Guggulsterone Inhibits NF-κB and IκBα Kinase Activation, Suppresses Expression of Anti-apoptotic Gene Products, and Enhances Apoptosis*

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Guggulsterone, derived from Commiphora mukul and used to treat obesity, diabetes, hyperlipidemia, atherosclerosis, and osteoarthritis, has been recently shown to antagonize the farnesoid X receptor and decrease the expression of bile acid-activated genes. Because activation of NF-κB has been closely linked with inflammatory diseases affected by guggulsterone, we postulated that it must modulate NF-κB activation. In the present study, we tested this hypothesis by investigating the effect of this steroid on the activation of NF-κB induced by inflammatory agents and carcinogens. Guggulsterone suppressed DNA binding of NF-κB induced by tumor necrosis factor (TNF), phorbol ester, okadaic acid, cigarette smoke condensate, hydrogen peroxide, and interleukin-1. NF-κB activation was not cell type-specific, because both epithelial and leukemia cells were inhibited. Guggulsterone also suppressed constitutive NF-κB activation expressed in most tumor cells. Through inhibition of IκB kinase activation, this steroid blocked IκBα phosphorylation and degradation, thus suppressing p65 phosphorylation and nuclear translocation. NF-κB-dependent reporter gene transcription induced by TNF, TNFR1, TRADD, TRAF2, NIK, and IKK was also blocked by guggulsterone but without affecting p65-mediated gene transcription. In addition, guggulsterone decreased the expression of gene products involved in anti-apoptosis (IAP1, xIAP, Bfl-1/A1, Bcl-2, cFLIP, and survivin), proliferation (cyclin D1 and c-Myc), and metastasis (MMP-9, COX-2, and VEGF); this correlated with enhancement of apoptosis induced by TNF and chemotherapeutic agents. Overall, our results indicate that guggulsterone suppresses NF-κB and NF-κB-regulated gene products, which may explain its anti-inflammatory activities.

Of the 121 prescription drugs in use today for cancer treatment, 90 are derived from plant species. Almost 74% of these were discovered by investigating a folklore claim (1, 2). Taxol is perhaps the most recent example. Between 1981 and 2002, 48 of 65 drugs approved for the therapy of cancer were natural products, were based on natural products, or mimicked natural products in one form or another (3). These phytochemicals are commonly called chemotherapeutic or chemopreventive agents. These phytochemicals may fight disease through suppression of the inflammatory response. Dysregulated inflammation is the cause of a great many diseases, including cancer (4, 5). It stands to reason, then, that suppression of inflammation, whether by phytochemicals or other means, should delay the onset of disease (1, 2).

One phytochemical that has aroused considerable interest is guggulsterone (4,17(20)-pregnadiene-3,16-dione), a plant steroid derived from the gum resin (guggulu) of the tree Commiphora mukul. The resin has been used in Ayurvedic medicine for centuries to treat a variety of ailments, including obesity, bone fractures, arthritis, inflammation, cardiovascular disease, and lipid disorders (6, 7). The anti-arthritic and anti-inflammatory activity of gum guggul was demonstrated as early as 1960, by Gujar et al. (8). Sharma et al. showed its activity in experimental arthritis induced by mycobacterial adjuvant (9). The effectiveness of guggul for treating osteoarthritis of the knee has also been demonstrated (10). Recent studies have shown that guggulsterone is an antagonist for bile acid receptor farnesoid X receptor (11, 12). Other studies have shown that guggulsterone enhances transcription of the bile salt export pump (13), thus regulating cholesterol homeostasis.

An understanding of the molecular mechanisms underlying guggulsterone is just now emerging. In 2003, Meselhy et al. (14) showed that guggulsterone can suppress inflammation by inhibiting inducible nitric-oxide synthetase expression induced by lipopolysaccharide in macrophages. Because most inflammatory diseases are mediated through the activation of NF-κB, 1 a nuclear transcription factor (15, 16), we hypothesize that it is involved in the activity of guggulsterone. Because expression of inducible nitric-oxide synthetase requires NF-κB activation, Meselhy’s demonstration that guggulsterone down-regulates the expression of inducible nitric-oxide synthetase supports this hypothesis.

NF-κB is present in all cells in a resting state in the cyto-

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1 The abbreviations used are: NF-κB, nuclear factor-κB; IκB, inhibitory subunit of NF-κB; TNF, tumor necrosis factor; SEAP, secretory alkaline phosphatase; IκK, IκBα kinase; COX-2, cyclooxygenase-2; MMP-9, matrix metalloproteinase-9; TNFR, TNF receptor; TRADD, TNFR-associated death domain; TRAF, TNFR-associated factor; NIK, NF-κB-inducing kinase; PMA, phorbol myristate acetate; PBS, fetal bovine serum; EMSA, electrophoretic mobility shift assay; TPCK, t-1-tosylamido-2-phenylethyl chloromethyl ketone; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide; PARP, polyadenosine ribose polymerase.

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plasm; only when activated and translocated to the nucleus is the sequence of events leading to activation initiated (15, 16). Currently NF-κB is considered a family of Rel-domain-containing proteins, namely Rel A (also called p65), Rel B, c-Rel, p50 (also called NF-κB1), and p52 (also called NF-κB2). Similarly, a family of anchorin-domain-containing proteins has been identified that keeps the NF-κB in its inactive state within the nucleus. These include IκBa, IκBγ, IκBγ, IκBe, bcl-3, p105, and p100. Most carcinogens, inflammatory agents, and tumor promoters, including cigarette smoke, phorbol ester, okadaic acid, H2O2, and TNF have been shown to activate NF-κB.

Under resting conditions, NF-κB consists of a heterotrimer of p50, p65, and IκBe in the cytoplasm. The phosphorylation, ubiquitination, and degradation of IκBa and phosphorylation of p65 leads to the translocation of NF-κB to the nucleus where it binds to specific response elements in the DNA. The phosphorylation of IκBa is catalyzed by IKK, which consists of three subunits IKKα, IKKβ, and IKKγ. Gene deletion studies have shown that IKKβ is essential for NF-κB activation by most agents. The identity of the kinase that induces the phosphorylation of p65 is controversial, but IKKβ, protein kinase C, and protein kinase A have been implicated (for references see Refs. 17 and 18). NF-κB has been shown to regulate the expression of a number of genes whose products are involved in tumorigenesis (15–18). These include anti-apoptotic genes (e.g. ciap, survivin, traf, bcl-2, and bcl-xl); cox2; mmp-9; genes encoding adhesion molecules, chemokines, and inflammatory cytokines; and cell cycle regulatory genes (e.g. cyclin d1).

Furthermore, NF-κB has been implicated in obesity (19), hyperlipidemia (20), atherosclerosis (21), osteoarthritis (22), and bone loss (23), all of which can be modulated by guggulsterone. In the present report we investigated the effect of guggulsterone on NF-κB activation induced by a variety of inflammatory agents and carcinogens. The aim of the study was to determine whether guggulsterone can suppress NF-κB activation induced by inflammatory agents and carcinogens and block NF-κB-regulated gene expression that mediates inflammation and carcinogenesis. We found that guggulsterone inhibited activation of NF-κB through suppression of IκBa kinase, IκBa phosphorylation, and degradation, p65 nuclear translocation, and NF-κB-dependent reporter gene expression. Guggulsterone also abrogated the expression of NF-κB-regulated gene products that inhibit apoptosis and promote inflammation and tumor metastasis.

**EXPERIMENTAL PROCEDURES**

**Materials**—Z-Guggulsterone, obtained from Steraloids, Inc. (Newport, RI), was dissolved in MeSO or a 100 mM stock solution and stored at −20 °C. Bacteria-derived human tumor necrosis factor (TNF), purified to homogeneity with a specific activity of 5 × 106 units/mg, was kindly provided by Genentech, Inc. (South San Francisco, CA). Penicillin, streptomycin, RPMI 1640 medium, FBS, and LipofectAMINE 2000 were kindly provided by Genentech, Inc. (South San Francisco, CA). Penicil- lin, streptomycin, RPMI 1640 medium, FBS, and LipofectAMINE 2000 were obtained from Invitrogen. The following polyclonal antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA): anti-p65, against the epitope corresponding to amino acids mapping within the amino-terminal domain of bHLH, anti-p50, and anti-p52, against a peptide 15 amino acids long mapping at the nuclear localization sequence region of NF-κB p50; anti-IκBa, against amino acids 297–317 mapping at the carbboxyl terminus of IκBa/MAD-3; anti-c-Rel, anti-cyclin D1 against amino acids 1–295, which represents full-length cyclin D1 of human origin; anti-MMP-9, anti-polyadenosine ribose polymerase (PARP); anti-IAP1; anti-IAP2; anti-Bcl-2; anti-Bcl-1; anti-G1A; and anti-TRAF1; anti-COX-2, anti-MMP9, and anti-IAP antibodies were obtained from BD Biosciences, and phospho-specific anti-IκBa (Ser23) antibody was from Cell Signaling (Beverly, MA). Anti-IKKα and anti-IKKβ antibodies were kindly provided by Imgenex (San Diego, CA).

**Cell Lines**—The cell lines used in our studies included human non-small cell lung carcinoma (H1299) cells and human lung epithelial cell carcinoma (A549) cells, both kindly provided by Dr. Reuben Lotan (The University of Texas M. D. Anderson Cancer Center). Human leukemia (Jurkat) and myelogenous leukemia (KBM-5) cells were obtained from the American Type Culture Collection (Manassas, VA). A549, Jurkat, and H1299 cells were cultured in RPMI 1640 medium, and KBM-5 cells were grown in Iscove’s modified Dulbecco’s modified EMEM medium. For Jurkat and A549 cells, RPMI 1640 was supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin; for KBM-5 cells, Dulbecco’s modified Eagle’s medium was supplemented with 15% FBS plus penicillin and streptomycin.

**NF-κB Activation**—To determine NF-κB activation by TNF, which has a well established role in inflammation, tumor proliferation, promotion, invasion, and metastasis (24), we carried out EMSA essentially as previously described (25). The dried gels were visualized, and radioactive bands were quantitated using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) using ImageQuanNT software. The fold activation, over that of untreated control lane, was then calculated.

**Western Blot Analysis**—To determine the effect of guggulsterone on TNF-dependent IkBe degradation, and phosphorylation, cytoplasmic extracts were prepared as previously described (26) from H1299 cells (2 × 106/ml) that had been pretreated with 50 μM guggulsterone for 4 h and then exposed to 0.1 nM TNF for various times. For determining the phosphorylation of IkBa, H1299 cells (2 × 106/ml) were first treated with 100 μM N-acetyl-Leu-Leu-norleucinal (a proteasome inhibitor) for 1 h, then treated with 50 μM guggulsterone for 4 h or TNF for various times. The cytoplasmic micrograms of cytoplasmic protein was resolved on 10% SDS-PAGE gel, transferred into a nitrocellulose membrane, blocked with 5% nonfat milk, and probed with specific antibody against IkBa, phosphorylated IkBa, p65, and phosphorylated p65. To determine the expression of cyclin D1, COX-2, MMP-9, cIAP1, xIAP, TRAF1, Bcl-2, Bfl-1, cFLIP, and survivin in whole cell extracts of treated cells (2 × 106 cells in 2 ml medium), 30–50 μg of protein was resolved on SDS-PAGE and probed with Western blot with specific antibodies as per the manufacturer’s recommended protocol. The blots were washed, exposed to horseradish peroxidase-conjugated secondary antibodies for 1 h, and finally detected by ECL reagent (Amersham Biosciences). The bands were quantitated using a Personal Densitometer Scan version 1.30 using ImageQuanNT software version 3.3 (Amersham Biosciences).

**Immunolocalization of NF-κB p65**—The effect of guggulsterone on cigarette smoke-induced nuclear translocation of p65 was examined by an immunocytochemical method using an epifluorescence microscope (Labophot-2, Nikon, Tokyo, Japan) and a Photometrics Coolscan CF color camera (Nikon, Lewisville, TX) as described previously (27).

**IKK Assay**—To determine the effect of guggulsterone on TNF-induced IKK activation, we analyzed IKK by a method essentially as described previously (28).

**NF-κB-dependent Reporter Gene Transcription**—The effect of guggulsterone on TNF-induced NF-κB-dependent reporter gene transcription in H1299 cells was measured as previously described (29).

**COX-2 Promoter-dependent Reporter Luciferase Gene Expression**—COX-2 promoter activity was examined as described elsewhere (28). To further examine the effect of guggulsterone on COX-2 expression, A293 cells were seeded at a concentration of 1.5 × 105 cells per well in six-well plates. After overnight culture, the cells in each well were transfected with 2 μg of DNA consisting of COX-2 promoter-luciferase reporter plasmid, along with 6 μl of LipofectAMINE 2000 according to the manufacturer’s protocol. The COX-2 promoter (−375 to +59), which was amplified from human genomic DNA by using the primers 5′-GACTGCTTTATTATTATTT3′ (sense) and 5′-GCTGCTGGAGGAGGTCCTGGACGTGTC-3′ (antisense), was kindly provided by Dr. Xiao-Chun Xu (M. D. Anderson Cancer Center). After a 6-h exposure to the transfection mixture, the cells were incubated in medium containing guggulsterone for 12 h. The cells were exposed to TNF (0.1 nM) for 24 h and then harvested. Luciferase activity was measured by using the Pro-mega luciferase assay system according to the manufacturer’s protocol and detected by using Monolight 2010 (Analytical Luminescence Laboratory, San Diego, CA). All experiments were performed in triplicate and repeated at least twice to prove their reproducibility.

**Cytotoxicity Assay**—Cytotoxicity was assayed by the modified tetra- zolium salt 3(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) assay as described previously (27).

**Cell Viability Analysis**—Cells were treated with the compounds of interest for 24 h. After treatment, cells were detached from the culture plates by a dissociation solution (trypsin-EDTA) and counted with a cell counter. To determine the growth effects of the test compounds on cell growth, cells were stained with the dye 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT). The MTT assay was performed as described previously (27).
Live and Dead Assay—To measure apoptosis, we used the Live and Dead assay (Molecular Probes), which determines intracellular esterase activity and plasma membrane integrity. This assay employs calcein, a polyanionic dye, which is retained within the live cells and provides green fluorescence (30). It also employs the ethidium monomer dye (red fluorescence), which can enter the cells only through damaged membranes and bind to nucleic acids but is excluded by the intact plasma membrane of live cells. Briefly, 1/H11003 105 cells were incubated with 10/H9262 M guggulsterone for 24 h and then treated with 1 nM TNF for 16 h at 37 °C. Cells were stained with the Live and Dead reagent (5/H9262 M ethidium homodimer, 5/H9262 M calcein-AM) and then incubated at 37 °C for 30 min. Cells were analyzed under a fluorescence microscope (Labophot-2).

RESULTS

The guggulsterone used in these studies, which has a steroidal-like structure (Fig. 1A), was dissolved in Me2SO. The concentration of guggulsterone used and the duration of exposure had minimal effect on the viability of these cells as determined by trypan blue dye exclusion test (data not shown).

Guggulsterone Blocks NF-κB Activation Induced by TNF, Interleukin-1β, Okadaic Acid, Cigarette Smoke Condensate, and H2O2—Because TNF, interleukin-1β, PMA, H2O2, and okadaic acid activate NF-κB, we examined the effect of guggulsterone on the activation of NF-κB by these agents in H1299 cells. A DNA-binding assay (EMSA) showed that guggulsterone suppressed the NF-κB activation induced by all these agents (Fig. 1B). These results suggest that guggulsterone acted at a step in the NF-κB activation pathway that is common to all these agents.

Inhibition of NF-κB Activation by Guggulsterone Is Not Cell Type-specific—Because some reports suggest that distinct signal transduction pathways mediate NF-κB induction in epithelial and lymphoid cells (31), we determined whether guggulsterone inhibited NF-κB activation in three cell types. Guggulsterone completely inhibited TNF-induced NF-κB activation in lung epithelial cell carcinoma (A549), T cell leukemia (Jurkat), and myeloid leukemia (KBM-5) cells (Fig. 2A), indicating a lack of cell type specificity.

Guggulsterone Inhibits Constitutive NF-κB Activation—We next tested the effect of guggulsterone on NF-κB activation in human multiple myeloma (U266) and head and neck squamous cell carcinoma (MDA 1986) tumor cells, which both express constitutively active NF-κB (27, 32). Guggulsterone completely inhibited this constitutively active NF-κB (Fig. 2B).

The Suppression of NF-κB by Guggulsterone Is Dose- and Time-dependent—Guggulsterone inhibited TNF-mediated

![Diagram](image-url)
NF-κB activation in a dose-dependent manner, with maximum inhibition occurring at 50 μM (Fig. 3A). The minimum time required for complete inhibition of NF-κB activation was 4 h (Fig. 3B).

Suppressed NF-κB Consists of Both p50 and p65—When nuclear extracts from TNF-activated cells were incubated with antibodies to the p50 (NF-κB1) and the p65 (RelA) subunit of NF-κB, the resulting bands were shifted to higher molecular masses (Fig. 3C), suggesting that the TNF-activated complex consisted of p50 and p65. Neither preimmune serum nor irrelevant antibody had any effect. Addition of excess unlabeled NF-κB (cold oligonucleotide; 100-fold) caused complete disappearance of the band, whereas mutated oligonucleotide had no effect on the DNA binding.

Guggulsterone Does Not Directly Affect Binding of NF-κB to the DNA—Although TPCK (the serine protease inhibitor), herbimycin A (protein tyrosine kinase inhibitor), and caffeic acid phenyl ethyl ester directly modify NF-κB to suppress its activation (33–35), EMSA showed that guggulsterone did not modify the DNA-binding ability of NF-κB proteins prepared from cells by treatment with TNF (Fig. 3D). Therefore, guggulsterone must inhibit NF-κB activation by a different mechanism.

Guggulsterone Inhibits TNF-dependent IκBα Degradation—Because IκBα degradation is normally a condition for translocation of NF-κB to the nucleus (36), we determined whether the guggulsterone’s inhibition of TNF-induced NF-κB activation was due to inhibition of IκBα degradation. We found that TNF induced IκBα degradation in control cells as early as 10 min, but in guggulsterone-pretreated cells TNF had no effect on IκBα degradation (Fig. 4A, upper panel).

Guggulsterone Inhibits TNF-dependent IκBα Phosphorylation—We next determined whether guggulsterone affected TNF-induced IκBα phosphorylation, another condition for NF-κB translocation. Western blot analysis using antibody that detects only the serine-phosphorylated form of IκBα indicated that TNF induced IκBα phosphorylation as early as 5 min, and guggulsterone almost completely suppressed it (Fig. 4A, middle panel). Thus guggulsterone inhibited TNF-induced

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**A. Inducible NF-κB:**

<table>
<thead>
<tr>
<th>Human lung epithelial cell carcinoma (A549)</th>
<th>Human myelogenous leukemia (KBM-5)</th>
<th>Human leukemia (Jurkat)</th>
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<td><img src="image1" alt="EMSA image" /></td>
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**B. Constitutive NF-κB activation:**

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<th>Multiple myeloma cells</th>
<th>Head and Neck cancer cells</th>
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<td><img src="image4" alt="EMSA image" /></td>
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Fig. 2. Guggulsterone suppresses inducible and constitutive NF-κB activation. A, suppression of inducible activation is not cell-type specific. Two million A549, Jurkat, or KBM-5 cells were pre-treated with 50 μM guggulsterone for 4 h and then treated with 0.1 nM TNF for 30 min. The nuclear extracts were then prepared and assayed for NF-κB by EMSA as described under “Experimental Procedures.” B, two million multiple myeloma and head and neck squamous cell carcinoma cells were exposed to 50 μM guggulsterone for 4 h, and then nuclear extracts were prepared and assayed for NF-κB by EMSA as described under “Experimental Procedures.” The numbers at the bottom of each lane indicate -fold activation over that of untreated control.
Inhibition of TNF-induced NF-κB by Guggulsterone

NF-κB activation by inhibiting phosphorylation and degradation of IκBα.

Guggulsterone Inhibits TNF-induced IKK Activation—Because guggulsterone inhibits the phosphorylation of IκBα, we tested the effect of guggulsterone on TNF-induced IKK activation, which is required for TNF-induced phosphorylation of IκBα. As shown in Fig. 4B (upper panel), guggulsterone completely suppressed TNF-induced activation of IKK. TNF or guggulsterone had no direct effect on the expression of either IKKa (middle panel) or IKKβ (lower panel) proteins. However,
we did find that guggulsterone inhibited the phosphorylation of GST IκBα (at a 50 μM concentration) by directly interfering with IKK activity (Fig. 4C).

**Guggulsterone Inhibits TNF-induced Phosphorylation and Nuclear Translocation of p65**—We also tested the effect of guggulsterone on TNF-induced phosphorylation of p65, because phosphorylation is also required for transcriptional activity of p65 (37). As shown in Fig. 5A, guggulsterone suppressed p65 phosphorylation almost completely. Likewise, Western blot analysis (Fig. 5B) and immunocytochemistry (Fig. 5C) indicated that guggulsterone abolished TNF-induced nuclear translocation of p65.

**Guggulsterone Represses TNF-induced NF-κB-dependent Reporter Gene Expression**—Although we showed by EMSA that guggulsterone blocked NF-κB activation, DNA binding alone does not always correlate with NF-κB-dependent gene transcription, suggesting that there are additional regulatory steps (38). Transient transfection of H1299 cells with the NF-κB-regulated SEAP reporter construct followed by stimulation with TNF produced an almost 13-fold increase in SEAP activity over vector control activity (Fig. 6A). TNF-induced SEAP activity was abolished by dominant-negative IκBα, indicating specificity. When the cells were pretreated with guggulsterone, TNF-induced NF-κB-dependent SEAP expression was inhibi...
Guggulsterone Represses TNF-induced COX-2 Promoter Activity—We next determined whether guggulsterone affected COX-2 promoter activity, which is regulated by NF-κB (41). As shown in Fig. 6A, guggulsterone significantly reduced the TNF-induced COX-2 promoter activity in a dose-dependent manner.

Guggulsterone Inhibits TNF-induced COX-2, MMP-9, and VEGF Expression and TNF-induced Cyclin D1 and c-myc Expression—Guggulsterone abolished, in a dose-dependent fashion, the TNF-induced expression of COX-2, MMP-9, and VEGF (Fig. 7A), which are known to be NF-κB-regulated gene products (41–43). Expression of the NF-κB-regulated gene products cyclin D1 and c-Myc (44, 45) was also abolished by guggulsterone (Fig. 7B).
Guggulsterone Inhibits TNF-induced Activation of Anti-apoptotic Gene Products—NF-κB up-regulates the expression of a number of genes implicated in facilitating tumor cell survival, including cIAP1, xIAP, Bfl-1, Bcl-2, TRAF1, cFLIP, and survivin (46–54). We found that guggulsterone inhibited the TNF-induced expression of all of these proteins (Fig. 8).

Guggulsterone Potentiates the Cytotoxic Effects of TNF and Chemotherapeutic Drugs—Because NF-κB-regulated products suppress TNF- and chemotherapy-induced apoptosis (55, 56), we examined the effects of guggulsterone on the apoptotic effects of TNF and the chemotherapeutic drugs paclitaxel and doxorubicin. Guggulsterone enhanced the cytotoxic effects of TNF, Taxol, and doxorubicin (Fig. 9A) and the caspase-induced cleavage of PARP activated by TNF (Fig. 9B). The Live and Dead assay also showed that TNF-induced apoptosis was significantly enhanced by guggulsterone (Fig. 9C).

DISCUSSION

In this study, we have demonstrated that guggulsterone suppressed NF-κB activated by carcinogens (phorbol ester, okadaic acid, and cigarette smoke condensate) and inflammatory stimuli (hydrogen peroxide, TNF, and interleukin-1β) through inhibition of IKK, IκBα phosphorylation, and IκBα degradation, which led to abrogation of p65 phosphorylation and nuclear translocation. NF-κB-dependent reporter gene transcription induced by TNF, TNFR1, TRADD, TRAF2, NIK, and IKK, was also blocked. The expression of gene products involved in anti-apoptosis (IAP1, xIAP, Bfl-1/A1, Bcl-2, cFLIP, and survivin), proliferative genes (cyclin d1 and c-Myc), and metastatic genes (MMP-9, COX-2, and VEGF) was down-regulated by guggulsterone, and this down-regulation correlated with enhancement of apoptosis induced by TNF and chemotherapeutic agents.

The present study was undertaken to investigate the potential mechanism for the anti-inflammatory effects of guggulsterone, which has been used to treat obesity, diabetes, hyperlipidemia, atherosclerosis, and osteoarthritis. Although guggulsterone can antagonize the farnesoid X receptor and decrease the expression of bile acid-activated genes (11), these effects do not explain its anti-inflammatory attributes. Because activation of NF-κB has been found in most inflammatory diseases, we hypothesized that
Guggulsterone modulates NF-κB activation. The results of the study validate this hypothesis. Very little is known about the mechanism of action of guggulsterone. Our results clearly indicate that guggulsterone can suppress NF-κB activation induced by a wide variety of agents, suggesting that the site of action of guggulsterone is common to all these agents. We identified IKK as a target site: cells that were exposed to guggulsterone failed to activate IKK in response to TNF. Surprisingly, incubation of IKK with guggulsterone was sufficient to suppress its activity, suggesting that guggulsterone is a direct inhibitor of IKK. Ours is the first report to suggest that a steroid can suppress NF-κB activation through inhibition of IKK activity.

Although steroids exhibit anti-inflammatory activity, whether it is through suppression of NF-κB is less clear. Auphan et al. (57) showed that glucocorticoids inhibit NF-κB activation and that this inhibition is mediated through induction of the IκBα, which traps activated NF-κB in inactive cytoplasmic complexes. Interestingly, estradiol was found to activate NF-κB (58).

By NF-κB gene reporter assay, we found that guggulsterone suppressed NF-κB activation induced by TNF, TNFRI, TRADD, TRAF2, NIK, and IKK but not that activated by p65. Although this pathway is restricted to TNF-induced NF-κB activation, these results again confirm that IKK is a potential target of guggulsterone. Whether guggulsterone suppresses NF-κB activation through other mechanisms cannot, however, be completely ruled out by the results of our study.

Various tumor cell types express constitutively active NF-κB, and it is critical for their proliferation (18, 27, 32). The potential mechanism of constitutive activation of NF-κB is not fully understood. Overexpression of IκBα without inhibition of NF-κB activity and mutations in the ikbα gene in Reed-Sternberg cells (59) and enhanced IκBα degradation in mature murine B-cell lines (60) have been demonstrated as potential mechanisms. Our laboratory showed that constitutive expression of TNF in T-cell lymphoma (32) and interleukin-1 in acute myelogenous leukemia (61) are potential mechanisms. We found that human multiple myeloma cells and head and neck squamous cell carcinoma cells also express constitutive NF-κB,
and guggulsterone suppressed the activation. It is very likely that this inhibition also occurs through inhibition of IKK.

That activation of NF-κB regulates genes that control proliferation and metastasis of cancer has been well established (17, 18, 43). Our results demonstrate that guggulsterone can suppress the expression of COX-2, MMP-9, VEGF, cyclin D1, and c-Myc, all regulated by NF-κB. We found that COX-2 promoter activity was also significantly down-regulated by this steroid. The lack of complete inhibition of COX-2 promoter activity by guggulsterone suggests that this promoter is also regulated by transcription factors other than NF-κB. These results imply that guggulsterone has a potential to suppress proliferation of tumor cells and their metastasis.

Our results indicate that guggulsterone also suppresses the expression of numerous anti-apoptotic gene products, all known to be regulated by NF-κB activation. The overexpression of IAP1, xIAP, Bcl-1/A1, bcl-2, cFLIP, and survivin has been found in numerous tumors and has been linked to survival, chemoresistance, and radioresistance. Because most of these gene products are down-regulated by guggulsterone, we believe that guggulsterone has potential as an agent for overcoming radioresistance and chemoresistance. Indeed, guggulsterone potentiated the apoptotic effects of TNF and Taxol and doxorubicin. Thus guggulsterone, which is a pharmacologically safe agent (6), could be used as an anticancer agent on its own, a preventive agent, or an enhancer of chemotherapy/radiotherapy activity. Our demonstration, that guggulsterone suppresses NF-κB and NF-κB-regulated gene expression, may
Inhibition of TNF-induced NF-κB by Guggulsterone

explain its effects against atherosclerosis, diabetes, osteoarthritis, and other inflammatory diseases.

Cancer is a hyperproliferative disorder characterized by the up-regulation of genes responsible for transformation, proliferation, invasion, angiogenesis, and metastasis. Most of these activities are influenced by the aberrant activity of NF-κB; therefore, the main objective of this study was to evaluate the efficacy of guggulsterone as an inhibitor of NF-κB. Our study for the first time demonstrates that guggulsterone can suppress NF-κB activation induced by various carcinogens, inflammatory agents, and tumor promoters. This effect was not cell type-specific and equally pronounced in cells where NF-κB is constitutively active. TNF-induced activation of IKK and phosphorylation and degradation IκBα were also inhibited by guggulsterone. Guggulsterone was quite effective in blocking the phosphorylation and translocation of TNF-induced p65, which led to the abrogation of NF-κB-dependent reporter gene expression. TNF-induced NF-κB reporter activity induced by TNFR, TRADD, TRAF, NIK, and IKKβ, but not that activated by p65, was also blocked.

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REFERENCES