Inhibition of Tumor Necrosis Factor-α-induced Nuclear Translocation and Activation of NF-κB by Dehydroxymethylepoxyquinomicin*

We previously designed and synthesized an NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), that showed anti-inflammatory activity in vivo. In the present study we looked into its mechanism of inhibition. DHMEQ inhibited tumor necrosis factor-α (TNF-α) and 12-O-tetradecanoylphorbol-13-acetate-induced transcriptional activity of NF-κB in human T cell leukemia Jurkat cells. It also inhibited the TNF-α-induced DNA binding of nuclear NF-κB but not the phosphorylation and degradation of IκB. Moreover, DHMEQ inhibited the TNF-α-induced nuclear accumulation of p65, a component of NF-κB. It also inhibited TNF-α-induced nuclear transport of green fluorescent protein-tagged p65. On the other hand, DHMEQ did not inhibit the nuclear transport of Smad2 and large T antigen. Also, it did not inhibit TNF-α-induced activation of JNK but synergistically induced apoptosis with TNF-α in Jurkat cells. Taken together, these data indicate that DHMEQ is a unique inhibitor of NF-κB acting at the level of nuclear translocation.

NF-κB is the transcription factor that binds to the κB sequence (1). It promotes transcription of cytokines such as IL-1, IL-2, IL-6, IL-8, TNF-α, interferon-γ, and viral proteins (4). NF-κB is a heterodimer mainly consisting of p65 (Rel A) and p50 proteins. The p65 and p50 proteins have a homologous sequence at their N-terminal regions (5, 6), and NF-κB can form various heterodimers or homodimers. The NF-κB family proteins include p65, p50, p52, c-Rel, Rel B, v-Rel, Dorsal, Dif, and Relish (7).

NF-κB is activated by extracellular signals such as TNF-α (8, 9), IL-1, lipopolysaccharide, UV, and phorbol esters (10). Signal transduction pathways from the TNF-α receptor to NF-κB activation have been extensively studied (7). TNF-α receptors (TNFRs) include 55-kDa TNFR I and 75-kDa TNFR II (11, 12). The ligand mainly acts through TNFR I, inducing its trimerization. For activation of NF-κB, TNFR-associated death domain protein recruits receptor-interacting protein and TNFR-associated factor-2 (TRAF2) (13). TRAF2 activates MEKK3, which is a mitogen-activated protein kinase. MEKK3 then activates IκB kinase (IKK), which induces the phosphorylation of the inhibitor of NF-κB (IκB). Phosphorylation of IκB induces its ubiquitination and degradation by proteasomes. Liberated NF-κB molecules then enter the nucleus where they bind to the κB site of DNA.

TNF-α is an apoptosis-inducing factor (14, 15) as suggested by its name, and the TNF receptor contains the death domain, like Fas (16–18). However, TNF-α does not induce apoptosis in most cases, because TNF-α also induces activation of NF-κB, which was found to inhibit apoptosis (19–21). Excess apoptosis was observed in the liver of IKK-β knockout mice (22).

Low molecular weight inhibitors of NF-κB should be useful as immunosuppressants and as anti-inflammatory, antiviral, and anticancer agents. Such inhibitors from natural sources include panepoxydine, cycloepoxydine, and gliotoxin. Panepoxydine was isolated from the basidiomycete Lentinus crinitus. It inhibited TNF-α-induced activation of NF-κB in a promoter-reporter assay using COS-7 cells (23). Panepoxydine was suggested to inhibit TNF-α or TPA-induced phosphorylation and degradation of IκB. Cycloepoxydine was also isolated from a deuteromycete strain as an NF-κB inhibitor in COS-1 cells (24). Gliotoxin produced by fungi was also reported to inhibit the activation of NF-κB by preventing the degradation of IκB (25).

Synthetic adenyl carbocyclic nucleoside (26) and o,o′-bismyristoyl thiamine disulfide (27) were also reported to inhibit NF-κB activation by unknown mechanisms. More recently, a peptide that inhibits the interaction of IκB complex and NF-κB essential modifier was designed for the inhibition of NF-κB activation (28). However, further effective NF-κB inhibitors of low molecular weight are necessary for therapeutic development. In the course of our search for inhibitors of NF-κB function, we have designed new NF-κB inhibitors of low molecular weight from the structure of the antibiotic epoxyquinomicin C.
Inhibition of NF-κB Translocation by DHMEQ

EXPERIMENTAL PROCEDURES

Materials—DHMEQ was synthesized in our laboratory as described before (29). Recombinant human TNF-α, TPA, and N-acetyl-Leu-Leu-norleucinal (ALLN) were purchased from Sigma. Rabbit polyclonal anti-IκB-α and mouse polyclonal anti-p65 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-Smad2 antibody was purchased from Transduction Laboratories (Lexington, KY). TransFast transfection reagent was purchased from Promega (Madison, WI). c-Jun-GST fusion protein was purchased from Upstate Biotechnology (Lake Placid, NY).

Cell Culture—Jurkat cells were grown in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 5% heat-inactivated fetal bovine serum (JRH Biosciences, Lenexa, KS), 100 μg/ml kanamycin, 100 units/ml penicillin G, 30 μg/ml l-glutamine, and 2.25 g/liter NaHCO3. COS-1 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 200 μg/ml kanamycin, 100 units/ml penicillin G, 600 μg/ml l-glutamine, and 2.25 g/liter NaHCO3.

κB/Luciferase Reporter Gene Assay—Jurkat cells were transfected with 2 μg of DNA consisting of tandem κB repeats and the luciferase gene by the DEAE-dextran method. The transfected cells were seeded into 12-well plates at 1 × 105 cells/well. Chemicals dissolved in Me2SO and TNF-α were added at 14 and 16 h, respectively. Six hours after the TNF-α or TPA addition the cells were harvested and lysed, and the lysate was used for the luciferase assay with luciferin substrate buffer (200 μM Tricine-NaOH (pH 8.0), 1.07 mM magnesium carbonate hydroxide, 2.67 mM MgSO4, 0.1 mM EDTA, 33.3 mM DTT, 270 μM CaCl2, 470 μM luciferin, 530 μM ATP). Luminescence was measured with a Lumat 9501 (Berthold). Each value was normalized by the transfection efficiency obtained from the β-actin promoter/β-galactosidase assay.

Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared according to the method of Andrews and Faller (31). In brief, Jurkat cells treated or not with DHMEQ were incubated for 2 h at 37 °C and then incubated for 30 min in the absence or presence of TNF-α (20 ng/ml). The cells were harvested, washed with phosphate-buffered saline (PBS), suspended in 400 μl of buffer A (10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM MgCl2, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF), and incubated on ice for 15 min. Nuclei were pelleted by centrifugation for 5 min at 14,000 rpm, resuspended in 40 μl of buffer C (50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 25% glycerol (v/v)), incubated on ice for 20 min, and centrifuged for 5 min at 14,000 rpm at 4 °C. The supernatant was used as a nuclear extract. The binding reaction mixture containing 5 μg of protein of nuclear extract, 2 μg of poly(dI-dC), and 32P-labeled probe was incubated for 20 min at room temperature. DNA-protein complexes were separated from free DNA on 4% native polyacrylamide gel (0.25 μM TBE buffer. The DNA probe used for NF-κB binding was the double-stranded oligonucleotide containing the κB site from the mouse κ light chain enhancer (5′-ATGTGGAGGGACTTTCCAGG-3′).

Western Blotting—Jurkat cells were lysed with lysis buffer (20 μM Tris (pH 8.0), 150 mM NaCl, 2 mM EDTA, 100 μM NaF, 400 μM Na2VO4, 1% Nonidet P-40, 1 μg/ml leupeptin, 1 μg/ml antipain, and 1 μM PMSF). Each extract (100 μg of protein) was fractionated on a polyacrylamide-SDS gel and then transferred to a polyvinylidene difluoride membrane (Amersham Biosciences). The membrane was incubated overnight at 4 °C for blocking in TBS buffer (20 μM Tris-HCl (pH 7.6), 137 mM NaCl) containing 5% skim milk. After having been washed three times with 0.1% Tween 20 in TBS the membrane was incubated for 1 h at room temperature with each antibody in TBS buffer. After three more washes with the TBS-Tween buffer, the membrane was incubated for 1 h at room temperature with anti-rabbit IgG sheep antibody linked to horseradish peroxidase (Amersham Biosciences). Immunoreactive proteins were visualized by the ECL detection system (Amersham Biosciences).

Nuclear and Cytoplasmic Fractionation for Detection of p65—Jurkat cells (2 × 106) were lysed with 40 μl of buffer A for 20 min on ice and then centrifuged at 14,000 rpm for 5 min. The supernatant was used for the cytoplasmic extract. After the pellet had been washed with buffer A, it was lysed with 40 μl of buffer C for 20 min and centrifuged at 14,000 rpm for 5 min. The supernatant was used for the nuclear extract. The cytoplasmic extract (5 μg of protein) and the nuclear extract (10 μg of protein) were electrophoresed on 10% polyacrylamide-SDS gels and then transferred to a polyvinylidene difluoride membrane. The membranes were incubated for 1 h at room temperature for blocking in TBS buffer containing 5% skim milk. After three washes with TBS-Tween buffer, the membranes were incubated for 1 h at room temperature with anti-rabbit IgG sheep antibody linked to horseradish peroxidase. Immunoreactive proteins were visualized by the ECL detection system.

Immunofluorescence Assay—For the localization of p65, COS-1 cells were seeded onto glass coverslips in 12-well plates at 5 × 105 cells/well. The next day, cells were transfected with 0.5 μg of GFP-tagged p65 plasmid by the lipofection method. After 2 days, cells were pretreated with various concentrations of DHMEQ for 2 h and stimulated with 20 ng/ml TNF-α for 30 min. Then the cells were washed twice with cold Ca2+-,Mg2+-free PBS (PBS’), fixed with 3.7% formaldehyde in PBS’ for several hours, and then permeabilized with 0.1% Triton X-100 in PBS for 10 min. Cells were washed three times with PBS’ and stained with 10 ng/ml Hoechst 33258 for 5 min. After having been washed three times with PBS’, the cells were photographed through B2 and UV filters at a magnification of ×200. Then the number of cells with p65 in the nucleus or cytoplasm was scored.

For the localization of Smad2, the cells were seeded onto glass coverslips in a 12-well plate at 1.5 × 105 cells/well. The next day, cells were transfected with 3 or 10 μg of DHMEQ for 2 h and stimulated with 20 ng/ml TNF-α for 1 h. The cells were then washed twice with cold PBS’ and fixed with 3% formaldehyde for several hours. Next the cells were permeabilized with 1% Triton X in PBS’ for 8 min. After having been washed three times with PBS’, the cells were stained with anti-Smad2 antibody for 60 min at room temperature. Then, they were washed with TBS buffer (0.1 M Tris-HCl (pH 7.5), 0.15 M NaCl, 0.05% Tween 20). Cells were three times stained and stained with anti-mouse secondary antibody for 1 h at room temperature. After three more washes with TNT buffer, the cells were stained with TSA-Direct-Green (PerkinElmer Life Sciences) reagent and 10 ng/ml Hoechst 33258 for 5 min. After three final washes with TUNT buffer, the cells were photographed through B2 and UV filters at a magnification of ×200.

JNK In Vitro Kinase Assay—Jurkat cells were lysed with 200 μl of WCE buffer (20 mM HEPES (pH 7.5), 75 mM NaCl, 2.5 mM MgCl2, 0.1 mM EDTA, 0.5 mM DTT, 20 mM β-glycerophosphate, 0.1 mM Na2VO4, 0.05% Triton X-100, 2 μg/ml leupeptin, 100 μg/ml PMSF). The lysates were incubated on ice for 30 min with protein G-agarose (Oncogene, Boston, MA). After centrifugation, the supernatant was incubated on ice for 1 h with anti-JNK1 antibody and further incubated for 1 h with protein G-agarose. The kinase reaction was performed by incubation of the JNK preparation for 20 min at 30 °C in 40 ml of JNK kinase buffer (20 mM HEPES (pH 7.6), 20 mM MgCl2, 2 mM DTT, 20 μM ATP, 3 μCi of [γ-32P]ATP, 1.5 μg of GST-c-Jun). The reaction mixture was applied on SDS-PAGE, and phosphorylated GST-c-Jun protein was visualized by autoradiography.

Trypan Blue Dye Exclusion—Jurkat cells were seeded into 24-well plates at 5 × 105 cells/well. Cells were pretreated with various concentrations of DHMEQ for 2 h and then stimulated with 20 ng/ml TNF-α for 16 h. Cells were stained with trypan blue, and the number of stained cells was counted.

Hoechst 33258 Staining—Jurkat cells were seeded into a 12-well plate at the concentration of 5 × 105 cells/well, pretreated with 10 μg/ml DHMEQ for 2 h, and stimulated with 20 ng/ml TNF-α for 6 h. After 6 h,
with 10 ng/ml Hoechst 33258 for 5 min. After three final washes with PBS, the cells were spun by centrifugation onto glass coverslips, fixed with 3% formaldehyde in PBS for 10 min. Next, the cells were washed three times with PBS and stained with 10 ng/ml Hoechst 33258 for 5 min. After three final washes with PBS, the cells were photographed through a UV filter at a magnification of ×200.

RESULTS

Inhibition of TNF-α and TPA-induced NF-κB Activation—TNF-α induces activation of NF-κB in human T cell leukemia Jurkat cells. Racemic DHMEQ inhibited TNF-α-induced NF-κB activation monitored by the promoter/luciferase reporter gene at a dose-dependent 1–10 μg/ml, as shown in Fig. 2A. It also inhibited TPA-induced NF-κB activation at similar concentrations (Fig. 2B). TPA is considered to activate IκK through activation of protein kinase C. Therefore, DHMEQ was suggested to inhibit NF-κB activation at a level downstream of IκK.

Inhibition of Cellular NF-κB Binding to κB DNA—Jurkat cells were examined for binding to the [32P]-labeled κB oligonucleotide in the EMSA assay. DHMEQ inhibited the DNA binding at 1–10 μg/ml, as shown in Fig. 3A. TNF-α induced the DNA binding at a time-dependent 10–60 min, which was completely inhibited by 10 μg/ml DHMEQ (Fig. 3B). The unlabelled DNA probe and anti-p65 antibody diminished and supershifted, respectively, the band for the NF-κB/DNA complex. When DHMEQ was added after the nuclear extract preparation of TNF-α-treated cells, it did not inhibit the mobility shift even at 100 μg/ml, as shown in Fig. 3C. Therefore, it should not inhibit the binding of NF-κB to the κB DNA directly.

Effect of DHMEQ on IκB Degradation—TNF-α induces phosphorylation and proteasome-mediated degradation of IκB to release NF-κB from the inactivated complex. TNF-α-induced phosphorylation of IκB in 5 min and degradation in 30 min. Thereafter, IκB synthesis was reactivated possibly by NF-κB in 90 min. NF-κB is known to bind to the IκB promoter (4). DHMEQ at 10 μg/ml did not inhibit the TNF-α-induced phosphorylation and degradation of IκB, as shown in Fig. 4A. However, IκB was not re-synthesized after 90 min in the presence of DHMEQ. This is because inhibition of NF-κB activity should inhibit regeneration of IκB mRNA. On the other hand, ALLN, a proteasome inhibitor, clearly inhibited the degradation without inhibiting phosphorylation. The lack of inhibition was confirmed with COS-1 cells, in which DHMEQ at 3 and 10 μg/ml showed no effect on IκB phosphorylation and degradation (Fig. 4B). Thus, DHMEQ did not inhibit degradation of IκB at a concentration that inhibited activation of NF-κB.

Inhibition of Nuclear Translocation of NF-κB by DHMEQ—DHMEQ diminished NF-κB activity in the nucleus (Fig. 3) but did not induce cell death or apoptotic morphology in Jurkat cells. However, DHMEQ increased apoptotic sensitivity to TNF-α-induced nuclear translocation of NF-κB from the cytoplasm to the nucleus in Jurkat cells. As shown in Fig. 5, TNF-α induced the accumulation of the p65 NF-κB subunit in the nucleus in 30 min. DHMEQ at 3–10 μg/ml decreased the amount of nuclear NF-κB, whereas it increased the amount of cytoplasmic NF-κB. Thus, DHMEQ inhibited the TNF-α-induced nuclear translocation of NF-κB.

Effect of DHMEQ on Smad2 Nuclear Translocation and JNK Activation—TGFB-β is known to induce nuclear translocation of Smad proteins after phosphorylation by the TGF-β receptor. Therefore, we studied whether DHMEQ would also inhibit TGF-β-induced Smad2 translocation. As shown in Fig. 7A, it did not affect the translocation of Smad2. Large T antigen is a typical nuclear protein and is known to be translocated to the nucleus immediately after its synthesis in the cytoplasm. DHMEQ had no effect on the nuclear accumulation of large T antigen either (data not shown). These results suggest that DHMEQ is a specific inhibitor of NF-κB nuclear translocation.

TNF-α activates three signaling pathways, i.e. those involving NF-κB, JNK (32), and apoptosis. Therefore, we next studied the effect of DHMEQ on JNK activation by analysis of the kinase activity. As shown in Fig. 7B, TNF-α-induced activation of JNK at 15 min, and DHMEQ at 10 μg/ml did not inhibit this activation. Preincubation with DHMEQ for 2 h weakly increased the basal JNK level at time 0.

Enhancement of Apoptotic Sensitivity by DHMEQ—TNF-α alone did not induce cell death or apoptotic morphology in Jurkat cells. However, TNF-α synergistically induced apoptotic morphology with DHMEQ in 6 h, as shown in Fig. 8A. After 16 h, cell death observed by the dye exclusion assay was induced by TNF-α only in the presence of 3 or 10 μg/ml DHMEQ (Fig. 8B). Thus, DHMEQ increased apoptotic sensitivity to TNF-α in Jurkat cells, suggesting that it selectively inhibited the signaling pathway for NF-κB activation.

DISCUSSION

The number of known roles of NF-κB is rapidly increasing. For instance, erythropoietin was shown to inhibit apoptosis of neuronal cells by activating JAK2 to increase the tyrosine phosphorylation of IκB (33). On the other hand, NF-κB is often activated in breast cancer cells possibly through the activation of CK2 (34). Activation of IκK and NF-κB was also reported in human melanoma cell lines (35). Activation of NF-κB would provide apoptotic resistance in these neoplastic tumors. NF-κB

FIG. 2. Inhibition of TNF-α- and TPA-induced activation of NF-κB by DHMEQ. Jurkat cells were pretreated with DHMEQ for 2 h and stimulated or not with 20 ng/ml TNF-α (A) or 100 ng/ml TPA (B) for 6 h. The κB reporter was assayed in terms of luciferase activity. White bars, stimulated; dark bars, nonstimulated. The values are means ± S.D. of quadruplicate determinations. The data indicate the -fold increase in luciferase activity.
is also constitutively activated in hormone-refractory prostate cancer cells (36), and genetic introduction of degradation-resistant IκB induced cell death selectively in these cancer cells (37). We confirmed that DHMEQ inactivated NF-κB in constitutively activated bladder carcinoma cells. Therefore, NF-κB inhibitors may be useful for chemotherapy for both neoplastic diseases in which NF-κB is activated and for inflammation.

Translocation of nuclear proteins containing the classic nuclear localization signal (NLS) such as SV40 T antigen is initiated by the formation of a complex consisting of the cargo protein and carrier proteins called importins (38–40). Importin family members include the adaptor protein, importin-α, and the carrier protein, importin-β. Importin-α/β complex recognizes and interacts with the classic NLS, and the importins transport the cargo proteins to the nucleus through the nuclear pore. Importins dissociate from the cargo proteins in nucleus. In nonstimulated cells, NF-κB interacts with IκB, which masks the NLS of NF-κB (41, 42). Degradation of IκB thus allows exposure of the NLS of NF-κB. Both p65 and p50 have the classic type of NLS; therefore, NF-κB uses the general nuclear translocation system (43).

DHMEQ did not inhibit TNF-α-induced phosphorylation and degradation of IκB-α, but it did inhibit the nuclear translocation of NF-κB in both cell lines. Therefore, the molecular target of the drug is likely to be involved in the protein complex for nuclear translocation. Because DHMEQ did not inhibit the translocation of other proteins such as Smad2 and large T antigen, it is unlikely that the drug blocks the general transport machineries such as importins and nuclear pore. Importins dissociate from the cargo proteins in nucleus. In nonstimulated cells, NF-κB interacts with IκB, which masks the NLS of NF-κB (41, 42). Degradation of IκB thus allows exposure of the NLS of NF-κB. Both p65 and p50 have the classic type of NLS; therefore, NF-κB uses the general nuclear translocation system (43).

DHMEQ did not inhibit TNF-α-induced phosphorylation and degradation of IκB in Jurkat and COS-1 cells, but it did inhibit the nuclear translocation of NF-κB in both cell lines. Therefore, the molecular target of the drug is likely to be involved in the protein complex for nuclear translocation. Because DHMEQ did not inhibit the translocation of other proteins such as Smad2 and large T antigen, it is unlikely that the drug blocks the general transport machineries such as importins and nuclear pore. Instead, it may bind to p65 or p50. A biotin-labeled analogue of DHMEQ is now being prepared for the detection of the target molecule.

Most NF-κB inhibitors, including panepoxydone, are suggested to act as upstream targets of IκB degradation (23–25). Interestingly, both panepoxydone and DHMEQ contain the cyclohexylepoxydone structure, but their mechanism of action is completely different. Lin et al. (44) designed and synthesized an inhibitor of NF-κB nuclear translocation called SN50 by conjugating the NLS of p50 to the amino acid sequence of the cell membrane-permeable motif. SN50 is considered to block the intracellular recognition system for the NLS of NF-κB. A similar mechanism cannot be excluded for DHMEQ. But SN50, a peptide consisting of 26 amino acids, is completely different in size and structure from DHMEQ.

**Fig. 3.** Inhibition of TNF-α-induced DNA binding of nuclear NF-κB by DHMEQ. A, Jurkat cells were cultured with various concentrations of DHMEQ in the presence or absence of TNF-α for 30 min. Then the nuclear extract was assayed by EMSA. B, the cells were incubated with 20 ng/ml TNF-α with or without 10 μg/ml DHMEQ. C, the nuclear extract was prepared from TNF-α-treated Jurkat cells. DHMEQ was added before the incubation of nuclear extract with DNA.

**Fig. 4.** Effect of DHMEQ on TNF-α-induced phosphorylation and degradation of IκB-α. A, Jurkat cells were treated with TNF-α alone or also with DHMEQ or ALLN and for the indicated periods. The lysates were fractionated on a 12.5% polyacrylamide gel. B, COS-1 cells (6 × 10⁵) were cultured with 20 ng/ml TNF-α in the presence or absence of DHMEQ for the indicated periods.

**Fig. 5.** Inhibition of TNF-α-induced nuclear translocation of NF-κB by DHMEQ. Jurkat cells were treated with DHMEQ and TNF-α for 30 min. The nuclear and cytosolic fractions were prepared as described under “Experimental Procedures” and fractionated on a 10% polyacrylamide gel.

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2 A. Ariga, J.-i. Namekawa, N. Matsumoto, J.-i. Inoue, and K. Umezawa, unpublished results.
It is not known whether DHMEQ has any selectivity among NF-κB/H9260B subunits. However, because DHMEQ almost completely inhibited the NF-κB/H9260B activity in human umbilical vein endothelial cells and human bladder carcinoma cells in which NF-κB/H9260B is constitutively activated in addition to Jurkat and COS-1 cells, it is likely to inhibit a variety of NF-κB subunit dimers.

It was recently reported that NF-κB down-regulates JNK. TNF-α-induced JNK activation was enhanced in IKK- and Rel A(p65)-deficient mouse embryonic fibroblasts (45, 46). Therefore, DHMEQ might up-regulate JNK in TNF-α-treated Jurkat cells. However, we found that DHMEQ alone activated JNK in a time-dependent manner, and it was difficult to show the NF-κB-mediated activation of JNK by DHMEQ. DHMEQ was found to inhibit rheumatoid arthritis in a mouse model (29). Because its weaker structural analogue, DHM3EQ, did not inhibit the in vivo model, the anti-inflammatory effect should be due to the inhibition of NF-κB. DHMEQ was derived from the weak antibiotic epoxyquinomicin, which showed no toxicity in mice. DHMEQ also showed no prominent toxicity in mice in the rheumatoid arthritis experiment or in other in vivo experiments. This agent, acting by a unique mechanism, may thus be a candidate for anti-inflammatory or anticancer therapy.
REFERENCES

1. Sen, R., and Baltimore, D. (1986) Cell 46, 705–716
2. Shaeov, A. N., Collart, M. A., Vassalli, P., Nedospasov, S. A., and Jongeneel, C. V. (1990) J. Exp. Med. 171, 35–47
3. Collart, M. A., Bauerle, P., and Vassalli, P. (1990) Mol. Cell. Biol. 10, 1498–1506
4. Pahl, H. L. (1999) Oncogene 18, 6853–6866
5. Nabel, G., and Baltimore, D. (1987) Nature 326, 711–713
6. Griffin, G. E., Leung, K., Folks, T. M., Kunkel, S., and Nabel, G. J. (1989) Nature 339, 70–73
7. Ghosh, S., May, M. J., and Kopp, E. B. (1998) Annu. Rev. Immunol. 16, 253–280
8. Osborn, L., Kunkel, S., and Nabel, G. J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2336–2340
9. Israel, A., Le Bail, D., Hata, D., Piette, J., Kieran, M., Logeat, F., Wallach, D., Fellous, M., and Kourilsky, P. (1989) EMBO J. 8, 3793–3800
10. Sen, R., and Baltimore, D. (1986) Cell 47, 921–928
11. Rothe, J., Gehr, G., Loetscher, H., and Lesslauer, W. (1992) Immunol. Res. 11, 81–90
12. Tartaglia, L. A., and Goeddel, D. V. (1992) Immuno. Today 13, 151–153
13. Aggarwal, B. B. (2000) Annu. Rev. Immunol. 18, 214–234
14. Carswell, E. A., Old, L. J., Kassel, R. L., Green, S., Fiore, N., and Williamson, B. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3666–3670
15. Old, L. J. (1985) Science 228, 630–632
16. Cleveland, J. L., and Ihe, J. D. (1995) Cell 81, 479–482
17. Hsu, H., Xiong, J., and Goeddel, D. (1995) Cell 81, 495–504
18. Shoji, S., Okada, K., Okada, Y., and Kourilsky, P. (1989) EMBO J. 8, 3793–3800
19. Antwerp, D. J. V., Martin, S. J., Kafri, T., Green, D. R., and Verma, I. M. (1991) Science 251, 379–382
20. Seg, A. A., and Baltimore, D. (1996) Science 274, 782–784
21. Wang, C. Y., Mayo, M. W., and Baldwin, A. S., Jr. (1996) Science 274, 784–787
22. Li, Q., Van Antwerp, D., Mecerio, F., Lee, K. F., and Verma, I. M. (1998) Science 284, 231–235
23. Erkel, A., Anke, T., and Sterner, O. (1996) Biochem. Biophys. Res. Commun. 226, 214–221
24. Gehrt, A., Erkel, G., Anke, T., and Sterner, O. (1998) J. Antibiot. (Tokyo) 51, 455–463
25. Pahl, H. L., Kraus, B., Schulze-Osthoff, K., Decker, T., Tranckner, E. B., Vogt, M., Myers, C., Parks, T., Waring, P., Muhlbaier, A., Czernilofsky, A. P., and Bauerle, P. A. (1996) J. Exp. Med. 183, 1829–1840
26. Xie, D., Wang, P., and Parmely, M. J. (2000) Biochem. Pharmacol. 60, 717–727
27. Shoji, S., Furushis, K., Ogata, A., Yamakata, K., Takahashi, K., Mokui, R., Uda, A., Harano, K., Matsusita, S., and Misumi, S. (1998) Biochem. Biophys. Res. Commun. 250, 745–753
28. May, M. J., D’Acquisto, F., Madle, L. A., Glueckner, J., Pober, J. S., and Ghosh, S. (2000) Science 289, 1559–1554
29. Matsumoto, N., Ariya, A., Oto-s, S., Nakamura, H., Agata, N., Hiran, S., Inoue, J., and Umezawa, K. (2000) Bioorg. Med. Chem. Lett. 10, 865–869
30. Umezawa, K., Ariya, A., and Matsumoto N. (2000) Anti-Cancer Drug Des. 15, 239–244
31. Andrews, N. C., and Faller, D. V. (1991) Nucl. Acids Res. 19, 2449–2455
32. Yuasa, T., Ohno, S., Hekl, J. H., and Kryssakis, J. M. (1998) J. Biol. Chem. 273, 22681–22692
33. Digicaylioglu, M., and Lipton, S. A. (2001) Nature 412, 641–647
34. Romieu-Murcet, R., Landesman-Bollag, E., Seldin, D. C., Traish, A. M., Mercurio, F., and Sonenshein, G. E. (2001) Cancer Res. 61, 3810–3818
35. Yang, J., and Richmond, A. (2001) Cancer Res. 61, 4901–4909
36. Palayoor, S. T., Younell, M. Y., Calderwood, S. K., Coleman, C. N., and Price, B. D. (1999) Oncogene 18, 7389–7394
37. Sumitomo, M., Tachihana, M., Oru, C., Asakura, H., Murai, M., Hayakawa, M., Miyajima, A., Kimura, F., Hayakawa, M., Nakamura, H., Takayanagi, A., and Shimizu, N. (1999) Hum. Gene Ther. 10, 37–47
38. Gorlish, D., and Kutay, U. (1999) Annu. Rev. Cell Dev. Biol. 15, 667–660
39. Yoneda, Y. (2000) Genes Cells 5, 777–781
40. Turpin, P., Ossarch-Nazar, B., and Dargegorn, C. (1999) FERS Lett. 452, 82–86
41. Ben, A. A., Ruben, N. M., Scheiman, R. I., Haskill, S., Rosen, C. A., and Baldwin, A. S. (1992) Genes Dev. 6, 1899–1913
42. Ganch, P. A., Sun, S. C., Greene, W. C., and Ballard, D. W. (1992) Mol. Biol. Cell 3, 1339–1352
43. Jans, D. A., Xiao, C. H., and Lam, M. H. C. (2000) Bioessays 22, 532–544
44. Lin, Y.-Z., Yao, S. Y., Veach, R. A., Forgerson, T. R., and Hawiger, J. (1995) J. Biol. Chem. 270, 44255–44258
45. Tang, G., Minemoto, Y., Dibbing, B., Purell, M., Li, Z., Karin, M., and Lin, A. (2001) Nature 414, 313–317
46. De Smaele, E., Zazzeroni, F., Papa, S., Nguyen, D. U., Jin, R., Jones, J., Cong, R., and Franzoso, G. (2001) Nature 414, 308–313
Inhibition of Tumor Necrosis Factor-α-induced Nuclear Translocation and Activation of NF-κB by Dehydroxymethylepoxyquinomicin

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