Communications to the Editor

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DIFFERENTIATION INDUCTION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS WITH COLLETOCHLORIN B AND ITS ANALOGUES

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Colletochlorin analogues are found to be highly potent inducers of differentiation of human promyelocytic leukemia cells. The differentiated cells are morphologically and functionally like mature monocytes. From the activity-structure relationship, it is concluded that a carbonyl residue attached to the benzene ring is essential for both differentiation and cytotoxicity.

KEYWORDS——differentiation-inducing factor; HL-60; colletochlorin analogue; retinoic acid

Colletochlorins and their analogues are characterized by multi-substituted (polyhydroxy)arenes having terpenoid side chains in common. They exhibit a variety of biological activities.[1] Recently, structurally related compounds are found to have a factor that induces differentiation of mouse erythroleukemia (B8) cells to hemoglobin-producing erythrocyte-like cells[2], and a morphogen factor that induces differentiation of *Dictyostelium discoideum*.[3] In relation to our studies of differentiation inducing factors,[4] we have screened various phenolic compounds having cell differentiation inducing activity and found that colletochlorin B and its synthetic intermediates readily induce human promyelocytic cells (HL-60).

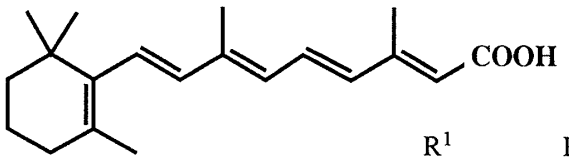
The HL-60 cell line has been maintained in continuous suspension culture. Cell suspensions of HL-60 were incubated at 37 °C in a humidified atmosphere of 5% carbon dioxide in air and were split every 6 days. Cell counts were determined by hemocytometer and viability was estimated by triypan blue dye exclusion. HL-60 were induced to differentiate by retinoic acid (RA) as a lead compound, and by compounds **1**-**7**. The compounds investigated were prepared according to the literature[1] and their structures are shown in Table I. Cells growing exponentially were harvested by centrifugation and resuspended at a density of 3.0 x 10^5 cells/ml in serum-free medium [RPMI 1640 supplemented with 5 μg of insulin/ml and 5 μg of transferrin/ml]. Induction of differentiation was initiated by adding a test compound to cells growing in defined medium. The compound was dissolved in absolute ethanol and added to the culture medium so that the final concentration of ethanol was less than 0.1%. Cells were cultured with the compound for 4 days and assayed by the nitroblue tetrazolium (NBT) reduction method, because differentiated HL-60 produces superoxide anions when stimulated with an appropriate agent such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Namely, approximately 2.0 x 10^5 cells per ml of medium with 20% fetal bovine serum were incubated for 25 min at 37 °C with an equal volume of 0.2% NBT dissolved in phosphate-buffered saline containing 200 ng of freshly diluted TPA per ml. Cytospin slide preparations were prepared and stained with Wright-Giemsa, and the proportion of cells containing formazan deposits in a minimum of 200 cells was counted by light microscopy.[5]

A measurement of the percentage of differentiated cells (%D) is commonly used in the HL-60 system to assess the relative activity of various compounds. As pointed out by Haces et al.,[6] this assay is meaningful if the cytotoxicity is low. Otherwise, preexisting differentiated cells might be contained in a portion of an apparent increase of %D under cytotoxic conditions, especially if cytotoxicity is effected predominantly against growing nondifferentiated cells. Thus, the higher the viability (%V), the more reliable the %D value will be. Therefore, viability determination is essential for an accurate assessment of differentiation inducers which are also cytotoxic. On this ground for cytotoxicity against growing tumor cells especially, viability can be used as an indicator of screening of antitumor agents in a manner similar to the use of the P-388 test for cytotoxicity.[7]

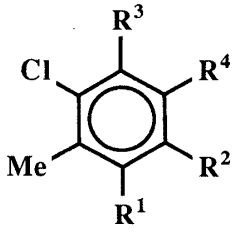
RA is known to be the most potent inducer of differentiation of HL-60.[8] As a typical experiment, the results of colletochlorin B (**2**) in comparison with RA is shown in Fig 1. At lower doses (< 2.5 x 10^{-8} M), differentiation occurred rather than cytotoxicity; at higher conentrations, cytotoxicity increased. The %D and %V curves of compounds **1** and **7** were similar to those of RA and **2**. Compounds **3**, **4**, **5**, and **6** gave %D curves similar to RA but were less cytotoxic.

The VD_{50} and ED_{50} of compounds **1**-**7** are shown in Table II. The VD_{50} is the dose resulting in 50% viability, and ED_{50} is the dose giving 50% effectiveness of differentiation. By comparing these values, we can assess the compounds from graphs of %V and %D. The VD_{50} values of compounds (**1**, **2**, and **7**) are about 50-120 nM, similar to the value of RA; those of other compounds (**3**, **4**, **5**, and **6**) are higher than 1 μM. Therefore, the cytotoxicity of these compounds appears to depend on the functional groups

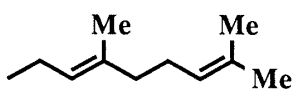
Table I. Structure of RA, Colletochlorin B and its Analogues



All-trans-retinoic acid (RA)



	R^1	R^2	R^3	R^4
1	CHO	OMe	OH	Geranyl
2	CHO	OH	OH	Geranyl (colletochlorin B)
3	COOMe	OMe	OMe	Br
4	COOMe	OH	OH	Br
5	COOMe	OMe	OMe	Geranyl
6	COOH	OMe	OMe	Br
7	CHO	OH	OMe	Geranyl

Geranyl = 

attached to the benzene ring (CHO > COOMe = COOH). Compounds with the CHO group are the most cytotoxic to HL-60 cells. The long terpenoid side chain and other functional residues (Br, OMe, OH) do not affect the cytotoxicity. In contrast, the ED_{50} values of all compounds are roughly 0.9-14 nM. In particular, compound **2** exhibits a value comparable to RA. Since ED_{50} values are almost independent of the long terpenoid side chain (R^4), Br, OMe, or OH substituent (R^2, R^3), the key functionality for differentiation inducing activity may be attributed to the benzene ring connected with a carbonyl residue: the activity order is CHO > COOMe > COOH.

Based on the structure-activity relationship, the compounds tested fall into two groups. Group I (RA, **1**, **2**, and **7**) has both cytotoxicity and differentiation-inducing activities against HL-60 cells. Group II (**3**, **4**, **5**, and **6**) has only differentiation-inducing activity. As RA is known to be too toxic for clinical use, in vivo testing of the compounds in Group II will be worthwhile, as a differentiating agent without cytotoxicity is ideal.

We have described here that colletochlorin B and its analogues are highly potent as a new inducer for differentiation.[9)]

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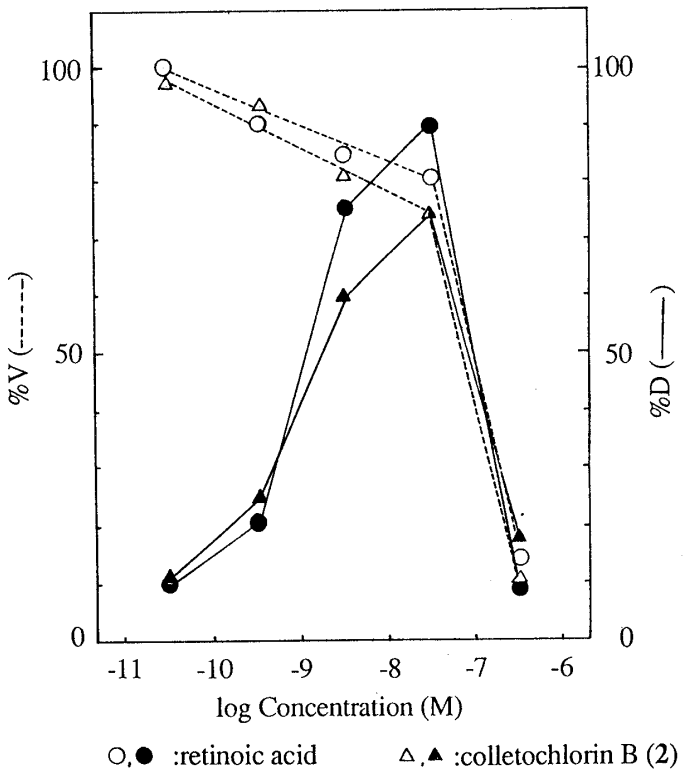

Fig 1. Dose-Response Curves of %V and %D

Table II. VD_{50} and ED_{50} of RA, Colletochlorin B and Its Analogues

	VD_{50} (nM)[a]	ED_{50} (nM)[b]
RA	58	0.71
2	55	0.93
1	100	1.85
7	116	1.14
3	>1000	2.5
4	>1000	3.6
5	>1000	2.5
6	>1000	13.8

[a] The dose for 50% viability. [b] The dose for 50% effectiveness of differentiation.

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9) Under the conditions we could not get 100% differentiation activity of HL-60 by these compounds alone: maximum %D was 85. Priming of HL-60 by a drug such as RA or cycloheximide may be possible as described.[10]
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