ENHANCEMENT OF PHORBOL ESTER-INDUCED PRODUCTION OF TUMOR NECROSIS FACTOR- α BY 2,6-DIMETHYLPHENYLPHthalIMIDE

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The effect of phenyl- and benzylphthalimide analogs on tumor necrosis factor (TNF)- α production by a human leukemia cell line, HL-60, stimulated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was investigated. Though non-substituted phenyl- and benzyl-phthalimide had no effect on TNF- α production after TPA-induction, introduction of methyl group(s) onto the phenyl group resulted in the appearance of potent enhancing activity on TNF- α production.

KEYWORDS 2,6-dimethylphenylphthalimide; tumor necrosis factor- α ; structure-activity relationship

Regulation of tumor necrosis factor (TNF)- α production *in vivo* is of interest because of the pleiotropic biological actions elicited by TNF- α , including anti-tumor activity,¹⁾ tumor promotion activity,²⁾ and replication-enhancing activity on human immunodeficiency virus type 1 (HIV-1).³⁾ Because TNF- α is one of the cytokines produced by stimulated monocyte/macrophages, chemical modulation of TNF- α production might represent a new strategy for tumor/AIDS chemotherapy. N(α)-Phthalimidoglutarimide (1: thalidomide) was first reported to be an inhibitor of TNF- α production.⁴⁾ Recently, we reported that the direction of the effect on TNF- α production elicited by thalidomide is cell type-specific; i.e., thalidomide enhanced TPA-induced TNF- α production by human leukemia cell lines including HL-60, K562 and U937,⁵⁾ while it repressed TNF- α production by normal human peripheral blood cells. However, thalidomide is not attractive as a clinical agent or a research agent because of its teratogenicity and its instability under physiological conditions. To develop a practical TNF- α production-regulator, structural modification of thalidomide would be mandatory.

Recently, we reported that phenethylphthalimide (2) possesses more potent enhancing activity than thalidomide on TPA-induced TNF- α production by HL-60 cells.⁶⁾ We also reported that phenyl- (3) or benzyl-phthalimide (4) had no effect on TNF- α production. In this paper, we describe the discovery of TNF- α production-enhancing activity of alkylated phenyl-/benzylphthalimides and the structure-activity relationships.

Thalidomide and phenyl-, benzyl-, and phenethylphthalimides were prepared as described previous-

1

2

3: R₁ = R₂ = H
9: R₁ = CH₃, R₂ = H
10: R₁ = R₂ = CH₃

4: R = H
5: R = o-CH₃
6: R = m-CH₃
7: R = p-OCH₃
8: R = p-NO₂

ly.⁶⁾ Other phthalimides were prepared by condensation reaction of phthalic anhydride with appropriately substituted anilines or benzylamines. The structures of the prepared compounds are shown above.

For assaying the effects of the prepared compounds on TPA-induced TNF- α production, exponentially growing HL-60 cells (2×10^5 cells per ml) were seeded in growth medium containing TPA (1 or 10 nM) in the presence or absence of compounds (0.1 - 100 μ M). After incubation for the indicated period, the cell culture supernatant was assayed for TNF- α concentration using a human TNF- α ELISA system (Amersham). The methods of assay and quantification were as recommended by the supplier. The results are shown in Table I and Fig. 1, where 100% was defined as the amount of TNF- α produced by HL-60 cells in the presence of 1 nM TPA alone.

Table I. Enhancing Effects of Phthalimide Analogs (100 μ M) on TPA-Induced TNF- α Production by HL-60 Cells

Compound	Amount of TNF- α %	mp °C	Elemental analysis
<u>1</u>	182	277-279	Calcd. for $C_{13}H_{10}N_2O_4$ (Racemate): C, 60.47; H, 3.88; N, 10.85. Found: C, 60.75; H, 3.91; N, 11.10.
<u>2</u>	209	129	Calcd. for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.67; H, 5.18; N, 5.52.
<u>3</u>	97	208-209	Calcd. for $C_{14}H_9NO_2$: C, 75.33; H, 4.06; N, 6.27. Found: C, 75.22; H, 4.16; N, 6.55.
<u>4</u>	122	115	Calcd. for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.67; H, 4.70; N, 5.89.
<u>5</u>	179	143	Calcd. for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.44; H, 5.32; N, 5.68.
<u>6</u>	152	117	Calcd. for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 71.85; H, 4.81; N, 5.36.
<u>7</u>	118	126	Calcd. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.85; H, 4.81; N, 5.36.
<u>8</u>	124	174	Calcd. for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.92. Found: C, 63.98; H, 3.58; N, 9.95.
<u>9</u>	109	180	Calcd. for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 76.03; H, 4.72; N, 5.86.
<u>10</u>	218	203-204	Calcd. for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.47; H, 5.31; N, 5.63.

First we investigated the effect of the prepared compounds at 100 μ M on TPA-induced TNF- α production by HL-60 cells (Table I). As reported,^{5, 6)} thalidomide (1) and phenethylphthalimide (2) showed TNF- α production-enhancing activity, though phenyl- (3) or benzylphthalimide (4) showed no effect. Recently, we reported that (S)-methylthalidomide possesses TNF- α production-enhancing activity, which is more potent than that of thalidomide.⁷⁾ Therefore, we introduced a methyl group into the phenyl group of benzylphthalimide (4), whose methylene spacer is one unit shorter than that of the active phenethylphthalimide (2). As expected, introduction of a methyl group at the *ortho* position, i.e., *o*-methylbenzylphthalimide (5), resulted in the appearance of potent TNF- α production-enhancing

Fig. 1. Enhancing-Effects of 1, 2 and 10 on TPA-Induced TNF- α Production by HL-60 Cells

activity. The methyl group had to be at the ortho-position for potent activity. Introduction of a methyl group at the meta position (m-benzylphthalimide: 6) resulted in the appearance of only weak activity, and introduction at the para position did not affect the activity of the molecule. Introduction at the para position of an electron-donating group such as a methoxy group (p-methoxybenzylphthalimide, 7) or an electron-withdrawing group such as a nitro group (p-nitrobenzylphthalimide, 8) had almost no effect on the activity.

It is noteworthy that o,o-dimethylphenylphthalimide (10) showed the most potent TNF- α production-enhancing activity among the compounds investigated. Though introduction of one methyl group at the ortho position (o-methylphenylphthalimide, 9) did not cause the appearance of potent activity, introduction of two methyl groups at the two ortho positions (compound 10) resulted in potent activity. The dose dependency curves of the activity elicited by o,o'-dimethylphenylphthalimide (10) as well as compounds 1 and 2 are shown in Fig. 1.

As has been recently reported,⁶⁾ compound 2 showed higher activity than 1 at the concentration of 100 μ M while the former is weaker than 1 at lower concentrations (0.1 - 10 μ M). Compound 10 showed a similar dose dependency curve to that of 2. This difference between the shapes of the dose dependency curves might result from differences in the cellular uptake, the metabolic/chemical stability of the compounds, or the molecular mechanism of action. The structure-activity relationships are under further study.

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