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Suppression of RelA/p65 transactivation activity by a lignoid manassantin isolated from *Saururus chinensis*

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Abstract

In our search for NF-κB inhibitors from natural resources, we have previously identified two structurally related dilignans, manassantin A and B as specific inhibitors of NF-κB activation from *Saururus chinensis*. However, their molecular mechanism of action remains unclear. We here demonstrate that manassantins A and B are potent inhibitors of NF-κB activation by the suppression of transciptional activity of RelA/p65 subunit of NF-κB. These compounds significantly inhibited the induced expression of NF-κB reporter gene by LPS or TNF-α in a dose-dependent manner. However, these compounds did not prevent the DNA-binding activity of NF-κB assessed by electrophoretic mobility shift assay as well as the induced-degradation of IκB-α protein by LPS or TNF-α. Further analysis revealed that manassantins A and B dose-dependently suppressed not only the induced NF-κB activation by overexpression of RelA/p65, but also transactivation activity of RelA/p65. Furthermore, treatment of cells with these compounds prevented the TNF-α-induced expression of anti-apoptotic NF-κB target genes Bfl-1/A1, a prosurvival Bcl-2 homologue, and resulted in sensitizing HT-1080 cells to TNF-α-induced cell death. Similarly, these compounds also suppressed the LPS-induced inducible nitric oxide synthase expression and nitric oxide production. Taken together, manassantins A and B could be valuable candidate for the intervention of NF-κB-dependent pathological condition such as inflammation and cancer.

Keywords: *Saururus chinensis*; Lignoids; NF-κB; RelA/p65; Transactivation; Apoptosis

1. Introduction

NF-κB represents a family of eukaryotic transcription factors participating in the regulation of various cellular genes involved in the immediate early processes of immune, acute-phase, and inflammatory responses as well as genes involved in cell survival [1]. In most cell types, the pleiotropic-inducible form of NF-κB is a heterodimer composed of p50 and RelA (previously termed p65) [2]. RelA/p65 contains a C-terminal transactivation domain in addition to the N-terminal Rel-homology domain, thus serving as a critical transactivation subunit of NF-κB. p50 lacks a transactivation domain, and it is believed to serve as a regulatory subunit modulating the DNA binding affinity of RelA/p65 [3,4]. The p50–RelA/p65 NF-κB heterodimer is normally sequestered in the cytoplasmic compartment by physical association with inhibitory proteins, including IκB-α and related proteins [5,6]. The latent cytoplasmic NF-κB RelA–p50 complex can be posttranslationally activated by a variety of cellular stimuli, which trigger site-specific phosphorylation of IκB-α by a multisubunit IκB kinase [7–9]. The phosphorylated IκB-α becomes rapidly ubiquitinated and degraded by the proteasome complex [10,11]. Following IκB-α degradation, the NF-κB heterodimer is rapidly translocated to the nucleus, where it activates the transcription of target genes.

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Abbreviations: NF-κB, nuclear factor κB; AP-1, activator protein-1; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; PMA, phorbol myristyl acetate; MEKK-1, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-1; NIK, NF-κB inducing kinase; IKK, IκB kinase; IKC, IκB kinase complex; EMSA, electrophoretic mobility shift assay; NO, nitric oxide; iNOS, inducible nitric oxide synthase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, (4′, 6-diamidino-2-phenylindole); TUNEL, terminal uridine nick end-labeling.

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NF-κB regulates the transcription of various inflammatory cytokines, such as interleukin-1, -2, -6, and -8 and TNF-α, as well as genes encoding cyclooxygenase-II, iNOS, immunoreceptors, cell adhesion molecules, hematopoetic growth factors, and growth factor receptors [12]. In addition to regulating the expression of genes important for immune and inflammatory responses, NF-κB also controls the transcription of genes that confer resistance to death-inducing signals. Candidate target genes include those encoding the caspase inhibitors c-IAP1, c-IAP2, and X-IAP, the TNF receptor-associated factors TRAF1 and TRAF2, the zinc finger protein A20, the immediate-early response gene IEX-1L, and the prosurvival Bcl-2 homolog Bfl-1/A1 [13–16]. Therefore, pharmacological inhibition of NF-κB could be a valuable strategy to modulate the inflammatory processes as well as cell death.

As part of our continuing search for NF-κB inhibitors from natural products, two structurally related dilignans, manassantin A and B, were identified as NF-κB inhibitors from *Saururus chinensis* (Saururaceae), which has been used in Korean folk medicine for the treatment of edema, jaundice, and gonorrhea [17,18]. We here describe that the lignoids inhibited not only NF-κB by the suppression of transactivation activity of RelA/p65 subunit without affecting the induced degradation of IκB-α and DNA-binding activity of NF-κB, but also the induced expression of NF-κB target genes such as Bfl-1/A1 and iNOS.

2. Materials and methods

2.1. Cell culture and chemicals

RAW264.7, HeLa, and HT-1080 cells were maintained in Dulbecco's modified Essential medium (Invitrogen Corporation) supplemented with penicillin (100 units/mL)–streptomycin (100 μg/mL) (Invitrogen Corporation) and 10% heat-inactivated fetal bovine serum (Hyclone). Manassantins A and B were isolated from dried root of *S. chinensis* as described previously [18] and their structures are shown in Fig. 1. The purity of manassantins A and B was over 98% in a HPLC analysis. Physicochemical and spectral data of these compounds were comparable to previously reported values [19].

2.2. Measurement of NO and cell viability assay

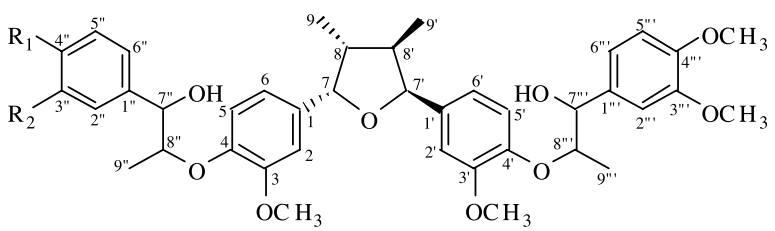
Nitric oxide was determined by measuring the amount of nitrite from cell culture supernatant as previously described [20]. In brief, RAW264.7 cells (2×10^5 cells/well) were stimulated for 24 hr with or without 1 μg/mL of LPS (Sigma Chemical Co.) in the absence or presence of the compounds tested (final concentration of ethanol was less than 0.2%). A hundred μL of cell culture supernatant was reacted with 100 μL of Griess reagent [21]. Cell viability was measured with MTT (Sigma Chemical Co.)-based colorimetric assay as previously described [22].

2.3. Plasmids, transfections and luciferase assay

A pNFκB-Luc and an expression plasmid for RelA/p65 have been previously described [23]. A pAP-1-Luc was obtained from Stratagene. A vector encoding a fusion protein between the DNA binding domain of Gal4 and activation domains of RelA/p65, Gal4-RelA$^{268–551}$, was constructed by inserting cDNA for Gal4-RelA$^{268–551}$ into pFA-CMV (Stratagene). A 5XGal4-luciferase reporter gene was obtained from Stratagene. Transfections were performed using Lipofectamine plus reagent (Invitrogen Corporation). Luciferase activity was measured using luciferase assay system according to the instructions of the manufacturer (Promega). Luciferase activity was determined in Microlumat Plus luminometer (EG&G Berthold) by injecting 100 μL of assay buffer containing luciferin and measuring light emission for 10 s. The results were normalized to the activity of β-galactosidase expressed by cotransfected *lacZ* gene under the control of a constitutive promoter.

2.4. NF-κB and AP-1 activity assay

NF-κB activity was determined as previously described with minor modifications [23]. The cells were transiently



	R_1	R_2
Manassantin A	$-OCH_3$	$-OCH_3$
Manassantin B	$-OCH_2O-$	

Fig. 1. Chemical structures of manassantins A and B isolated from *Saururus chinensis*.

transfected with a pNFκB-Luc plasmid. Twenty-four hours after transfection, the cells were stimulated with the corresponding stimulant for 8 hr in the presence or absence of various concentrations of the compounds, and the luciferase activity was determined. In certain experiment, a pNFκB-Luc plasmid and an expression for RelA/p65 were cotransfected into HeLa cells, the cells were treated with various concentrations of the compounds and luciferase activity was determined. For AP-1 activity, HeLa cells were transiently transfected with a pAp-1-Luc plasmid. Twenty-four hours after transfection, the cells were stimulated with 50 ng/mL of PMA for 8 hr in the presence or absence of various concentrations of the compounds, and the luciferase activity was determined.

2.5. Northern blot analysis

RNA was isolated from cells using RNeasy Mini Kits according to the instructions of the manufacturer (Qiagen). Ten μg of total RNA were resolved on 1% agarose-formaldehyde gel and transferred to a nylon membrane by capillary action. Membranes were probed and washed according to the instructions of the manufacturer (Boehringer Mannheim). ^{32}P-labeled probes were generated by the random priming method using Rediprime II (Amersham Pharmacia Biotec) and 50 μCi [α-^{32}P]dCTP (3000 Ci/mmol NEN). Unincorporated nucleotides were removed by purification through a G-25 spin column. The results were visualized by autoradiography.

2.6. Western blot analysis

Western blot analysis was performed as described previously [23]. Fifty μg of protein were analyzed by 7% SDS–polyacrylamide gel and the immunoblots were probed with antibodies for iNOS (Upstate Biotechnology, Inc.), or α-IκB-α (New England Biolabs Inc.). After binding of an appropriate secondary antibody coupled to horseradish peroxidase, proteins were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (Amersham Pharmacia Biotec).

2.7. Electrophoretic mobility shift assay (EMSA)

Electrophoretic mobility shift assay was performed as described previously [23]. In brief, 30 minutes prior to stimulation, cells were preincubated with the indicated concentrations of manassantin A at 37°. In following, cells were stimulated, harvested by centrifugation, washed twice with cold phosphate-buffered saline, and then nuclear extracts were prepared. Nuclear extracts were incubated for 20 min at room temperature with a gel shift binding buffer (5% glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 10 mM Tris–HCl, pH 7.5, 50 μg/mL poly(dI-dC) poly(dI-dC)) and ^{32}P-labeled oligonucleotide. The DNA–protein complex formed was separated on 4% native polyacrylamide gels, and the gel was transferred to Whatman 3 MM paper, dried, and exposed to X-ray film.

2.8. Detection of apoptosis

Apoptosis was detected using ApopTag Fluorescein *In Situ* Apoptosis Detection Kits (Serologicals Corp.), according to the instructions of manufacturer. Cells were viewed using a Zeiss microscope with the appropriate filters, and captured using Axiovision 2.0.

3. Results

3.1. Manassantins A and B inhibit NF-κB activation by TNF-α and LPS

In an effort to identify NF-κB inhibitors from anti-inflammatory herbal medicines, we have identified manassantins A and B from a traditional medical plant, *S. chinensis* (Fig. 1). To investigate the effect of the compounds on the induced NF-κB activation by various stimuli, we performed a NF-κB reporter assay. As shown in Fig. 2, manassantins A and B dose-dependently inhibited the TNF-α-induced expression of NF-κB reporter gene construct with IC_{50} values of 4.7 and 5.9 μM, respectively. These compounds also inhibited LPS-induced expression of NF-κB reporter gene construct with the similar extent. As shown in Fig. 2C, however, manassantins A and B weakly inhibited the PMA-induced expression of AP-1 reporter gene construct with IC_{50} values of 38 and 40 μM, respectively. Since these two compounds exhibited the similar inhibitory activity on NF-κB activation, we mainly described the studies for manassantin A.

3.2. Manassantin A does not interfere with the DNA-binding activity of NF-κB and degradation of IκB-α

To confirm that manassantin A inhibits NF-κB activation, we performed electrophoretic mobility shift assays (Fig. 3A). RAW264.7 cells were preincubated with various concentrations of manassantin A for 30 min prior to stimulation with 1 μg/mL LPS. After the stimulation, nuclear extracts were prepared and DNA-binding activity of NF-κB in the nuclear extracts was measured. RAW264.7 cells stimulated with LPS strongly induced DNA-binding activity of NF-κB. Interestingly, pretreatment of manassantin A did not inhibit DNA-binding activity of NF-κB induced by LPS. Similarly, manassantin A did not prevent the induced DNA-binding activity of NF-κB induced by TNF-α in HeLa cells (data not shown). Furthermore, manassantin A did not inhibit the induced degradation of IκB-α protein by LPS in RAW264.7 cells assessed by Western blot analysis (Fig. 3B).

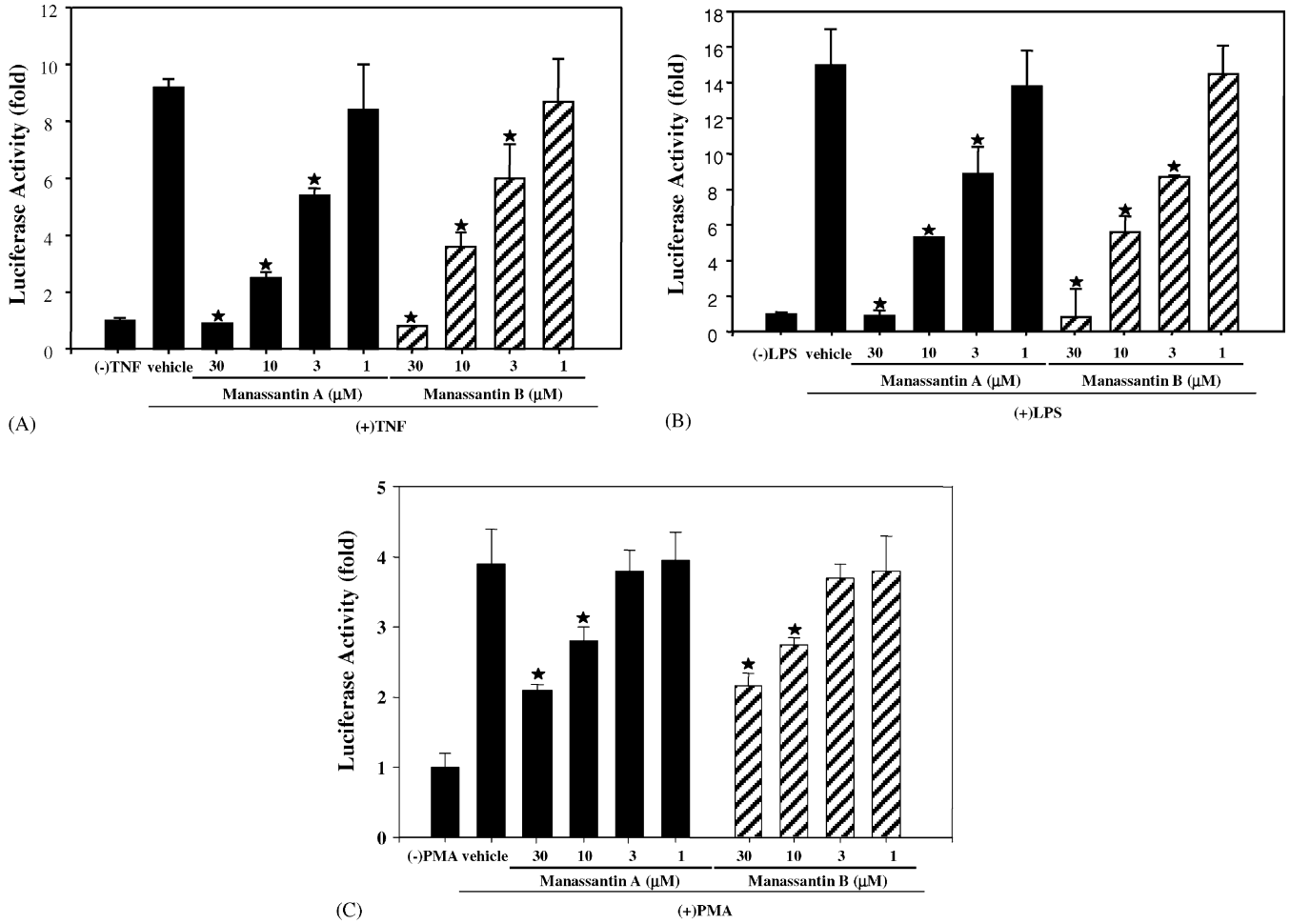


Fig. 2. Effect of manassantins A and B on NF-κB- and AP-1-dependent reporter gene expression by different stimuli. HeLa (A) and RAW264.7 (B) cells, which were transiently transfected with a NF-κB-dependent reporter gene, were grown for 1 day, pretreated for 30 min with the indicated concentrations of manassantin A or B followed by stimulation for 8 hr with TNF-α (A; 20 ng/mL), LPS (B; 1 μg/mL). HeLa (C) cells, which were transiently transfected with a AP-1-dependent reporter gene, were grown for 1 day, pretreated for 30 min with the indicated concentrations of manassantin A or B followed by stimulation for 8 hr with 50 ng/mL PMA. Luciferase activities were determined as described in Section 2; bar indicates the SDs. Statistical significance ($P < 0.001$) judged by paired Student's *t*-test is marked with an asterisk.

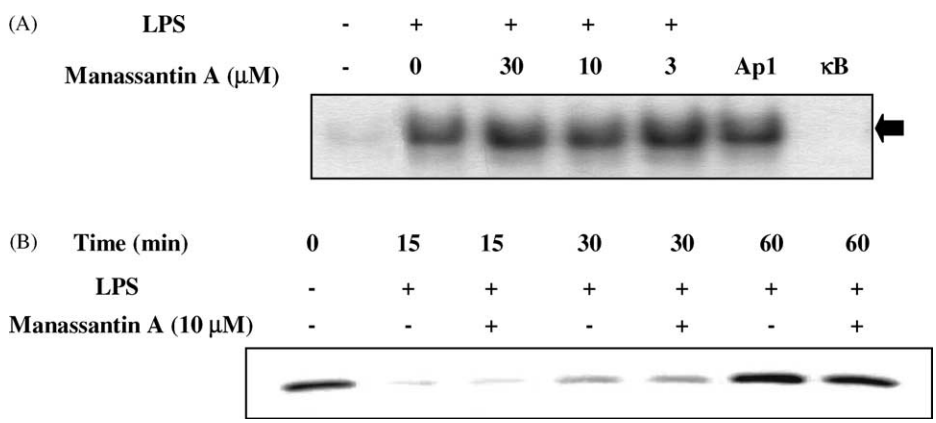


Fig. 3. Manassantin A does not inhibit DNA-binding activity of NF-κB. (A) RAW264.7 cells were preincubated for 30 min with the indicated concentrations of manassantin A and stimulated with LPS (1 μg/mL) for 90 min. Subsequently, nuclear extracts were prepared and tested for DNA-binding of activated NF-κB by EMSA as described in Section 2. In lane AP-1; a 100-fold excess of unlabeled AP-1 consensus oligonucleotide was added to the reaction mixture. In lane κB; a 100-fold excess of unlabeled NF-κB oligonucleotide was added to the reaction mixture. The *arrow* indicates the location of the DNA–NF-κB complex. (B) RAW264.7 cells were pretreated for 30 min with 10 μM of manassantin A prior to stimulation with LPS (1 μg/mL). Cells were harvested at the indicated time points and total cell extracts were prepared. IκB-α protein was detected by Western blot analysis.

3.3. Manassantin A inhibits transactivation of RelA/p65 subunit

To further investigate how manassantin A prevents NF-κB activation, we examined the effect of manassantin A on the induced NF-κB activity by overexpression of RelA/p65 subunit, which is a critical transactivation subunit of NF-κB [3,4]. HeLa cells were transiently transfected either with a NF-κB-dependent reporter gene alone or in combination with an expression vector encoding RelA/p65. As shown in Fig. 4A, NF-κB-dependent transcription induced by overexpression of RelA/p65 was significantly affected by manassantin A. This result led us to make a hypothesis that manassantin A could influence the transactivation activity of RelA/p65 subunit. To test this hypothesis, a plasmid encoding a fusion protein of the transactivating domains of ReA, Gal4-RelA$^{268–551}$, with the DNA binding domain of the yeast transcription

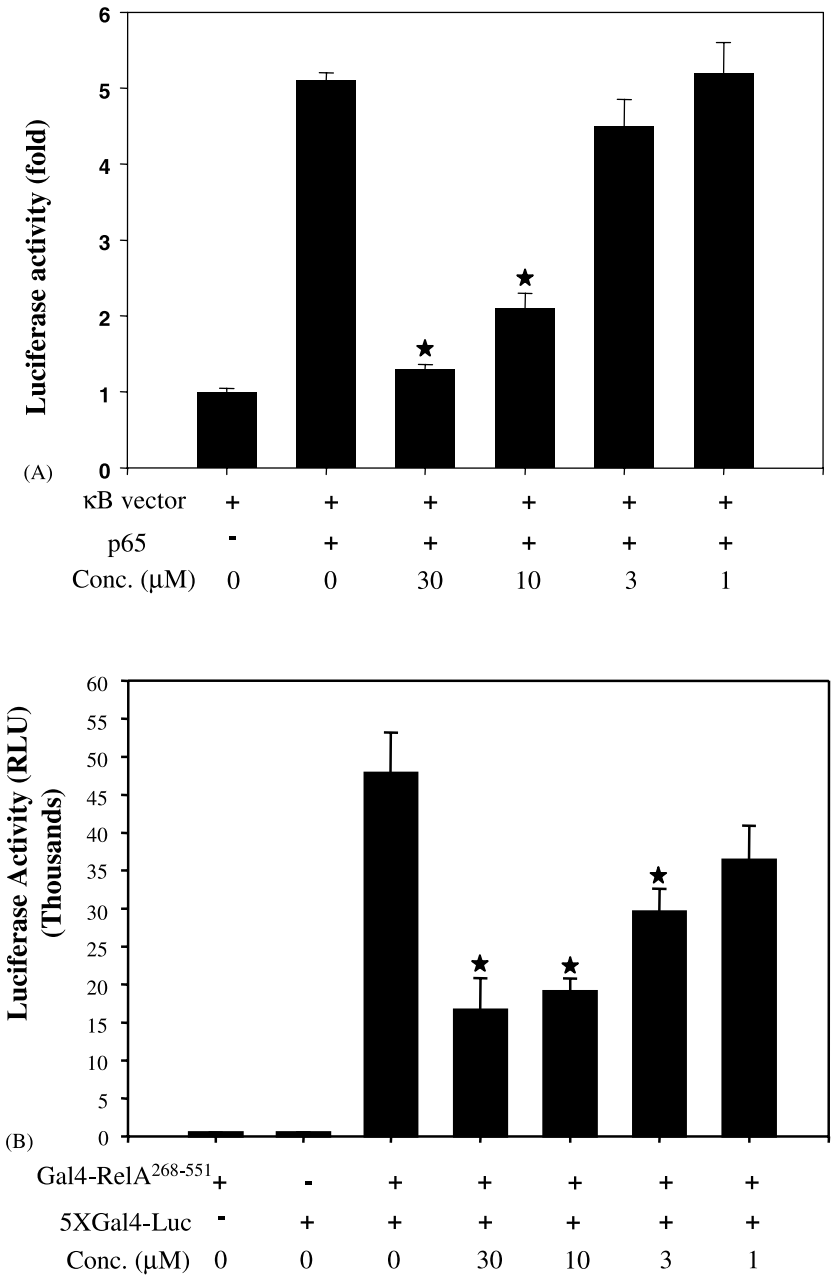


Fig. 4. Manassantin A inhibits NF-κB activation induced by overexpression of RelA and transcriptional activity of RelA subunit. (A) HeLa cells were transiently transfected with a NF-κB-dependent reporter gene together with expression vectors encoding RelA/p65/p65. The cotransfected cells were subsequently grown for 24 hr and for another 12 hr in the presence of the indicated concentrations of manassantin A, and the luciferase activity was determined as described in Section 2. (B) HeLa cells were transiently transfected with a 5XGal4-luciferase reporter gene alone or in combination with a plasmid encoding a fusion protein between the DNA binding domain of Gal4 and activation domains of RelA. After 24 hr, the cells were incubated for another 12 hr in the presence of the indicated concentrations of manassantin A, and the luciferase activity was determined as described in Section 2; bar indicates the SDs. Statistical significance ($P < 0.001$) judged by paired Student's *t*-test is marked with an asterisk.

factor Gal4, was transfected into HeLa cells along with a luciferase reporter containing upstream Gal4 binding sites. As shown in Fig. 4B, manassantin A blocked transcriptional activity of the transactivation domain of RelA/p65 in a dose-dependent manner. Taken together, our results suggested that manassantin A inhibits the activation of NF-κB by inhibiting transactivation activity of RelA/p65 subunit.

3.4. Manassantin A prevents the TNF-α-induced expression of Bfl-1/A1

To investigate whether manassantin A inhibits the induced expressions of NF-κB target genes involved in apoptosis and inflammation, we analyzed the induced expression of Bfl-1/A1 and inducible nitric oxide synthase. Firstly, we analyzed the effect of manassantin A on the

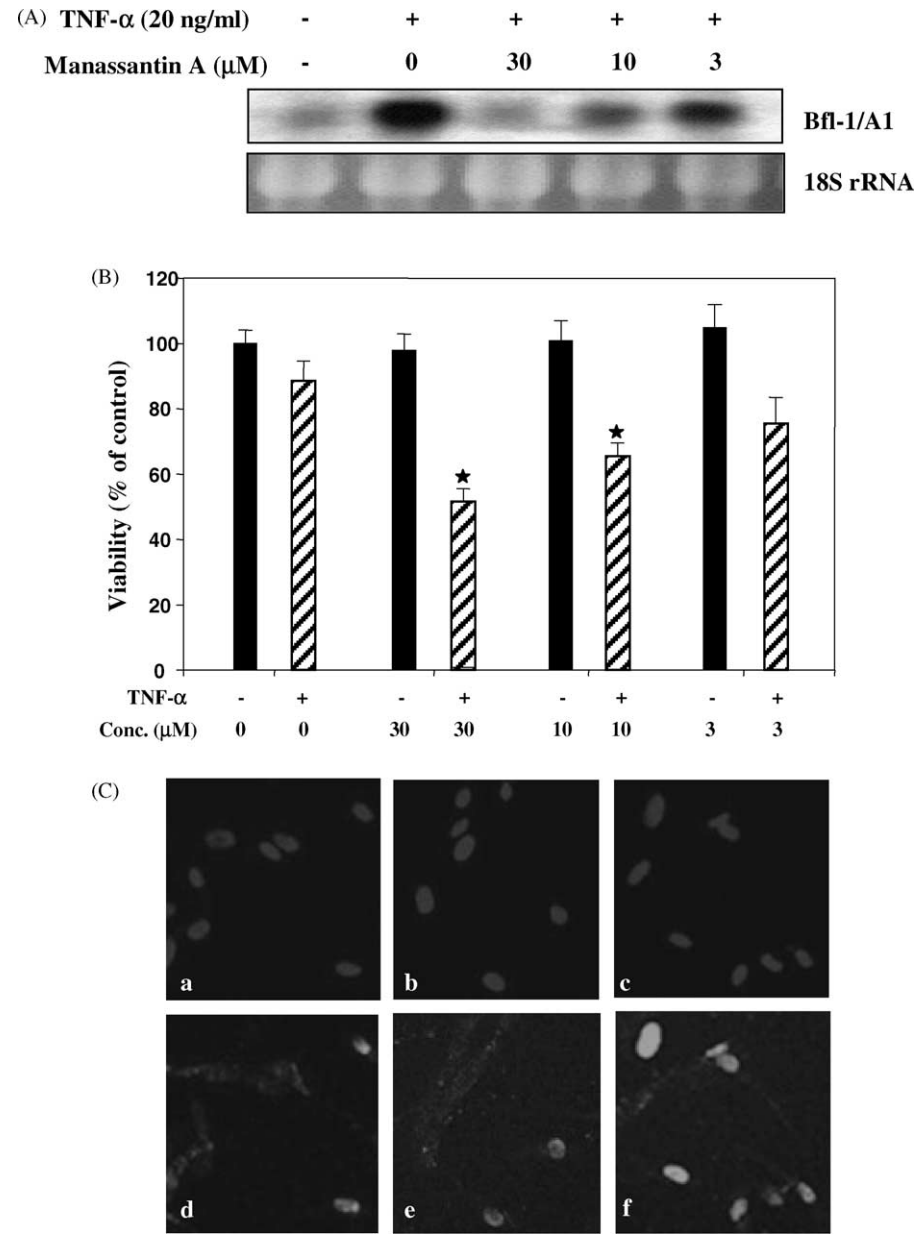


Fig. 5. Effect of manassantin A on the TNF-α-induced expression of Bfl-1/A1 and cell death in HT-1080 cells. (A) Expression of Bfl-1/A1. HT-1080 cells were pretreated for 30 min with the indicated concentrations of manassantin A and stimulated with TNF-α (20 ng/mL) for 3 hr. Subsequently, total RNAs were isolated and Northern blot analysis was performed as described in Section 2. Ethidium bromide staining 18S ribosomal RNA band on the gel was shown to demonstrate equal loading of RNA. (B) Effect of manassantin A on TNF-α-induced cell death. HT-1080 cells were pretreated with the indicated concentrations of manassantin A for 30 min and treated with TNF-α (20 ng/mL). After 48 hr incubation, cell viability was determined by MTT as described in Section 2. Mean values from two independent experiments performed in triplicated are shown; bar indicates the SDs. Statistical significance ($P < 0.001$) judged by paired Student's *t*-test is marked with an asterisk. (C) Detection of apoptosis. HT-1080 cells were pretreated with the indicated concentrations of manassantin A (10 μM) for 30 min and treated with TNF-α (20 ng/mL). After 48 hr incubation, a TUNEL assay was performed, and followed by counterstaining with DAPI. (a) DAPI staining of manassantin A treated, (b) DAPI staining of TNF-α treated, (c) DAPI staining of manassantin A plus TNF-α treated, (d) TUNEL assay of manassantin treated, (e) TUNEL assay of TNF-α treated, (f) TUNEL assay of manassantin A plus TNF-α treated.

induced expression of Bfl-1/A1 by TNF-α. After preincubation of HT-1080 cells with indicated concentrations of manassantin A for 30 min and subsequent stimulation with TNF-α for 3 hr, the induced expression of Bfl-1/A1 was analyzed by Northern blot. As shown in Fig. 5A, TNF-α induced a 10-fold increase of Bfl-1/A1 mRNA in HT-1080 cells, however, the induced-expression was blocked by manassantin A in a dose-dependent manner. Manassantin A almost completely inhibited the TNF-α-induced mRNA expression of Bfl-1/A1 at the concentration of 30 μM. Concentrations to inhibit Bfl-1/A1 expression are comparable with those of NF-κB inhibition.

3.5. Manassantin A sensitizes TNF-α-induced apoptosis

We further investigated whether this compound sensitizes TNF-α-induced cell death in HT-1080 cells (Fig. 5B). Cells were incubated with 20 ng of TNF-α for 48 hr either in the presence or absence of manassantin A and then examined for cell viability by the MTT method. TNF-α-induced cell death in HT-1080 cells was potentiated by manassantin A in a dose-dependent manner. TNF-α alone induced cell death in approximately 12% of cells and manassantin A alone did not induced cell death up to 30 μM. However, the combination of TNF-α and manassantin A significantly induced cell death. To confirm whether manassantin A potentiated TNF-α-induced apoptosis of HT-1080 cells, we performed a TUNEL assay. As shown in Fig. 5C, the combination of TNF-α and manassantin A significantly induced TUNEL-positive HT-1080. The ratios of TUNEL-positive cells vs. entire cells were 18.4, 23.8, and 65.6% ($P < 0.01$ vs. TNF-α alone treated group, one-way ANOVA,) in TNF-α alone treated, manassantin A alone treated, and TNF-α plus manassantin A treated group, respectively (counted in seven random fields visualized by microscopy, 630×).

3.6. Manassantin A inhibits the expression of LPS-induced iNOS and production of nitric oxide

Next, we investigated the effect of manassantin A on the LPS-induced iNOS expression in RAW264.7 cells. After RAW264.7 cells were stimulated with 1 μg/mL of LPS for 6 hr in the presence or absence of various concentrations of

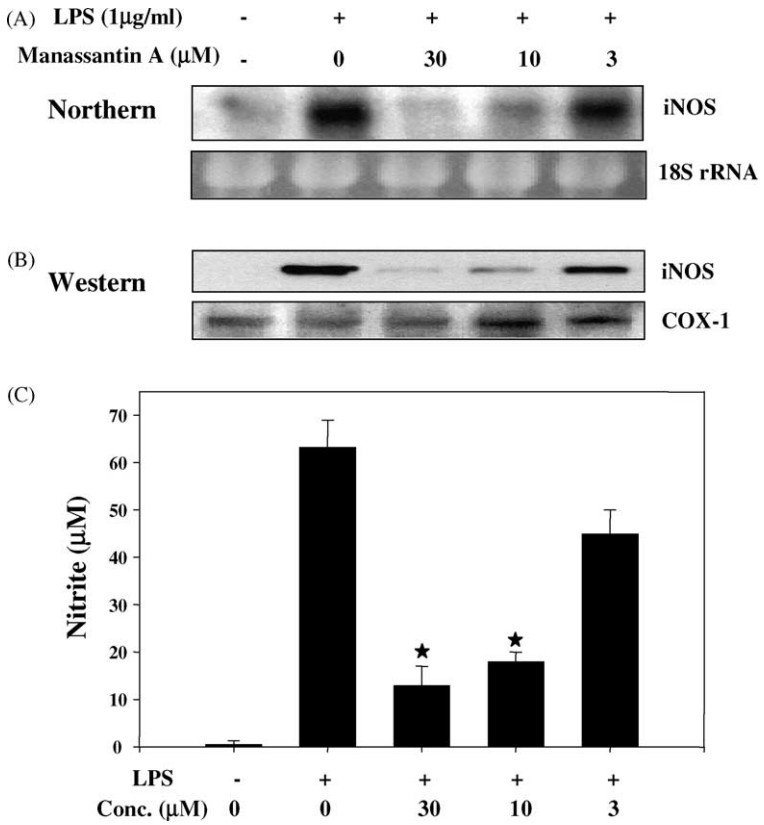


Fig. 6. Effect of manassantin A on the expression of iNOS mRNA and protein in LPS-stimulated RAW264.7 cells. RAW264.7 cells were incubated with or without LPS (1 μg/mL) in the presence of the indicated concentrations of manassantin A. (A) After incubation for 6 hr, total RNAs were isolated, and Northern blot analysis was carried out as described in Section 2. Ethidium bromide staining 18S ribosomal RNA band on the gel was shown to demonstrate equal loading of RNA. (B) After incubation for 18 hr, cellular proteins were prepared, and Western blot analysis was carried out as described in Section 2. To demonstrate equal loading of protein, the blot was stripped and reprobed with anti-COX-1 (cylooxygenase-1) antibody. (C) RAW264.7 cells were stimulated with 1 μg/mL of LPS for 18 hr and the media was exchanged with fresh one. The cells were incubated with indicated concentrations of manassantin A for another 18 hr and NO in the culture medium was measured as described in Section 2. Mean values from two independent experiments performed in triplicated are shown; bar indicates the SDs. Statistical significance ($P < 0.001$) judged by paired Student's *t*-test is marked with an asterisk.

manassantin A, total RNAs were prepared and then Northern blot analysis was performed. As shown in Fig. 6A, manassantin A suppressed the LPS-induced iNOS mRNA expression in a dose-dependent manner. Manassantin A almost completely inhibited the LPS-induced mRNA expression of iNOS at the concentration of 30 μM. We further confirmed the effect of manassantin A on LPS-induced iNOS expression with immunoblot analysis. After RAW264.7 cells were stimulated for 18 hr with 1 μg/mL of LPS in the presence of various concentrations of manassantin A, total cell lysates were prepared, and then immunoblot analysis was performed. As shown in Fig. 6B, LPS-induced 130 kDa of iNOS protein expression was suppressed by manassantin A in a dose-dependent manner. Consistent with the above results, manassantin A significantly inhibit the production of NO in LPS-stimulated RAW264.7 cells. As shown in Fig. 6C, RAW264.7 cells spontaneously released detectable levels of NO (1.5 ± 0.8 μM, mean ± SD). Upon stimulation, the production of NO was significantly increased up to 63.2 ± 5.8 μM for 24 hr. Manassantin A significantly inhibited LPS-induced NO production in a dose-dependent manner with IC_{50} value of 5.91 μM.

4. Discussion

The roots of *S. chinensis* have been used for the treatment of edema, jaundice, gonorrhea, and inflammatory disease in Korean traditional medicine. Despite of its various pharmacological activities, the molecular mechanism has not been sufficiently explained. In present study, we investigated how manassantins A and B, two major constituents of this plant, suppress NF-κB activation.

Manassantin A did not prevent induced degradation of IκB-α and DNA-binding activity of NF-κB following stimulation, but inhibit NF-κB activation by various stimuli and RelA/p65-overexpression. These observations lead us to formulate a hypothesis that manassantins may modify transactivation activity of RelA/p65 subunit of NF-κB. To test this hypothesis, we investigated whether these compounds suppress the transactivation activity of RelA/p65 subunit using Gal4 system. As expected, these compounds significantly suppressed the transcriptional activity of RelA/p65 subunit. Concentrations to inhibit transcriptional activity of RelA/p65 were comparable to those which these compounds inhibit NF-κB activation by various inducers. It is well known that RelA/p65 is a critical transactivation subunit of NF-κB [3,4]. Recent studies have demonstrated that the transcriptional activity of RelA/p65 subunit is regulated by posttranslational modifications such as phosphorylation and acetylation [24]. It was demonstrated that the phosphorylation status of RelA determines whether it associates with CREB binding protein/p300, which is a critical regulator of NF-κB [25]. In this regard, it is possible that manassantins could suppress transcriptional activity of RelA/p65 by modifying the phosphorylation or acetylation status of RelA/p65 subunit. Further studies remain to be elucidated how manassantins do regulate the transcriptional activity of RelA/p65/p65 subunit. It was reported that a crude methanol extract of the aerial part of the same plant inhibited LPS-induced iNOS expression in the RAW264.7 cells via inhibition of IκB degradation [26]. The methanol extract of the aerial part of the plant would contain flavonoids as major components and numerous other components unidentified yet. Manassantins have isolated from the root of the plant, therefore, the mechanism of inactivation of NF-κB by manassantin would be different from that of the methanol extract.

The relevance of most of NF-κB target genes makes this transcription factor an interesting therapeutic target for the identification of inhibitors. One group of NF-κB inhibitors exerts its inhibitory effects by antioxidative properties [27–29]. Another group of inhibitors interferes with the induced degradation of IκB family members by affecting the 26S proteosome or inhibiting IκB kinase complex [30,31]. Another group of inhibitors interferes with DNA-binding activity of NF-κB by directly targeting the NF-κB subunits [23,32]. Further group of inhibitors exerts their effects by impairing the transcriptional activity of NF-κB already bound to DNA. Examples are inhibitors of PG490 (triptolide), and, at least in some cell type, glucocorticoids [33,34]. Manassantins could be added to this group as an inhibitor of transactivation activity of NF-κB.

Several studies have demonstrated an essential role for NF-κB in apoptosis and inflammatory diseases. It is now clear that several downstream effectors of NF-κB activation have been known to involve in apoptosis and inflammation. Manassantin A clearly suppressed not only the induced expression of Bfl-1/A1 by TNF-α, but the induced expression of inducible nitric oxide synthase by LPS. Therefore, manassantins could serve as an interesting lead compound for the development of new, potent anti-inflammatory or anticancer agent.

Taken together, we have shown that manassantins inhibit the NF-κB signal cascade by preventing transcriptional activity of RelA/p65, and the induced expression of NF-κB target genes such as Bfl-1/A1 and inducible nitric oxide synthase. Furthermore, this study extends our understanding on the molecular mechanisms underlying the diverse biological activities of *S. chinensis* that is used in traditional medicine. Further studies remain to be elucidated how manassantins regulate the transactivation activity of RelA/p65 in detail.

Acknowledgments

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