Nuclear Factor κB Inhibition by Dibenzoylmethane Derivatives

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We established stable HeLa transformants with reporter genes containing nuclear factor (NF)-κB response elements to measure NF-κB inhibition. Tumor necrosis factor (TNF)-α treatment induced a 6-fold activation of NF-κB in HeLa$_{NF\text{-}\kappa B}$-3 cells. Three chloro-derivatives of fourteen dibenzoylmethane derivatives inhibited NF-κB activity.

Key words —— nuclear factor κB, dibenzoylmethane derivative, inhibitor, stable transformant

INTRODUCTION

Nuclear factor-κB (NF-κB) is a dimeric transcription factor that induces expression of genes involved in the inflammatory process. In unstimulated cells, NF-κB is retained in the cytoplasm via interaction with its inhibitor, IκB.[1] In response to pro-inflammatory stimuli, IκB is phosphorylated by the IκB kinase complex, leading to its ubiquitination and subsequent proteasome-mediated degradation, allowing a dimmer of NF-κB to enter the nucleus. NF-κB then binds to its promoter recognition sequences to induce target gene transcription, including mediators of the immune and inflammatory response such as the inflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-2, IL-6, and IL-8.[2] Compounds that inhibit NF-κB are of great interest as lead structures for the treatment of acute and chronic inflammation.

Diacylmethanes possess an active methylene function, which forms carbanions or enolic ions in neutral solutions. These ions may inhibit mutagenesis and carcinogenesis, as we have shown for several diacylmethanes that inhibit the mutagenicity of 2-nitrofluorene, 2-naphthohydroxamic acid, benzo[*a*]pyrene, aflatoxin B_1 and methylnitrosourea in *Salmonella typhimurium*.[3] The diacylmethane derivative, 2,2′-dimethoxydibenzoylmethane, protects dopaminergic neurons against both endoplasmic reticulum (ER) stress and oxidative stress by preventing the formation of reactive oxygen species.[4]

Here we established stable transformants with an NF-κB response element to evaluate the ability of diacylmethane derivatives to inhibit NF-κB activity.

MATERIALS AND METHODS

Materials —— Dulbecco's Eagle's medium was purchased from Nissui (Tokyo, Japan). Calf serum was obtained from Boehringer Mannheim (Mannheim, Germany). Non-essential amino acids were purchased from Invitrogen (Carlsbad, CA, U.S.A.). pNF-κB-secreted alkaline phosphatase (SEAP) and pSV2-neo was purchased from BD Biosciences (San Jose, CA, U.S.A.) and the Japan Health Science Foundation (Osaka, Japan), respectively. Recombinant human TNF-α was obtained from PeproTech House (London, U.K.). Effectene^{TM} transfection reagent was from QIAGEN (Valencia, CA, U.S.A.). Great EscAPe SEAP Fluorescence Detection Kits were obtained from BD Biosciences.

Cell Culture —— HeLa cells were obtained from the Japan Health Science Foundation. Cells were cultured in Dulbecco's Eagle's medium containing 10% calf serum and 100 μM non essential amino

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acids at 37°C.

Stable Transformants with an NF-κB Response Element —— The day before transfection, 1.5×10^6 HeLa cells were seeded on a 100 mm dish. pNF-κB-SEAP (0.2 μg) and pSV2-neo (2.0 μg) were co-transfected with an Effectene™ transfection reagent according to the manufacturer's protocol. After 16 hr, the medium containing plasmids was exchanged to fresh growth medium with 50 μg/ml of G418 (Nakarai Tesque, Kyoto, Japan). Cells were subcultured several times for single-cell cloning in 96-well plates.

NF-κB Activity —— $HeLa_{NF\text{-}\kappa B}$-3 cells ($0.5 \times 10^4$) were seeded on a 96-well tissue culture plate. After 2 hr, aliquots of test sample solution were added to each well. After 1 hr, 10 μl of 800 ng/ml TNF-α was also added into 200 μl of each well. After 24 hr, media was assayed for SEAP activity according to the manufacturer's protocol.

RESULTS AND DISCUSSION

To efficiently evaluate the ability of compounds to inhibit NF-κB, we generated stable transformants of HeLa cells with an NF-κB response element containing an SEAP reporter gene. We observed TNF-α-induced activation of SEAP in 4 of 12 clones obtained by single-cell cloning (Fig. 1), with $HeLa_{NF\text{-}\kappa B}$-3 cells showing the highest activity, 6-fold, similar to transient transfection experiments (data not shown). And 10 μg/ml of 12-*O*-tetradecanoylphorbol 13-acetate (TPA) also activated NF-κB of $HeLa_{NF\text{-}\kappa B}$-3 cells. Its activity was 1.1 times as high as the activity by the stimulation with TNF-α. Furthermore we confirmed that 1 μM of parthenolide,[4] a known NF-κB inhibitor, inhibited 38% of NF-κB activity using the cloned cells.

Fourteen dibenzoylmethane derivatives (**1**–**14**) synthesized by Choshi *et al.* were evaluated for NF-κB inhibition at 20 μM (Table 1) using $HeLa_{NF\text{-}\kappa B}$-3 cells. Compounds **3**, **6**, **11**, and **14** showed 30%, 50%, 41%, and 27% inhibition, respectively. The structures of **1**, **3**, **6**, **12**, and **14** were substituted with fluorine (**12**), chlorine (**3**, **6**, and **14**) or bromine (**1**) of an electron-withdrawing group on two benzene rings, except for **11**, but **1** (8%) and **12** (0%) were not inhibitors. Thus, the substitution by chlorine(s) and the methoxy group affected NF-κB inhibition. Compounds **6** and **11** protect dopaminergic neurons against both oxidative and ER stress, which can activate NF-κB.[5] Future work will involve the development of more active compounds.

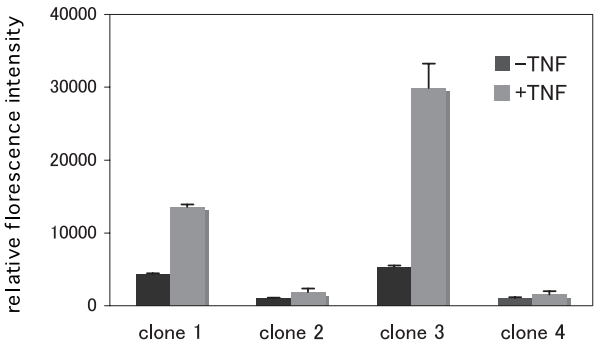
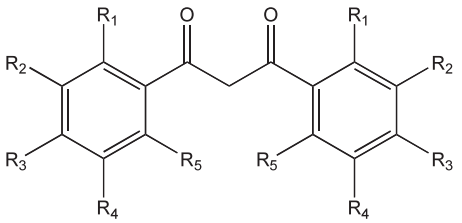


Fig. 1. NF-κB Activation with/without TNF-α Stimulation on Stable Transformant Cells Obtaining NF-κB Response Element in Genomic DNA

0.5×10^4 cells of each stable transformant cells were seeded into 96-well culture plates followed by the addition of 40 ng/ml of TNF-α to the culture medium. After 24 hr, SEAP activity based on NF-κB activity was evaluated using 25 μl of the recovered medium according to the manufacturer's protocol.

Table 1. Structures of Dibenzoylmethane Derivative and Those NF-κB Inhibitory Activities



	R_1	R_2	R_3	R_4	R_5	Inhibitory activity (%)[a]
1	H	H	Br	H	H	8
2	H	Me	Me	H	H	0
3	H	Cl	Cl	H	H	30
4	OMe	H	H	OMe	H	0
5	H	H	OMe	H	H	8
6	H	H	Cl	H	H	50
7	H	H	Me	H	H	14
8	OMe	H	OMe	H	H	0
9	OMe	OMe	OMe	OMe	H	10
10	OMe	H	H	H	H	18
11	H	OMe	H	H	H	41
12	F	H	F	H	H	0
13	H	H	OCH_2Ph	H	H	11
14	Cl	H	H	H	Cl	27

a) Inhibitory activity was evaluated at the concentration of 20 μM.

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