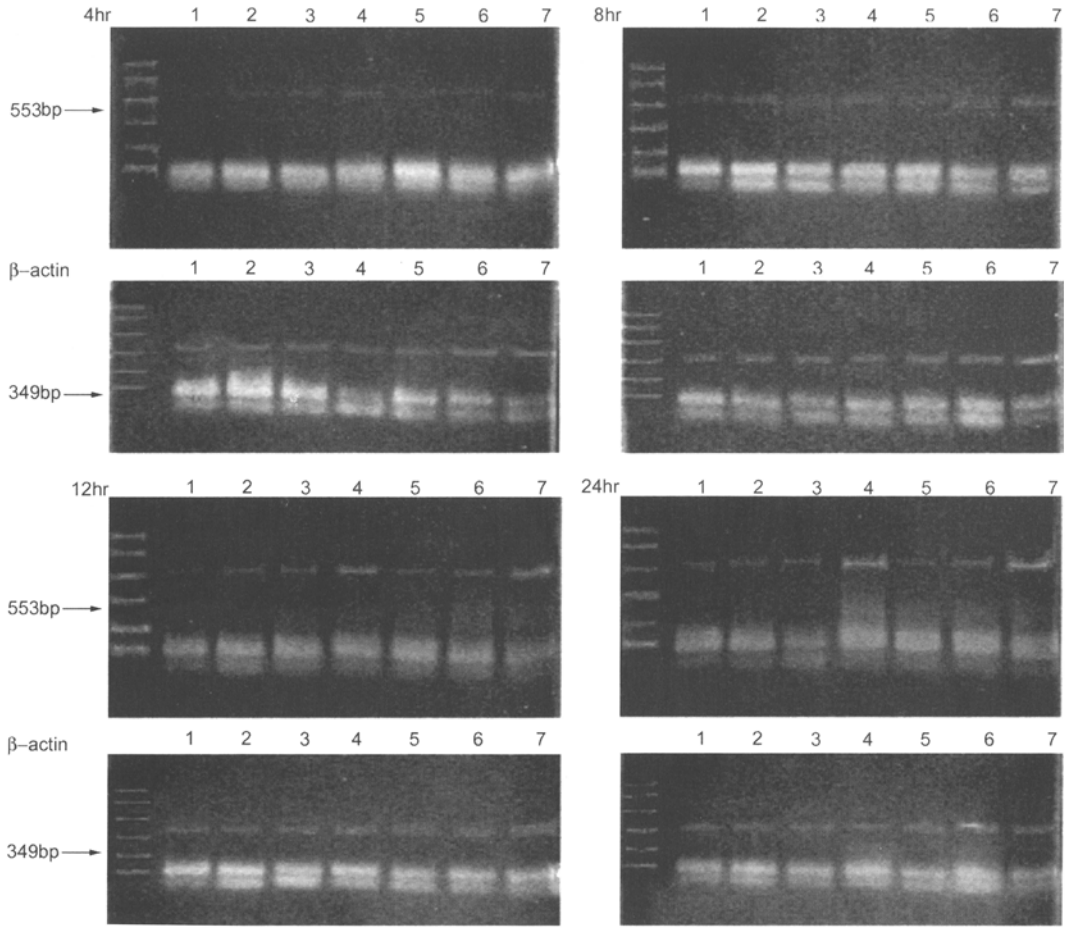

Fig. 3-1. The effect of lectin-conjugated praecoxin A inducing IL-12 mRNA in murine macrophage *in vitro*. Total RNA extracted from murine macrophage 4, 8, 12, 24 hr after applications of 1, 10, 100 μg/ml of lectin-conjugated praecoxin A, 10 μg/ml of lectin, 10 μg/ml of praecoxin A, was analyzed by RT-PCR for the identification and the induction of IL-12 mRNA and β-actin mRNA. 1 μg/ml of LPS was used as a positive control. 1 = Baseline signal, 2 = Lectin-conjugated praecoxin A 1 μg/ml, 3 = Lectin-conjugated praecoxin A 10 μg/ml, 4 = Lectin-conjugated praecoxin A 100 μg/ml, 5 = Lectin 10 μg/ml, 6 = Praecoxin A 10 μg/ml, 7 = LPS 1 μg/ml.

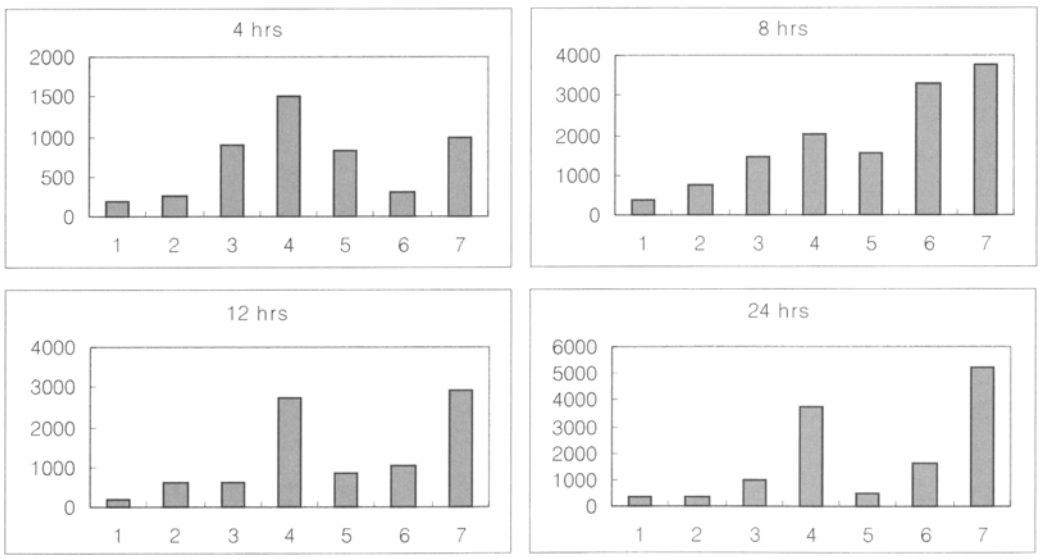

Fig. 3-2. The effect of lectin-conjugated praecoxin A inducing IL-12 mRNA in murine macrophage *in vitro* (density comparison).

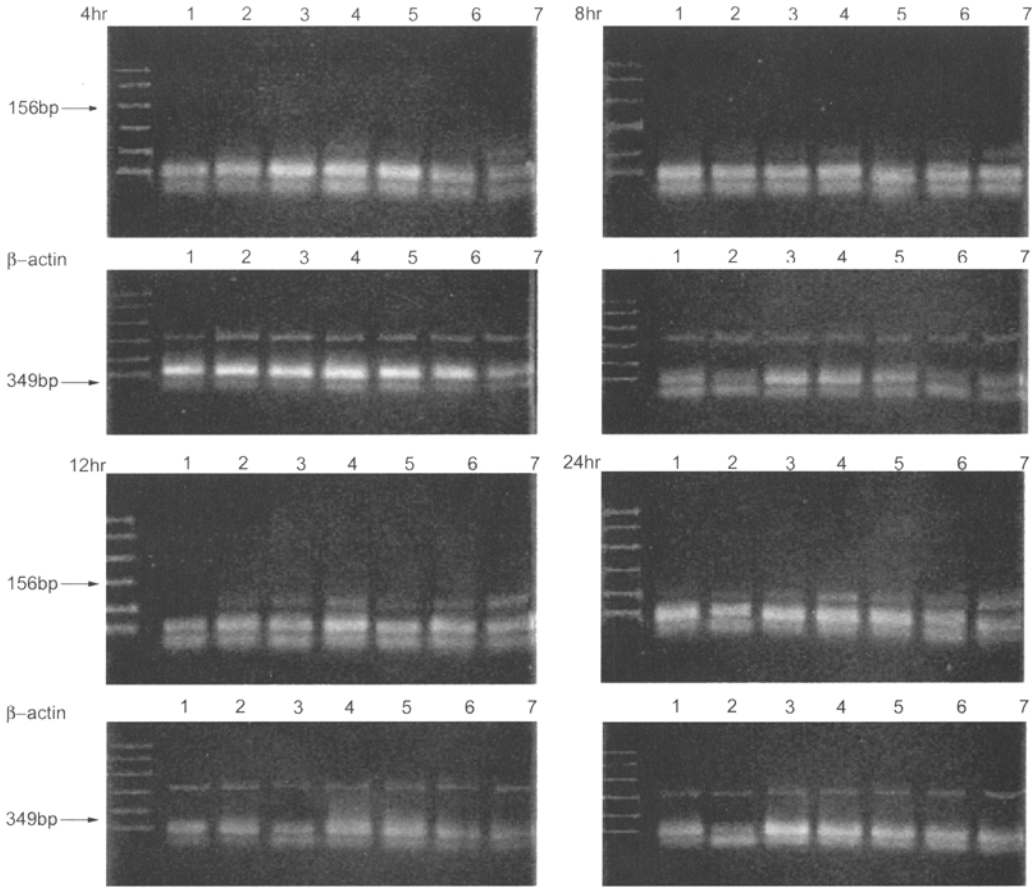

Fig. 4-1. The effect of lectin-conjugated praecoxin A inducing IL-6 mRNA in murine macrophage *in vivo*. Total RNA extracted from murine macrophage 4, 8, 12, 24 hr after injections of 1, 5, 10 mg/ml of lectin-conjugated praecoxin A, 5 mg/ml of lectin, 5 mg/ml of praecoxin A, was analyzed by RT-PCR for the identification and the induction of IL-6 mRNA and β-actin mRNA. 1 mg/ml of LPS was used as a positive control. 1 = Baseline signal, 2 = Lectin-conjugated praecoxin A 1 mg/ml, 3 = Lectin-conjugated praecoxin A 5 mg/ml, 4 = Lectin-conjugated praecoxin A 10 mg/ml, 5 = Lectin 5 mg/ml, 6 = Praecoxin A 5 mg/ml, 7 = LPS 1 mg/ml.

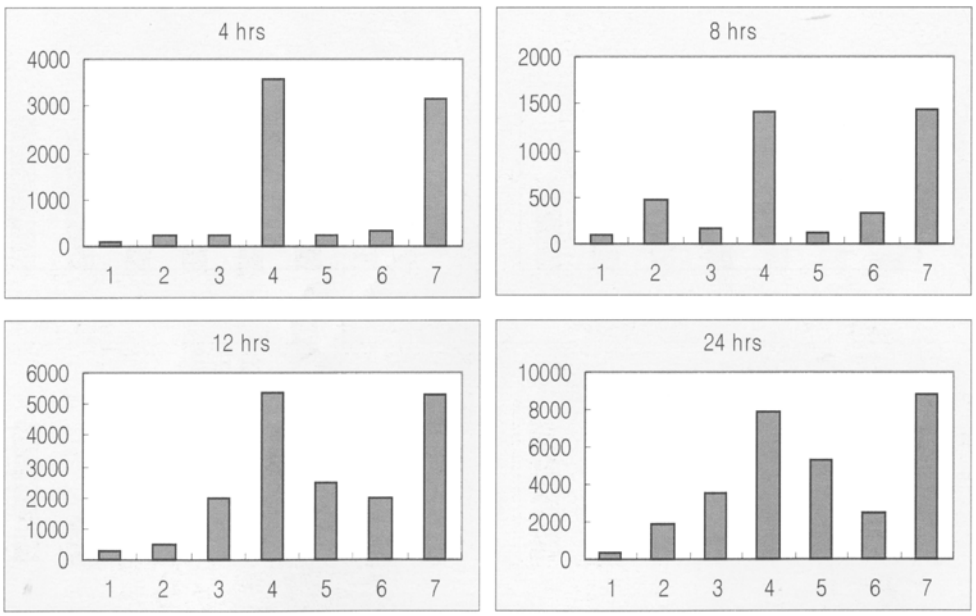

Fig. 4-2. The effect of lectin-conjugated praecoxin A inducing IL-6 mRNA in murine macrophage *in vivo* (density comparison).

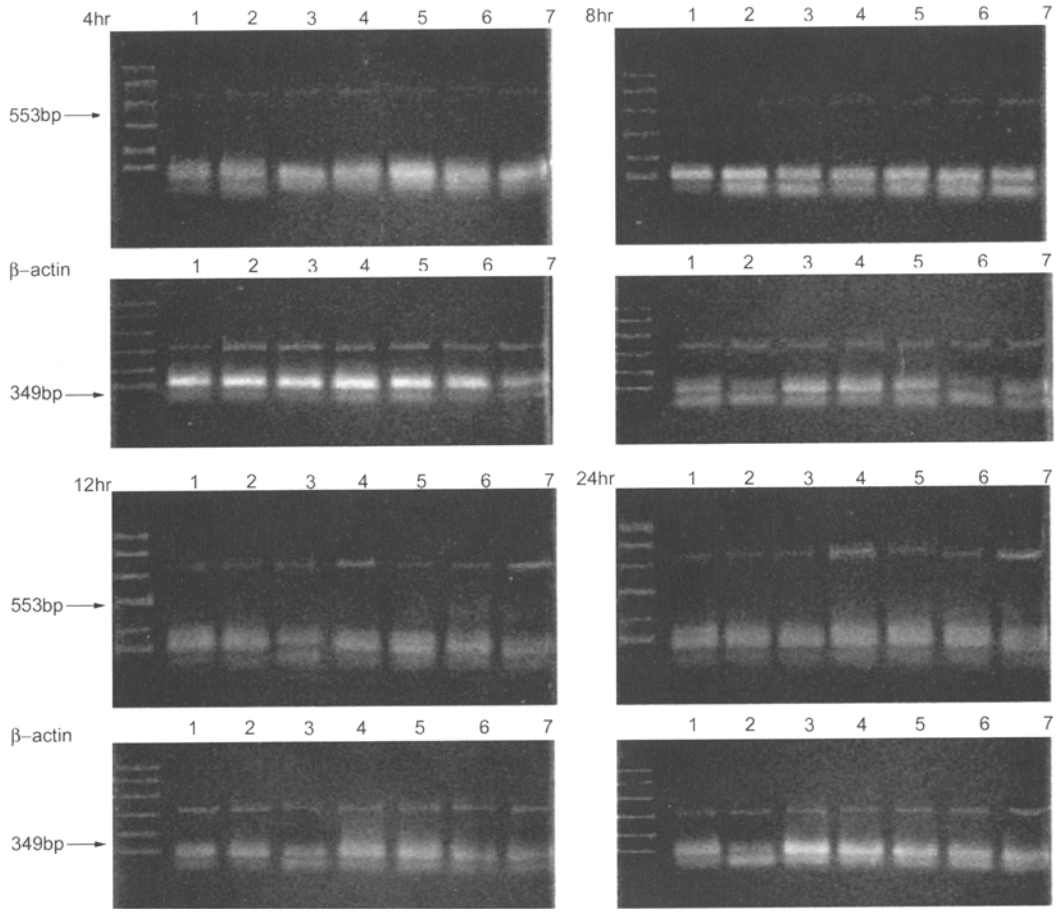

Fig. 5-1. The effect of lectin-conjugated praecoxin A inducing IL-12 mRNA in murine macrophage *in vivo*. Total RNA extracted from murine macrophage 4, 8, 12, 24 hr after injections of 1, 5, 10 mg/ml of lectin-conjugated praecoxin A, 5 mg/ml of lectin, 5 mg/ml of praecoxin A, was analyzed by RT-PCR for the identification and the induction of IL-12 mRNA and β-actin mRNA. 1 mg/ml of LPS was used as a positive control. 1 = Baseline signal, 2 = Lectin-conjugated praecoxin A 1 mg/ml, 3 = Lectin-conjugated praecoxin A 5 mg/ml, 4 = Lectin-conjugated praecoxin A 10 mg/ml, 5 = Lectin 5 mg/ml, 6 = Praecoxin A 5 mg/ml, 7 = LPS 1 mg/ml.

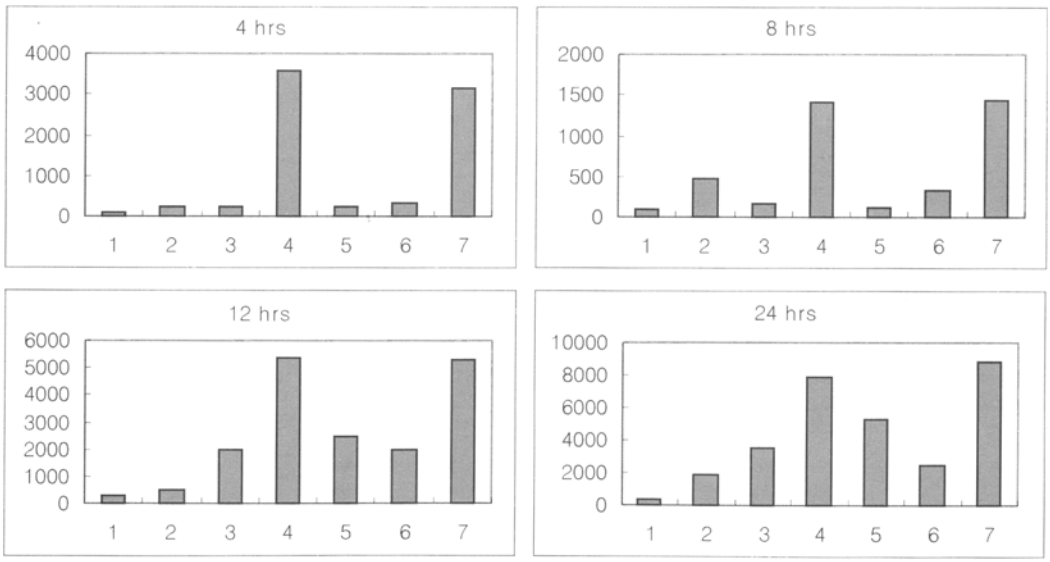

Fig. 5-2. The effect of lectin-conjugated praecoxin A inducing IL-12 mRNA in murine macrophage *in vivo* (density comparison).

praecoxin A 1 mg/kg and IL-12 was increased at 12 and 24 hour though their increases were weaker than that of LPS. In lectin-conjugated praecoxin A 10 mg/kg, IL-6 mRNA was increased at 24 hour and the increase was almost same with LPS. In contrast, IL-12 mRNA was increased at 12 and 24 hour in lectin-conjugated praecoxin A 5 mg/kg and lectin 5 mg/kg and also increased at 8, 12, 24 hour in praecoxin A 5 mg/kg though their increases were weaker than that of LPS. In lectin-conjugated praecoxin A 10 mg/kg, IL-12 mRNA was increased at 4, 12, 24 hour, and it was almost same with LPS except for 4 hour. Therefore, *in vivo,* lectin-conjugated praecoxin A is thought to increase IL-6, 12 mRNA expressions in accordance with times and doses in normal murine macrophage. This represents that *in vivo,* lectin-conjugated praecoxin A can act as an activating agent of macrophage. In addition, lectin and praecoxin A may also have a possibility to activate macrophage, *in vivo.*

In summary, there were no big differences observed between IL-6 and IL-12 mRNA in same doses of lectin-conjugated praecoxin A, lectin and praecoxin A both *in vitro* and *in vivo*. Additionally, IL-6 and IL-12 were also expressed even in small amounts at baseline of lectin-conjugated praecoxin A, lectin, praecoxin in normal murine macrophage.

Moreover, increase of IL-12, a T cell growth and differentiation factor may induce Th1 cell and inactivate the CTL differentiation, and lastly reproduction of NK, LAK cell may be activated.

Considering the results mentioned above, both *in vitro* and *in vivo,* IL-6 and IL-12 were increased when adding lectin-conjugated praecoxin A to normal murine macrophage, and this may cause an increase of activation of NK cell, CTL, B cell and LAK cell. In addition, non-conjugated lectin and praecoxin A single dose showed to slightly increase the release of IL-6, IL-12. However, lectin-conjugated praecoxin A, a conjugation of lectin and praecoxin A, showed better results in IL-6, IL-12 mRNA expression than that of the same concentration in lectin or praecoxin A. In light of this result, an outstanding increase on immuno-activity effect is an assignment of future to be assessed.

In addition, as lectin has been known to have a tumor cell specificity in functional aspect (Kim *et al.*, 2001; Ogawara *et al.*, 1987; Ogawara *et al.*, 1985) while Praecoxin A has been known to have an anti-tumor effect, we can expect that lectin-conjugated praecoxin A may show more effective anti-tumor effect with good specificity than praecoxin A and lectin alone, which means to lead a synergic effect. In future, the study on the anti-tumor effect of lectin-conjugated praecoxin A remains to be identified in various fields.

In conclusion, the results of this study suggested that lectin-conjugated praecoxin A can play a certain role in activating macrophage and may have immuno-activity effect in addition to a direct anti-tumor effect. Furthermore, lectin-conjugated praecoxin A had an equal effect to lectin and praecoxin A in immuno-activity effect, and thus we believe that this study will be a path of future study on the tumor specificity of lectin-conjugated praecoxin A over immuno-activity reaction in the body.

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