ENHANCEMENT OF 12-O-TETRADECANOYLPHORBOL-13-ACETATE-INDUCED TUMOR NECROSIS FACTOR α PRODUCTION BY PHENETHYLPHthalIMIDE ANALOGS

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Effects of phenyl-, benzyl-, phenethyl-, and phenylpropylphthalimides on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced tumor necrosis factor (TNF)- α production by human leukemia cell line HL-60 were examined. Among the four phthalimide derivatives, only phenethylphthalimide showed potent enhancing effect on TNF- α production.

KEYWORDS phthalimide; tumor necrosis factor α ; structure-activity relationship

Tumor necrosis factor (TNF)- α is a pleiotropic cytokine secreted by activated monocyte/macrophages, and is an important mediator of the tumor-killing activity of monocyte/macrophages.¹⁾ TNF- α has a direct anti-tumor effect, and also stimulates host immune anti-tumor response.²⁾ Thus, TNF- α was considered to be a promising anti-tumor agent. However, high doses of TNF- α elicit unfavorable biological effects such as tissue inflammation and a lethal endotoxic shock-like syndrome.^{2, 3)} Therefore, chemicals which regulate TNF- α production are expected to be useful biological response modifiers (BRM's).

Recently, we reported that N(α)-phthalimidoglutarimide (1: thalidomide) acts as a cell-type specific regulator of TNF- α production.⁴⁾ In human leukemia cell lines including HL-60, K562 and U937, 1 showed enhancing activity on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced production of TNF- α ,⁴⁾ though 1 has been reported to inhibit TNF- α production by normal human peripheral blood cells and some other cell lines stimulated with TPA or lipopolysaccharides.⁵⁾ We have also reported that only the (S)-form of α -methyl-N(α)-phthalimidoglutarimide (2: methylthalidomide), but not its (R)-enantiomer, has a more potent enhancing effect than 1 on TPA-induced TNF- α production by HL-60 cells.⁶⁾

In order to develop superior thalidomidal BRM's, we prepared phthalimide analogs bearing a phenyl group (compounds 3 - 10) and examined their effects on TPA-induced TNF- α production by HL-60 cells.

Compounds 1 and 2 were prepared as described previously.^{4, 6)} Compounds 3 - 6 were prepared by condensation of phthalic anhydride with appropriate amines in good yields. Our previous results indicating that 2 shows much higher activity than 1 prompted us to introduce a methyl group onto compound 5; i.e., 7 and 8, which were prepared by condensation with phthalic anhydride of 2-amino-3-

phenylpropane (prepared by successive treatments of benzaldehyde with nitroethane in the presence of *n*-butylamine, and with LiAlH_4) and 1-amino-3-phenylpropane (prepared by reduction of 2-cyanoethylbenzene), respectively. To obtain information concerning the active conformation(s) of 5, we also prepared two rigid compounds, 9 and 10. Compound 9 was prepared by elimination of HBr (by DBU treatment) from β -bromophenethylphthalimide, which was prepared by bromination of 5 with NBS in the presence of benzoyl peroxide. Compound 10 was prepared by separation of a photoisomerized mixture of 9. Effects on TPA-induced $\text{TNF-}\alpha$ production by HL-60 cells of the prepared compounds were measured according to the previously described method.^{4, 6)} Briefly, exponentially growing HL-60 cells (2×10^5 cells/ml) were treated with 1 nM TPA in the presence or absence of the compounds (concentration indicated in Fig. 1 and Table I) at 37 °C for 24 h. Then the concentration of $\text{TNF-}\alpha$ in the cell culture supernatant was measured by the use of a human $\text{TNF-}\alpha$ ELISA system (Amersham Co.). $\text{TNF-}\alpha$ production by HL-60 was TPA-dependent,⁴⁾ and the concentration of $\text{TNF-}\alpha$ produced in the presence of 1 nM TPA alone was defined as 100%. All the samples were assayed in duplicate. The results are basically reproducible, and a typical set of data (mean values of duplicate) is shown in Table I and Fig. 1. None of the examined compounds themselves showed any effect on cell growth or $\text{TNF-}\alpha$ production in the concentration range examined under the experimental conditions used.

Table I. Enhancing Effects of Phthalimide Analogs ($100 \mu\text{M}$) on TPA-Induced $\text{TNF-}\alpha$ Production by HL-60 Cells

Compound	Amount of $\text{TNF-}\alpha$ %	mp °C	Elemental analysis
<u>1</u>	188	277-279	Calcd. for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_4$ (racemate): C, 60.47; H, 3.88; N, 10.85. Found: C, 60.75; H, 3.91; N, 11.10.
<u>2</u>	324 [(S)-form] 105 [(R)-form]	248 249	Calcd. for $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_4$ (racemate): C, 61.75; H, 4.45; N, 10.29. Found: C, 61.64; H, 4.44; N, 10.31.
<u>3</u>	94	208-209	Calcd. for $\text{C}_{14}\text{H}_9\text{NO}_2$: C, 75.33; H, 4.06; N, 6.27. Found: C, 75.22; H, 4.16; N, 6.55.
<u>4</u>	117	115	Calcd. for $\text{C}_{15}\text{H}_{11}\text{NO}_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 75.67; H, 4.70; N, 5.89.
<u>5</u>	253	129	Calcd. for $\text{C}_{16}\text{H}_{13}\text{NO}_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.67; H, 5.18; N, 5.52.
<u>6</u>	103	48	Calcd. for $\text{C}_{17}\text{H}_{15}\text{NO}_2$: C, 76.98; H, 5.66; N, 5.28. Found: C, 76.73; H, 5.47; N, 5.26.
<u>7</u>	219	48-51	Calcd. for $\text{C}_{17}\text{H}_{15}\text{NO}_4$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.76; H, 5.64; N, 5.40.
<u>8</u>	192	85-86	Calcd. for $\text{C}_{17}\text{H}_{15}\text{NO}_4$: C, 76.96; H, 5.70; N, 5.28. Found: C, 76.74; H, 5.60; N, 5.48.
<u>9</u>	145	184	Calcd. for $\text{C}_{16}\text{H}_{11}\text{NO}_2$: C, 77.10; H, 4.45; N, 5.62. Found: C, 77.18; H, 4.50; N, 5.66.
<u>10</u>	105	120	Calcd. for $\text{C}_{16}\text{H}_{11}\text{NO}_2$: C, 77.10; H, 4.45; N, 5.62. Found: C, 77.08; H, 4.54; N, 5.77.

Fig. 1. Enhancing Effects of 1 and 5 on TPA-Induced TNF- α Production by HL-60 Cells

As shown in the Table I, among the four simple compounds, i.e., 3 - 6, only 5 showed apparent activity which was more potent than that of 1. The result suggests that the spatial position of the phenyl ring in relation to the phthalimide moiety is crucial for the activity. Introduction of a methyl group into 5, i.e., 7 and 8, caused slight repression of the activity. The methylation might cause conformational change of the biologically active phenethylphthalimide skeleton. Of the two rigid compounds, 9 and 10, only the *E*-isomer (9) was active, though the activity was lower than that of the corresponding compound 5. The result suggests that the active conformation of 5 is *s-trans*-like.

Thus, we succeeded in the preparation of compounds with potent TNF- α production-enhancing activity; i.e., compounds 5, 7 and 8. The dose dependency curves (mean duplicate values) for 1 and 5 are shown in Fig. 1. The curves were basically reproducible, and the results indicate that 5 showed higher activity than 1 at 100 μ M whereas it showed only weak activity at 0.1 - 10 μ M compared with 1. We cannot yet explain this phenomenon. Possible explanations include (1) poor uptake of 1 by the cells at the high dose and/or (2) faster metabolic/chemical (hydrolysis, etc) decomposition of 5 as compared to 1. Further investigation of the structure-activity relationship is in progress.

REFERENCES

- 1) E.A.Carswell, L.J.Old, R.L.Kassel, S.Green, N.Fiore, B.Willamson, Proc.Natl.Acad.Sci.,USA., 72, 3666 (1975).
- 2) R.J.Debs, H.J.Fuchs, R.Philip, E.N.Brunette, N.Duzgunes, J.E.Shellito, D.Liggitt, J.Patton, Cancer Res., 50, 375 (1990).
- 3) T.Manda, F.Nishigaki, H.Hemmi, N.Ishida, Cancer Res., 48, 4250 (1988).
- 4) K.Nishimura, Y.Hashimoto, S.Iwasaki, Biochem.Biophys.Res.Comm., 199, 455 (1994).
- 5) E.P.Sampio, A.L.Moreira, E.N.Sarno, A.M.Malta, G.Kaplan, J.Exp.Med., 175, 1729 (1992).
- 6) K.Nishimura, Y.Hashimoto, S.Iwasaki, Chem.Pharm.Bull., 42, 1157 (1994).

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