Modification by Heme Oxygenase Inhibitor, Tin Protoporphyrin, of Cellular Differentiation of Human Myeloid Leukemia K562 Cell Line

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Human myeloid leukemia K562 cells can be induced to differentiate to mature cells bidirectionally, *i.e.*, hemin induces erythroid differentiation, while 12-*O*-tetradecanoylphorbol 13-acetate (TPA) induces differentiation to monocytes. TPA is also a potent inducer of heme oxygenase (HO), which catabolizes heme to biliverdin. We show here that TPA suppresses hemin-induced erythroid differentiation of K562 cells, while retinoids augment it. Further, an HO inhibitor, tin protoporphyrin (SnPP), suppresses TPA-induced K562 cell differentiation to monocytes. It was also found that co-treatment of K562 cells with SnPP and TPA induces erythroid differentiation of K562 cells, though SnPP alone or TPA alone does not induce erythroid differentiation, suggesting a role of HO in the directional switch of differentiation.

Key words K562 cell; heme oxygenase inhibitor; tin protoporphyrin; 12-*O*-tetradecanoylphorbol 13-acetate; retinoid

Cell differentiation of hematopoietic cells is dependent on a complex interplay of cytokines, growth factors, and heme oxygenase (HO), with the latter enzyme being considered to play a major regulatory role.¹⁾ HO (EC 1.14.99.3) degrades cellular heme to biliverdin, and has pleiotropic physiological roles.²⁾ 12-*O*-Tetradecanoylphorbol 13-acetate (TPA), a classical tumor promoter and a potent inducer of HO expression, has been shown to modulate hematopoiesis and has been widely used as a chemical inducer of cell differentiation of a number of cell lines, including human leukemia cell lines K562, HL-60, THP-1, KG-1 and U937.³⁾ Among them, human myeloid leukemia K562 cell line, established from a patient with chronic myeloid leukemia (CML) in terminal blast crisis,⁴⁾ is a unique cell line which can be induced to differentiate bidirectionally by hemin and TPA, *i.e.*, the former induces erythroid differentiation,⁵⁾ while the latter induces differentiation to mature monocytes.⁶⁾

Recently, we reported a comprehensive study comparing the effects of porphyrin analogs on erythroid differentiation of K562 cells and its modification by bioresponse modifiers.⁷⁾ Information obtained by this type of study is of importance because of the clinical potential of certain cell differentiation inducers and modifiers. In this study, we examined the role of HO in K562 cell differentiation by the use of a potent differentiation/HO-expression inducer, TPA, and a potent HO inhibitor, tin protoporphyrin (SnPP).⁸⁾

Assay of K562 cell differentiation was performed by the usual method, as described previously.⁷⁾ K562 cells, maintained at 37 °C in RPMI1640 supplemented with 5% fetal bovine serum in a 5% CO₂ humidified incubator, were incubated (4 × 10⁴ cells/ml) with or without a cell differentiation

inducer (hemin or/and TPA) in the presence or absence of a modifier (SnPP, retinoid or tubulin disruptors) for 4 days. The erythroid differentiation of the cells was scored by benzidine (BZD) staining,^{7,9)} and the monocyte differentiation was assessed in terms of the morphological change of the cells scored by microscopic analysis after Wright-Giemsa (WG) staining and NBT staining.^{7,10)} Cells which were judged to have differentiated to erythrocytes and monocytes are denoted in this paper as BZD+ and WG+ cells, respectively. Though the results (percentage of differentiated cell population) showed some variation from experiment to experiment, they were basically reproducible, and typical sets of data are shown in tables. Background values, *i.e.*, the number of BZD+ and/or WG+ cells found in the control cell culture (incubated without any chemical differentiation inducer or modifier) were subtracted from all observed values. None of the chemicals used in our experiment showed apparent cytotoxicity in the concentration ranges used.

First we examined the effect of TPA on hemin-induced erythroid differentiation of K562 cells (Table 1). The percentages of BZD+ cells (erythroid differentiation) induced by 10 μM and 25 μM hemin were about 40% and 60%, respectively. No cells differentiated to monocytes (WG+ cells) under these experimental conditions. The co-existence of 0.5 nM TPA caused a remarkable decrease in the percentage of BZD+ cells to less than 20%. This effect of TPA can be interpreted in terms of the TPA-induced HO activity, which degrades hemin, at least in part. TPA itself induced the appearance of WG+ cells (monocytic differentiation) amounting to 20–30% at 0.5 nM and 50–60% at 5 nM, and these values were not affected by the co-existence of hemin. On the other hand, retinoids (all-*trans* retinoic acid, ATRA, and a synthetic retinoid, Am80¹⁰⁾) and tubulin disruptors (colchicine, CLC, vinblastine, VBL, and rhizoxin, RZX) augmented hemin-induced erythroid differentiation, though we cannot explain these effects at this stage.

Next, we analyzed the effect of a potent HO inhibitor, SnPP, on TPA-induced K562 cell differentiation to mature monocytes (Table 2). SnPP inhibits HO activity completely at the dose of 10–50 μM,¹¹⁾ and it did not have any cell differentiation-inducing activity in the concentration range used. As mentioned above, TPA itself induced monocytic differentiation of K562 at 0.5 nM, generating approximately 20% WG+ cells. No BZD+ cells appeared under this condition. Co-addition of SnPP caused a decrease of the number of WG+ cells, with concomitant appearance of BZD+ cells, in a dose-dependent manner. In the presence of 50 μM SnPP plus 0.5 nM TPA, the percentages of WG+ cells and BZD+ cells were 15% (22% in the presence of TPA alone) and 16% (0–5% in the presence of TPA alone), respectively.

The results suggest that the HO inhibitor suppresses TPA-induced monocytic differentiation of K562, and raise the possibility that HO plays a critical role in determining the direction of TPA-induced K562 cell differentiation, *i.e.*, HO might direct K562 cells to differentiate to monocytes. If this were the case, K562 cell differentiation might be interpreted as follows, *i.e.*, (i) TPA might act as a general differentiation inducer, and HO induced by TPA might determine the direction of differentiation, and (ii) an HO inhibitor might switch the direction of differentiation induced by TPA from mono-

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Table 1. Effects of the HO-inducer TPA and Other Bioresponse Modifiers on Hemin-induced Erythroid Differentiation

Additive (conc.)	BZD+ cells (%)			WG+ cells (%)		
	Hemin: 0 μ M	10 μ M	25 μ M	Hemin 0 μ M	10 μ M	25 μ M
None	0	41	60	0	± 3	± 3
TPA (0.5 nM)	± 3	8	16	18	18	17
ATRA (1 μ M)	± 3	61	72	± 3	± 3	± 3
Am80 (1 μ M)	± 3	52	65	± 3	± 3	± 3
CLC (0.3 μ M)	± 3	56	60	± 3	± 3	± 3
VBL (10 nM)	± 3	53	66	± 3	± 3	± 3
RZX (1 nM)	± 3	60	70	± 3	± 3	± 3

Table 2. Effect of an HO Inhibitor, SnPP, on TPA (0.5 nM)-induced Monocytic Differentiation of K562 Cells

Concentration of SnPP (μ M)	In the presence of TPA		In the absence of TPA	
	WG+ cells (%)	BZD+ cells (%)	WG+ cells (%)	BZD+ cells (%)
0	22	± 3	0	0
1	21	6	± 3	± 3
5	22	6	± 3	± 3
10	21	8	± 3	± 3
25	18	14	± 3	± 3
50	15	16	± 3	± 3

cytes to erythrocytes by inhibiting the activity of HO.

Though further detailed studies are required, the above idea represents an attractive working hypothesis. Investiga-

tion of the role of HO in other chemically induced cell differentiation systems, including TPA-induced monocytic and retinoid-induced granulocytic differentiation of HL-60 cells, for example, is in progress.

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