Note

Disappearance of Differentiation-induction of Friend Leukemia Cells upon Racemization of Trichostatic Acid†

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Friend erythroleukemia cells have been used as a model of differentiation of normal erythroid cells since they can be induced *in vitro* to have various normal functions specific to erythroid cells such as hemoglobin biosynthesis.[1] Although the mechanism of the differentiation is unkown, suppression of their proliferation is assumed to be a prerequisite for their differentiation.[2~6] Many chemicals such as dimethylsulfoxide (DMSO)[7] and hexamethylene bisacetamide (HMBA)[8] are inducers.

We used the differentiation of Friend leukemia cells to search for new antitumor substances. Actinomycin V,[9] cosmomycins,[10,11] and trichostatic acid[12] were potent inducers of Friend cell differentiation, and may provide clues to elucidate the mechanism of Friend leukemia cell differentiation. We have been studying the relationship between differentiation-inducing activity and the structure of these compounds. This paper reports that the racemate of trichostatic acid prepared from (+)-trichostatic acid lost the ability to induce Friend leukemia cell differentiation while retaining its cytotoxicity.

The properties and culture conditions of Friend leukemia cells, F5-5 were described in our previous paper.[12] Cell differentiation was evaluated by the frequency of hemoglobin-positive cells.[13]

(+)-Trichostatic acid (Fig. 1) was racemized in 1 N NaOH at 30°C for 3 hr. The reaction product was purified by HPLC with a μBondapack C18 column using a solvent system of acetonitrile and 10 mM ammonium acetate buffer (35:65, pH 4.0). Circular dichroism (CD) and ^{1}H-NMR spectra of the product eluted at the same retention time as that of (+)-trichostatic acid were examined. The product showed the same spectra as those of (+)-trichostatic acid in ^{1}H-NMR. It, however, showed no significant peaks in the CD spectrum, while (+)-trichostatic acid had peaks at 243, 260, 295, 299, 307, and 349 nm (Fig. 2). These results indicate that trichostatic acid was successfully racemized.

We examined whether the (+) isomer of trichostatic acid is absolutely necessary for the differentiation-inducing activity. (+)-Trichostatic acid caused differentiation of F5-5 cells from 1.0 to 20 μM (Fig. 3). Differentiation of F5-5 cells was first observed at a concentration that slightly inhibited the cell growth and reached a maximum at the concentration that inhibited the

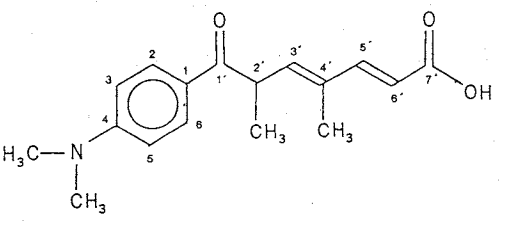

FIG. 1. Chemical Structure of Trichostatic Acid.

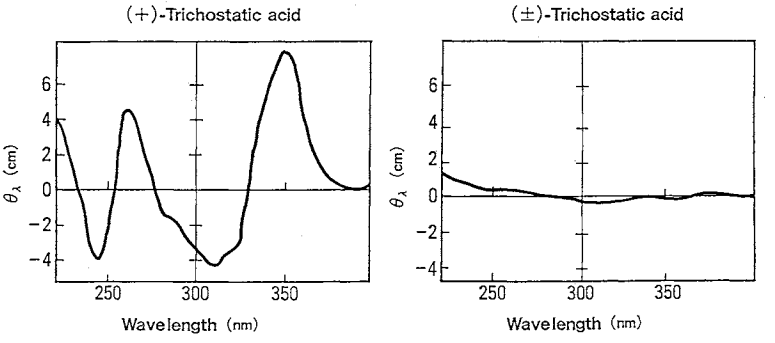

FIG. 2. CD Spectra of (+)- and (±)-Trichostatic Acid.

† This study was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, April, 1985, p. 221.

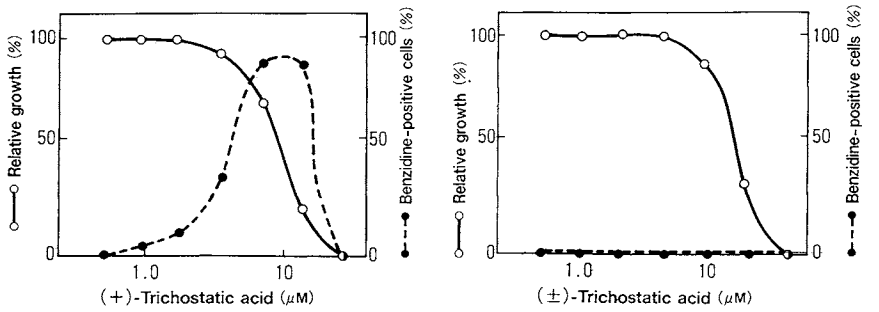

FIG. 3. Cytotoxicity and Differentiation-inducing Activity of (+)- and (±)-Trichostatic Acid.

A Friend cell line, F5-5, was grown at 2×10^4 cells/ml in the presence of various amounts of the compounds. On the 6th day, the percentage of benzidine-positive cells (---●---) and final cell number (—○—) were measured.

cell growth to half the level of the control. At the optimal concentration (8 ~ 18 μM), approximately 90% of the cells differentiated within 6 days. It inhibited cell proliferation and eventually became cytotoxic at concentrations above 36 μM.

The racemate of trichostatic acid completely lost differentiation-inducing activity from 0.45 to 36 μM, but retained cytotoxicity at concentrations at which (+)-trichostatic acid is cytotoxic (Fig. 3). The optical active center at 2′ position is considered to be necessary for the differentiation-inducing activity but not for cytotoxicity. During suppression of proliferation, (+)-trichostatic acid may act on the differentiation site by interacting with the gene necessary for terminal differentiation. To verify this hypothesis, we are separating (−)-trichostatic acid from the racemate to analyze the effect of the (−) isomer on the differentiation of F5-5 cells.

REFERENCES

1) P. A. Marks and R. A. Rifkind, *Annu. Rev. Biochem.*, **47**, 419 (1978).
2) R. C. Reuben, P. L. Khanna, Y. Gazitt, R. Breslow, R. A. Rifkind and P. A. Marks, *J. Biol. Chem.*, **253**, 4214 (1978).
3) Z.-X. Chen, J. Banks, R. A. Rifkind and P. A. Marks, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 471 (1982).
4) T. Murate, T. Kaneda, R. A. Rifkind and P. A. Marks, *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 3394 (1984).
5) S. Nomura and M. Oishi, *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 210 (1983).
6) T. Watanabe, S. Nomura and M. Oishi, *Exp. Cell Res.*, **159**, 224 (1985).
7) C. Friend, W. Scher, J. Holland and T. Sato, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 378 (1971).
8) R. C. Reuben, R. L. Wife, R. Breslow, R. A. Rifkind and P. A. Marks, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 862 (1976).
9) H. Morioka, M. Takezawa, H. Shibai, *Agric. Biol. Chem.*, **49**, 2835 (1985).
10) H. Morioka, Y. Etoh, I. Horino, M. Takezawa, T. Ando, K. Hirayama, H. Kano and H. Shibai, *Agric. Biol. Chem.*, **49**, 1951 (1985).
11) T. Tsuji, H. Morioka, M. Takezawa, T. Ando, A. Murai and H. Shibai, *Agric. Biol. Chem.*, **50**, 1697 (1986).
12) H. Morioka, M. Ishihara, M. Takezawa, K. Hirayama, E. Suzuki, Y. Komoda and H. Shibai, *Agric. Biol. Chem.*, **49**, 1365 (1985).
13) S. Orkin, F. I. Harosi and P. Leder, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 98 (1975).