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Biostatic activity of *Coix lacryma* seed extract on *Toxoplasma gondii* in macrophages

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Abstract: Water extract of *Coix lacryma* seeds (Co-Ex) was separated into several components; dissolved with Tris-Cl buffer and the supernatant (WC1), ammonium sulfate treatment supernatant (WC2) and the pellet (WC3), QAE column chromatography of WC1 and the peak portions; WC4, WC5 and WC6. Murine peritoneal macrophages in DMEM containing 10% heat-inactivated FCS were infected with tachyzoites of *Toxoplasma gondii*, RH strain, *in vitro*. By adding modulators such as Co-Ex, WC1,2,3,4,5,6 and LPS or IFN-γ for 24 hrs, toxoplasmastatic activity of macrophages was examined in relation to nitrite production. Nitrite production of macrophages was enhanced especially in the series of WC2, WC1 and the combination sample (WC1 + WC2 + WC3) by order, than other components or fractions (WC4, WC5, WC6) tested. Toxoplasmastatic actions such as percentage of the macrophages infected by *T. gondii* and fold increase of *T. gondii* in macrophages showed retroverse relations with the amount of nitrite production; *i.e.* as nitric oxide (NO) increased the phagocytic index of macrophages and the fold increase of tachyzoites in macrophages decreased. Nitrite (NO_2^-) production was increased by adding IFN-γ in all cases together with enhancement of biostatic effects. Through the results obtained, it is speculated that some components other than the non-proteinous and defatted components in *Coix lacryma* seeds may contribute to activate macrophages through induction of NO for the biostatic activity.

Key words: *Toxoplasma gondii*, macrophage, *Coix lacryma*, reactive nitrogen intermediates, fold increase

INTRODUCTION

Coix lacryma-jobi L. var *frumentacea* Makino is an annual plant which is popular in rural areas in Korea. The seed has been appreciated as a supplementary foodstuff and for medical purposes. According to literature, it is effective for purulent disease of the respiratory organs (Dong-E Bo-Gam p.1109, Poong-Yeonsa Co. Korea,1966), malignant diseases (Joong-Yak Daesa-Jeon II, p.2636, Rep. of China) or cutaneous warts (Honso-Hiko, p.163, Korin-Shoin,Japan, p.163). The seed contains ; water 8.5, protein crude 17.6, fat crude 7.2, starch 51.6 and ash 2.3 by percentile (Karigome, 1943). It is said that cutaneous warts disappear when the powdered seed is taken orally for several weeks. Kaneda *et al.* (1992), Hidaka *et al.* (1994) and Numata *et al.* indicated that *Coix* seed may modulate

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peripheral blood lymphocyte subsets and effective for viral diseases through enhancement of cytotoxic activity. However, since no scientific data in regard to the intracellular parasites were reported hitherto in literature, Soh *et al.* (1994) tried an experimental study with *Coix lacryma* seed extract, reporting some biostatic effect on the multiplication of *Toxoplasma gondii* in macrophages.

Acquired immunity to intracellular parasites is mainly dependent on mononuclear cells such as the macrophage, monocyte and lymphocyte. Hitherto, a number of reports indicated that cytotoxic effects against intracellular pathogens were due to the nitric oxide (NO) production of the host cells as reactive nitrogen intermediates (RNIs), together with cytokines such as interferon-γ (IFN-γ), interleukins (ILs) or biological response modifiers (BRMs) as activator or modulator (Jun *et al.*, 1993; Gazzinelli *et al.*, 1993). Along with the theoretical background,IFN-γ, bacterial lipopolysaccharide (LPS) and tumor necrosis factors (TNFs) have been popularly utilized for *in vitro* experiments with competent findings. Oh *et al.* (1995) reconfirmed that IFN-γ activated macrophages as primer in producing NO, and enhanced the production by combining with LPS as a trigger.

In regard to the natural herbs which may enhance the macrophage activity for production of cytotoxic chemicals such as reactive nitrogen intermediates (RNIs) or reactive oxygen intermediates (ROIs) have not yet been systematically evaluated. Soh *et al.* (1994) examined several herbs in anticipating whether any of the herbs hold potential to activate macrophages in producing nitric oxide (NO), and found that *Coix lacryma* was the promising biological response modifier (BRM) as modulator or synergizer in activating macrophages. The *Coix lacryma* water extract (Co-Ex) showed synergism with murine IFN-γ in activating murine peritoneal macrophages to produce nitrogen intermediates such as NO, consequenting biostatic effects to *T. gondii* in the cell. The present study aimed to elucidate the potential component in the water extract for enhancement of cytotoxic effects of macrophages.

MATERIALS AND METHODS

Herb

Dried seeds of *Coix lacryma* (Fig. 1) were peeled off and powdered. The powder, 200 gm, was put in 2.0 l of distilled water and kept at 5°C for 72 hours, stirred time to time followed by filtration with ordinary filter paper; high pressure saturation at 121°C for 15 minutes, centrifugation at 3,000 rpm for 1 min (sediment away); put talc, stir and separate by decompression method repeating several times until the filtrates become yellow. The filtrates were mixed with chloroform in a separation funnel, stirring for 5 min. After removing chloroform, hexane was poured in and left overnight; the filtrates were separated from hexane by heating at 80-90°C and filtered with a membrane filter. The yield of extract was about 3-4% of the raw powder. It was

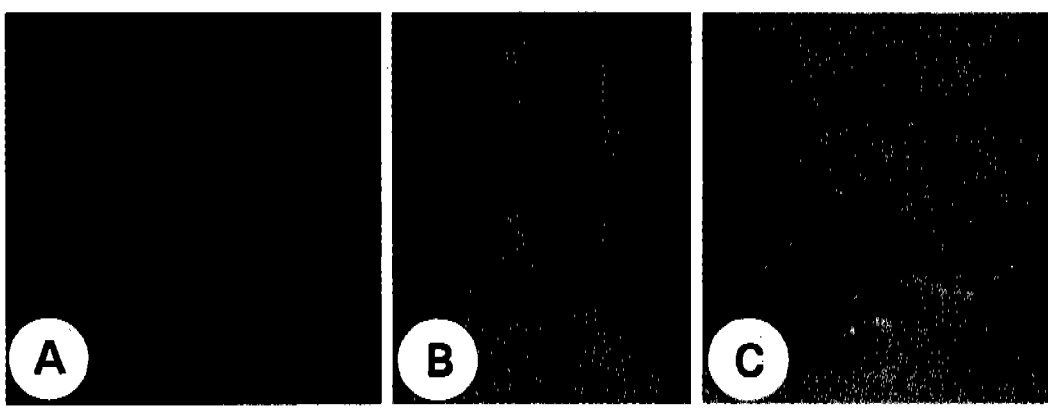

Fig. 1. *Coix lacryma-Jobi L.* var *frumentacea* Makino. **A.** plant; **B.** seeds; **C.** seeds-peeled off.

lyophilized and kept in a refrigerator until use as a water extract (Co-Ex)(Soh *et al.* 1994). Before use, Co-Ex, 30 mg, was dissolved in 1.0 ml distilled water, filtered through membrane filter (pore size 0.22 μm). For further fractionation of Co-Ex, the following procedures were done; Co-Ex, 15 gm, dissolved in 100 ml of 10 mM Tris-Cl (pH 7.4) stirred at times, centrifugation 20,000 rpm for 1 hr and collect the supernatant (WC-1). WC-1 was precipitated with 70% ammonium sulfate, centrifuge at 20,000 rpm for 1 hr, then separated the supernatant (WC-2) and pellet. The pellet was dissolved in 20 ml of 10 mM Tris-Cl (pH 7.4)(WC-3). WC-2 and WC-3 were dialyzed in 2 L of 10 mM Tris-Cl (pH 7.4) overnight to remove salts, then concentrated for 8 hrs by lyophilization. Another portion of WC-1 was put to QAE colum chromatography, and obtained several fractions, predicting oligopeptides or glycoprotein in process of time; peak1 (WC-4), peak2 (WC-5), peak3 (WC-6) (Fig. 2). The dialysate was applied to a 3.0 × 10 cm column of QAE preequilibrated with 0.01 M Tris-HCl, pH 7.4. The column was washed with same buffer and 0.1 N NaCl linear gradient was then generated using a gradient mixer. Each 3 ml fractions were pooled and concentrated, then dialysed overnight with 10 mM Tris-Cl (pH 7.4) to exclude salts, and concentrated for 8 hrs in lyophilizer. The amount of protein in each fraction was measured by the Bradford method (Bradford, 1976). For it, bovine serum albumin (BSA) (Giemsa) was used as standard material for the determination, and commercial Bio-Rad was used as stain for the quantitation.

Reagents

The reagents and materials for the experiment were purchased: murine rIFN-γ (1 × 10^6 units/mg) from Genzyme Co. (Munchen, F.R.G.); LPS (phenol extracted of *Salmonella enteritidis*), Dulbecco's modified Eagle's Medium (DMEM) and sodium nitrite from Sigma chemical Co. St Louis, MO, U.S.A.; fetal calf serum (FCS) and other tissue culture reagents from Gibco, Eggenstein, FRG; tissue culture flask and coverglass chamber from Nunc Inc., IL, U.S.A.

Macrophages

Murine peritoneal macrophages were utilized for experiment. The preparation method was essentially the same as that of the previous reports (Jun *et al.* 1993; Soh *et al.* 1994). Briefly, sterilized 3% thioglycollate, 2.0 ml, was injected intraperitoneally into locally bred adult BALB/c mice. Three days later, the peritoneal contents were lavaged with 10 ml of ice cold phosphate buffered solution (PBS) containing 50 U/ml heparin (pH 7.2) or Hank's buffered saline solution (HBSS) under pathogen free condition and centrifuged at 1,500 rpm for 5 min. When erythrocytes were recognised in suspension, hemolysis was done with lysis solution (NH_4Cl 8.26 g, $NaHCO_3$ 1.09 g, NaEDTA 0.037 g; 1000 ml dH_2O pH 7.2) followed by repeated washing with HBSS. The macrophages collected were put in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal calf serum (FCS). The macrophage suspension of 1 × 10^6

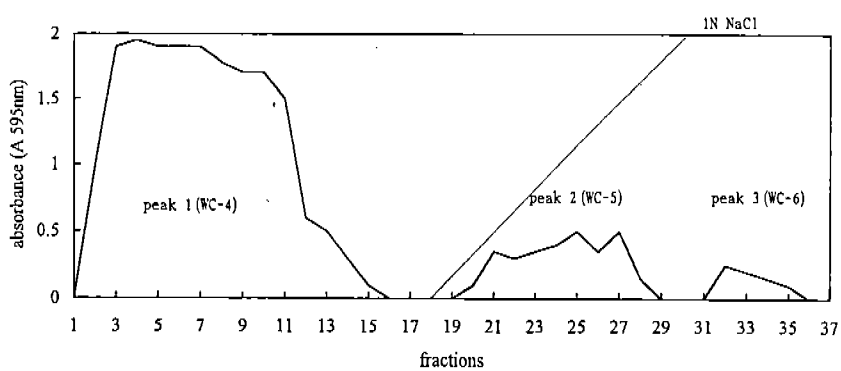

Fig. 2. QAE column chromatography of supernatant of *Coix lacryma* water extract.

cells/ml was plated on a coverglass (Nunc Inc.) and incubated at 37°C for 6 hours in 7.5% CO_2 incubator. For determination, the chamber was rinsed out cautiously preventing macrophages from being detached. DMEM with 10 % FCS at 37°C was added.

Toxoplasma gondii

Tachyzoites of *T. gondii*, RH strain (courtesy from Dept. of Parasitology, Catholic Medical College, Seoul) were maintained in BALB/C mice. The organisms were introduced intraperitoneally twice a week. To collect *T. gondii*, mice were killed by cervical dislocation, and 5 ml PBS or HBSS adding a few drops of antibiotics (penicillin G 10^6 unit, streptomycin 1 g/H_2O 5 ml) was injected intraperitoneally. By centrifugation at 800 rpm for 5 min., peritoneal fluid was removed and the organisms were washed. In due course, macrophages and some intact murine cells on the bottom of the tube were withdrawn. The supernatant was centrifuged again at 2,500 rpm for 15 min to collect *T. gondii*. The tachyzoites were resuspended in DMEM with 10% FCS, and inoculated, 1×10^5 cells/ml, immediately to the macrophage chamber (Soh *et al.* 1994).

Measurement of nitrite

Nitrite concentration in the supernatant of the cell culture of macrophages was assayed by a standard Griess reagent. At 24 hrs after treatment with the corresponding reagents, 100 μl of the supernatant of the culture medium (refer toxoplasmastatic assay in the following) was dropped into each well of the 96 well plate (ELISA Titertek Plate), and equal volume of Griess reagent (1:1, v/v, N-1-naphthylethylendiamine 0.1% in H_2O, sulfanilamide 1% in 5% H_3PO_4) was added. After incubation at room temperature for 10 min., the absorbance was measured spectrophotometrically at 540 nm in a Titertek multiskan (Flow Laboratories, Australia). Concentration of NO_2^- was determined by using sodium nitrite as the standard. Cell-free medium contained 5~8 μM of NO_2^- which was assayed in each experiment and the figure was subtracted from the value measured from cells. The measurements were expressed as micromoles of NO_2^- or NO per liter (μM/l).

Toxoplasmastatic activity

Macrophages were infected with *T. gondii* as previously described (Soh *et al.* 1994). In terms, the cells were suspended in medium at a concentration of 1×10^6 cells/ml, and each of aliquots of the suspension, 1 ml, was plated in a coverglass chamber. After adherence in the coverglass chamber for 6 hours at 37°C in 7.5% CO_2, adherent cells were incubated in medium with or without cytokines and reagents. After 24 hours the cells were washed and incubated for 30 min. at 37°C in 7.5% CO_2 incubator in 2 ml of medium containing 1×10^6 *T. gondii* 1 ml, followed by three washes with warm PBS to remove noningested *Toxoplasma*. The cells on one coverglass chamber were fixed in methanol and stained with Giemsa stain for determination of the number of ingested protozoa and the percentage of infected macrophages. After the determination at the start of the assay, fresh medium was added to the cells in the coverglass chamber which were then incubated for another 48 hours at 37°C in 7.5% CO_2 before fixation in methanol and Giemsa staining. Then the number of tachyzoites per 100 macrophages were counted microscopically, and at least 200 cells per coverglass chamber were examined. The ratio of the mean number of tachyzoites per 100 macrophages after 48 hrs of incubation to the mean number of tachyzoites per 100 macrophages at the beginning of the assay were expressed as fold increase (FI). Intracellular killing of *T. gondii* was defined as the reduction in percentage of infected macrophages over the course of the experiment *i.e.* percentile ratio of the number of tachyzoites infected macrophages after 48 hrs of incubation compared to the number at time zero.

RESULTS

Effect of *Coix lacryma* on the production of NO by macrophages

Previous to the regular trial, determination of the optimum concentration of the samples for nitric oxide (NO) production was examined.

Each sample was dissolved in distilled water at various concentrations. The original concentration was found as toxic to macrophages in medium, so that dilutions in two fold and ten fold were studied comparatively and found that the two fold dilution was the better way to keep macrophages vital and producing more nitrogen intermediates (Fig. 2). Based on the results, the following studies were carried out with two fold dilutions, amounting to 10 μl in each series of experiment. In the test, WC-2, WC-1 showed the higher rate of NO_2^- production compared to the pellet (WC-3), and QAE chromatography-fractioned group (WC-4,5,6) resulted similar figures.

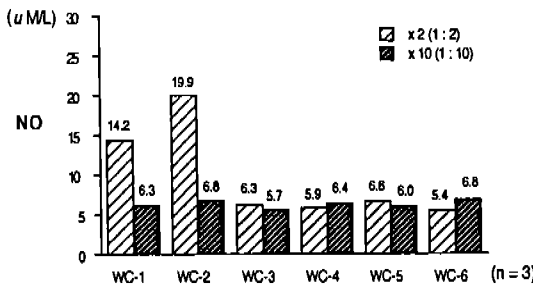

Fig. 3. Effect of *Coix lacryma* on the production of NO by macrophages.
WC-1, Co-Ex (water extract of *Coix lacryma* seeds) dissolved in Tris-Cl buffer. The supernatant; WC-2, WC-1 was precipitated with ammonium sulfate. The supernatant; WC-3, pellet of WC-2, dissolved in Tris-Cl. The supernatant; WC-4,5,6, peak fractions by QAE anion exchange chromatography of WC-1.

NO production in macrophages culture medium treated with various extracts of *Coix lacryma* or IFN and LPS was examined (Table 1). Among the samples, WC2, WC1, and a combined sample of (WC1 + WC2 + WC3) resulted in higher production of NO_2^- (uM/L) compared to WC3, WC4, WC5 and WC6. It is noteworthy that the combined sample resulted in the highest production from 41.9 in non-IFN-γ to 61.3 in rIFN-γ. In general the NO production resulted almost double in IFN-γ added group compared to the non-IFN group.

Effect of *Coix lacryma* in the toxoplasmastatic capacity of macrophage

Macrophages incubated for 24 hrs in DMEM

Table 1. Nitrite production of macrophages treated with the fractions of water extracts of *Coix lacryma* seed extracts for 24 hours in DMEM medium in which 10% FCS was added

Reagents[a] (dilution × 1)	NO_2^- (mM/l)/1·× 10^6 macrophages[b]	
	rIFN-γ(-)	rIFN-γ(+)
DMEM with 10% FCS***(control)	7.5 ± 0.8	14.2 ± 1.5
Co-E	13.5 ± 2.0	45.0 ± 9.4
WC1	22.7 ± 5.6	50.4 ± 6.6
WC2	26.2 ± 4.1	57.8 ± 4.6
WC3	8.5 ± 1.3	24.0 ± 5.0
WC1 + WC2 + WC3	41.9 ± 7.3	61.3 ± 5.0
WC4	9.1 ± 2.0	15.8 ± 13.3
WC5	6.6 ± 2.2	12.3 ± 10.2
WC6	7.9 ± 3.8	16.1 ± 8.9
LPS	12.3 ± 1.2	41.8 ± 6.3

[a]Co-Ex, *Coix lacryma* seed extract, 30 mg/ml; WC1, Co-Ex was dissolved in Tris-Cl. The supernatant; WC2, WC1 was precipitated with 70% ammonium sulfate. The supernatant; WC3, pellet of WC2, dissolved in Tris-Cl. The supernatant; WC1 + WC2 + WC3, combine WC1,2,3 at equal amount, and 30 mg/ml; WC4, 1st peak, fraction of WC-1 by QAE anion exchange chromatography; WC5, 2nd peak, fraction of WC-1 by QAE anion exchange chromatography; WC6, 3rd peak, fraction of WC-1 by QAE anion exchange chromatography; LPS, phenol extracted of *Salmonella enteritidis* 10 ng/ml; rIFN-γ, 10 U/ml, rIFN-γ(-), without interferon; rIFN-γ(+) , with interferon.

[b]The amount of NO_2^- released by 1 × 10^6 macrophages was measured after treatment with the corresponding reagents for 24 hours incubation. Values are the means ± SE of 5 experiments.

Table 2. Percentage of the macrophages infected by *T. gondii* in 48hr treatment with modulators from *Coix lacryma* seed extracts

Reagents	[a]PI (%)(Nr. = 5)	
	rIFN-γ(-)	rIFN-γ(+), 10 U/ml
DMEM with 10% FCS (control)	55.6 ± 10.0	38.0 ± 4.8
Co-Ex	46.0 ± 10.0	31.0 ± 7.6
WC1	47.4 ± 11.0	20.0 ± 4.6
WC2	46.0 ± 9.2	19.2 ± 4.6
WC3	51.6 ± 18.0	19.3 ± 3.2
WC1 + WC2 + WC3	47.8 ± 2.5	7.2 ± 2.0
WC4	69.0 ± 0.1	70.0 ± 0.2
WC5	76.0 ± 0.2	69.3 ± 0.1
WC6	59.6 ± 0.1	86.0 ± 0.3
LPS, 10 ng/ml	66.3 ± 2.0	14.4 ± 2.8

[a]PI, Percentage of the macrophages infected.

containing 10% heat-inactivated FCS with or without modulators were infected *in vitro* with tachyzoites of *T. gondii*, 1×10^6, for 30 min. Intracellular killing of tachyzoites was defined as percent of the macrophage infected by *T. gondii* (PI) based on the reduction in percentage of infected macrophages over the course of 48 hrs, i.e. the percentile ratio of the number of *T. gondii* infected macrophages after 48 hrs of incubation compared to the number at time zero. PI in control was 55 which was a little higher than the experimental series. Co-Ex, WC1, WC2, WC3 and the combined (WC1 + WC2 + WC3); 46-47 denoting that the Co-Ex or components are more effective in inhibition of phagocytosis of macrophages or killing of *T. gondii* in macrophages than pellet (WC3) and QAE group (WC4, WC5, WC6). The IFN-γ added group showed more notable differences between experimental groups and the control. Specially the combined group decreased from 47.8 to 7.2 (Table 2). To determine the fold increase (FI) of the ingested tachyzoites, the number in macrophages which were incubated in DMEM containing 10% heat-inactivated FCS for 24 hrs with or without modulators were infected *in vitro* with *T. gondii*, 1×10 ,for 30 min. The number of the *T. gondii* in macrophages, 100-200 cells, was counted at time zero, and was compared to the number in 48 hrs after another incubation (Table 3). In medium without modulator (control) the FI was 8.8, while in the IFN-γ added group, the decrease was spurred. Among the series, combination group (WC1 + WC2 + WC3) decreased from 7.8 to 1.4, and WC2 from 7.6 to 1.7, WC1 from 7.8 to 4.1, WC3 from 8.6 to 7.5, Co-Ex from 7.5 to 5.2, suggesting that the combination group and WC2 are the more promising synergizers to IFN-γ in activation of macrophages leading to biostatic action. In general, both of the PI of macrophages and FI in macrophages showed an inverse relation with the amount of the NO production, and the gap increased especially when IFN-γ was added.

DISCUSSION

T. gondii is an intracellular parasite taking two ways in cells, proliferation or disintegration resulting corresponding effects to the host; disease in the former and homeostasis in the latter (Gazzinelli *et al.*, 1993). The mechanism of the disintegration has been recognised to proceed from digestion by phagolysosome, generation of superoxide in mainly polymorphonuclear leucocytes or the formation of NO mainly in cells of mononuclear phagocytic system (MPS)(Hibbs *et al.*, 1988). Among MPS, the macrophage is known to play a key role in the course of the immune responses producing reactive nitrogen intermediates (RNIs). Especially the NO among RNIs in the cell gives hazard to the phagocytosed parasites by blocking electron

Table 3. Intracellular proliferation of *T. gondii* (fold increase: FI[a)]) after infection to macrophages for 30 minutes and incubated for 48 hrs.

Macrophages with	Intracellular proliferation of *T. gondii* (fold increase)(Nr. = 3)	
	rIFN-γ(-)	rIFN-γ(+), 10 U/ml
DMEM with 10% FCS (control)	8.8 ± 0.4	6.0 ± 0.5
Co-Ex	7.5 ± 0.9	5.2 ± 0.2
WC1	7.8 ± 0.4	4.1 ± 1.1
WC2	7.6 ± 0.8	1.7 ± 0.2
WC3	8.6 ± 0.6	7.5 ± 0.7
WC1 + WC2 + WC3	7.8 ± 0.3	1.4 ± 0.1
WC4	6.5 ± 0.2	8.8 ± 0.1
WC5	11.0 ± 0.1	9.9 ± 0.2
WC6	8.1 ± 0.3	6.5 ± 0.1
LPS, 10 ng/ml	12.1 ± 2.6	1.6 ± 0.2

[a)]FI: The ratio of the mean number of *T. gondii* tachyzoites per 100 macrophages after 48 hours of incubation to the mean number per 100 macrophages at the start of assay.

transport system of the mitochondria, inhibiting DNA synthesis which is important for survival and propagation of the intracellular pathogens (Hibbs *et al.*, 1988; Chung 1991). In brief, NO is so unstable that converts to nitrite (NO_2^-) and nitrate (NO_3^-) within 6-10 seconds (Oh *et al.*, 1995). In such a short moment, it suppresses DNA synthesis of the parasites hindering ribonucleotide reductase which is the regulatory enzyme in forming the basic structure of DNA; reduction of ribonucleotide to deoxyribonucleotide. Actually the reductase contains thiol and iron which are sensitive to NO (Drapier, 1989; Stubbe, 1990).

Based on such background, Jun *et al.* (1993) reported that murine microglia cells produced NO when the cells were activated by IFN-γ. In the same study, it was proved that the biological response modifier (BRM) such as bacterial lipopolysaccharide (LPS) enroled synergy to IFN-γ. Among several preliminary trials to find an effective BRM from among herbs which have been traditionally appreciated for treatment of tumors or infectious diseases, Soh *et al.* (1994) found that water extract of *Coix lacryma* seed activated macrophages *in vitro* by enhancing NO production, and inhibited propagation of the phagocytosed *T. gondii*. In continuation of the previous experiment, the present study was designed to elucidate the components of *Coix lacryma* seed extract in relation to NO production in macrophages and the inhibitory effect to intracellular parasites. Some reports indicated that IFN-γ plays a prime role but becomes more vital when tumor necrosis factor (TNF) or LPS are triggered (Drapier *et al.*, 1988, Ignarro 1990, Oh *et al.*, 1988).

Among the series of reagents or modulators tested in the present study, Tris-Cl treated sample (WC1) and ammonium sulfate treated sample (WC2) resulted in higher amount of nitrite production, specially compared to the pellet (WC3). The finding endorses the theory that a certain non-proteinous substance in water extract (Co-Ex) may hold a potential component for activation of macrophages, since fat substances were already excluded during process of Co-Ex preparation and most of the proteinous matters were denatured in the courses of fractionation under high temperatures. Then, polysaccharides shall also be considered as the candidate component, although further search shall be needed for final conclusion. It is noteworthy that nitrite production was almost doubled when the samples were combined (WC1 + WC2 + WC3) to compare the individual reagent; WC1, WC2 and WC3. NO amount was 41.9 μM/L in WC1+ 2 + 3, although 22.7 in WC1, 26.2 in WC2 and 8.5 in WC3 which indicated that the pellet holds negligible potential for activation of macrophages. Among the individual reagent, WC2 seems the most effective one which is non-protein or non-fat. It also synergizes

prominently with IFN-γ in activating macrophages to compare another components.

QAE (quartenary amino-acid exchange) chromatography samples (WC4, WC5, WC6) were expected to contain a part of proteinous substances, presumably glycoprotein or oligopeptide which are heat stable. But the nitrite productions were almost similar to the level of control; 6.6-9.1. Effective potential in producing RNI differed by BRM, but more in WC1, WC2, Co-Ex and the combined (WC1 + 2 + 3), but almost negligible in WC3, 4, 5, 6. When IFN-γ was combined with the fractions, the nitrite production was almost doubled or more in respective test compared to the samples without IFN-γ. It is conjectured that non-protein substances in *Coix lacryma* seeds may take place in activating macrophages as a primer, but more to IFN-γ leading to the biostatic action. For example, NO_2^- production (μM/L) was 14.2 in IFN-γ only, but 57.8 (μM/L) in WC2 with IFN-γ and 26.2 without IFN-γ. It is generally recognized that T.gondii takes two ways into host cells as described previously: phagocytosed by cells or automatically enter into cells. In case no defense mechanism is mobilized, intracellular propagation shall be enhanced leading to pathological effects. For the reason immunological activation of macrophage should be considered first in connection with the biostatic effect in present study.

Badger *et al.* (1987) reported that satisfactory level of the activation was achieved when macrophages were incubated with gamma interferon for 48-72 hours prior to the challenge with *T. gondii*. In the present study, taehyzoite positives (%) in WC1 ,WC2 and the combined sample (WC1 + WC2 + WC3) were 46-47, whereas 55-76 in the WC3 and QAE treated group (Table 2). When IFN-γ was added, the ratio reduced in respective trial. For example, PI (%) of [WC1 + WC2 + WC3] without IFN-γ was 47.8, but decreased to 7.2 when IFN-γ was added, although a slight difference was noticed in samples by QAE chromatography.

How the parasites in macrophages are eliminated in due course of the 48 hrs incubation period and the reason for the PI change is not yet clear. Once, Jones *et al.*, (1975) reported that cellular immune response appeared to be an inhibitor of *Toxoplasma* multiplication rather than a toxoplasmacidal one. Nevertheless, it might not be neglected that the cytokine, IFN-γ takes the part of a principal factor for the inhibition of proliferation or killing of *T. gondii* in macrophages. In the present study, 24 hr incubation with reagents was already potent to activate macrophages in producing RNIs (reactive nitrogen intermediates), resulting biostatic activity. Previously, Black *et al.*, (1987) and Suzuki *et al.*, (1988) also reported that gamma interferon activated murine peritoneal and alveolar macrophages in killing of *T. gondii* both *in vitro* and *in vivo*. They found that incubation with 500-200 units/ml rMuIFN-γ for 48-72hr prior to challenge with *T. gondii* resulted in the highest level of biostatic activation. It will be the pending subject to clarify the reason whether it is due to the destruction of phagocytosed organism or autolysis of the infected cells. At all events WC2 seems the most effective component among water extracts of *Coix lacryma* seeds in activation of macrophages leading to the biostatic action.

T. gondii in macrophages may multiply in case no impedimentary factors; such as biological response modifiers (BRM) or cytokines are involved. In the present study, inhibitory action was recognized with the candidate BRMs from *Coix lacryma* water extract or the various components, but enhancement was noticed by adding IFN-γ. Notable decreases of FI were recognized with the samples; of WC1, WC2 and the combination sample (WC1 + WC2 + WC3). The indice were; from 7.8 to 1.4 in WC1 + WC2 + WC3, from 7.6 to 1.7 in WC2 and from 7.8 to 4.1 in WC1, while from 8.6 to only 7.5 in WC3 suggesting the effective portion might not be involved in the pellet (WC3). Virtually, the combination (WC1 + WC2 + WC3) is to be the same with Co-Ex by the source. Nevertheless the former showed more biostatic effects than the latter. The rational explanation shall be obtained through extensive study, although it is speculated tentatively that some impediments in Co-Ex might be excluded in due course of the preparation for WC2 and

WC3 (Table 3). Such results suggest that some non-proteinous portions in water extract of *Coix lacryma* may serve as modulators to activate macrophages and inhibit *T. gondii* multiplication in the cells especially. The activation was highly enhanced by adding IFN-γ. From view point as synergizer to IFN-γ, WC1 or WC2 seemed almost same level compared with LPS (Table 2).

Through the results in the present study, it is speculated that the fate of *T. gondii* in macrophages is rather dependent upon RNI showing a retroverse relationship with the infection rate and fold increase. It is also clear that some non-proteinous component, presumably the carbohydrate portion or some other frations of *Coix lacryma* seeds may participate in activation of macrophages as primer or trigger with IFN-γ, enhancing nitrite production which are hazardous for the multiplication of *T. gondii* in cells. Continuous studies shall elucidate the most potential fraction in WC2, relaying to the reproducibility for *in vivo* trial.

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=초록=

율무씨 수침 추출물이 대식세포내 톡소포자충에 미치는 영향에 관한 실험적 연구

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율무 수침엑스(Co-Ex)가 대식세포내 톡소포자충(*Toxoplasma gondii*, RH strain)의 번식에 억제 작용이 있다는 보고(Soh *et al.*, 1994)에 이어 본 연구는 Co-Ex내의 유효성분(effective component)을 추구하기 위하여 이미 탈지조작과 단백질 변성과정을 거친 Co-Ex를 Tris-buffer solution에 녹인 후 원심과정으로 그 상청(WC1), 그리고 WC1을 ammonium sulfate 처리 후 원심한 상청(WC2)과 침사(WC3)를 분리하였고 WC1은 다시 column chromatography에 의하여 WC4, WC5, WC6로 분획하여 시료로 사용하였으며, 대식세포 활성에 기여하는 것으로 이미 알려진 IFN-γ, LPS 등을 참고자료로 하였다. 각 시험자료들의 대식세포 활성화로 인한 반응질소 중간산물(RNI) 생산량, 대식세포의 톡소포자충 감염상, 대식세포내 톡소포자충 번식상 등을 비교 조사하였다. 10% FCS 첨가 DMEM 배지에 배양하였고 이에 각종 시료(BRM)를 실험목적에 따라 배지에서 24시간 작용시킨 뒤 RNI를 조사하였고 이어 톡소포자충 1×10^6을 30분 감염시킨 뒤 새 배지에 옮겨 48시간 배양한 뒤 PI, FI 등을 조사하였다. NO 생산은 각례 대조(시료 비투여)에 비하여 높았고 IFN-γ 첨가는 생산량(μM/l)을 더욱 증가시켰다(예: Control 7.5, Control + IFN-γ 14.2, WC2 26.2, WC2 + IFN-γ 57.8). 포자충감염율(%)은 대조에 비하여 낮았고 IFN-γ 첨가시에는 더욱 낮아졌는데 IFN-γ 첨가예에서의 감염율은 대조 38.0인데 비하여 WC2 19.2, WC1 + WC2 + WC3 7.2로 나타났다. 세포당감염율도 같은 경향으로 INF-γ 첨가시험에서 대조 6.0(감염 대식세포 100개 중의 톡소포자충 평균수), WC2 1.7, WC1 + WC2 + WC3 1.4 등으로 나타났다. 결론적으로 율무엑스 수침엑스 중 WC2, WC1 등은 대식세포를 활성화하나 IFN-γ 첨가는 더욱 그 활성화를 높이며 그 중 WC2가 활성화에 주역을 하는 것으로 사료되는 바이다.

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