STIMULATION OF MAMMALIAN CELL PROLIFERATION BY LAVANDUCYANIN

Sir:

Lavanducyanin (Fig. 1) produced by *Streptomyces aeriouvifer* was isolated as an antitumor substance against P388 and L1210 cells[1]. It was found later that this compound stimulated cell growth of some mammalian cells applied under specific conditions with its concentrations being lower than the cytotoxic concentration level. The manner of growth stimulation of lavanducyanin was related to the early stage of the cell cycle. The results so far obtained suggest that lavanducyanin stimulates the cell proliferation in a similar manner as do growth factors. In this paper, we report the unique activity of lavanducyanin in human and murine cells expressed at low concentrations.

The cell growth stimulating activity of lavanducyanin was measured by the increase of cell numbers and amount of DNA synthesis which was assessed by measuring [^{3}H]thymidine incorporation into TCA-precipitable materials.

HeLa cells were seeded at the density of 2.5×10^5/ml to 96-wells plates containing MEM supplemented with 1% fetal calf serum. Since the activity of lavanducyanin was greatly affected by lot of the serum, we used the adaptable serum in all experiments. After cells adhesion, lavanducyanin at various concentrations was added to the medium. After 68 hours cultivation, the cells were incubated for 4 hours in MEM containing 1 μCi/ml [^{3}H]thymidine. As shown in Fig. 2, lavanducyanin stimulated strongly cell proliferation and DNA synthesis at the concentrations between $0.1 \sim 10$ ng/ml. When the cells were treated at a sub-optimum condition with the serum concentrations being 1% to 3%, lavanducyanin enhanced cell growth. However, when the serum concentration was increased to 10%, lavanducyanin did not enhance the growth of the cells (data not shown). Therefore it is suggested that lavanducyanin replaces a function of growth stimulating factors in the serum.

We then measured the time course of DNA synthesis in HeLa cells in the presence of lavanducyanin at the concentration of 10 ng/ml (Fig. 3). Without addition of lavanducyanin, DNA synthesis of the cells under the sub-optimum condition reached the maximum at about 18 hours. In the presence of lavanducyanin, the incorporation of [^{3}H]thymidine was strongly stimulated at the same time. This result indicates that the growth stimulation of lavanducyanin is strictly related to the specific stage of the cell cycle.

In order to investigate the action mechanism of lavanducyanin in more detail, we utilized BALB/c 3T3 cells (clone A31) which had been well studied on their cell proliferation mechanism[2]. BALB/c 3T3 cells were grown in DMEM containing 0.15%

Fig. 1. Structure of lavanducyanin.

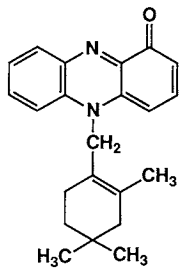
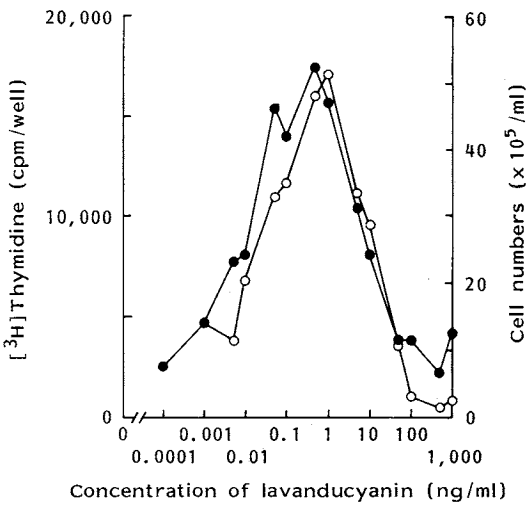


Fig. 2. Effects of lavanducyanin on DNA synthesis and cell proliferation in HeLa cells.

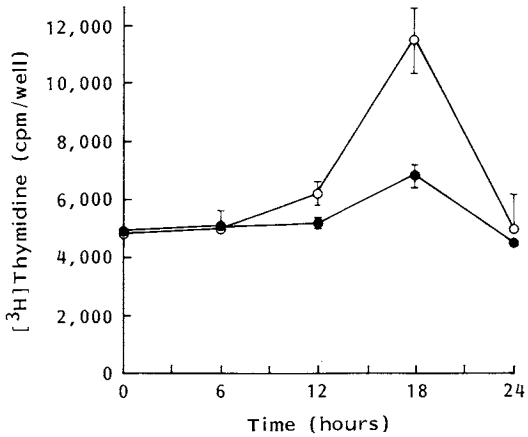
○ [^{3}H]Thymidine incorporation, ● cell numbers.



HeLa cells were seeded at 2.5×10^5/ml in 96-wells plates in MEM containing 1% fetal calf serum. After $2 \sim 3$ hours incubation at 37°C in a humidified atmosphere consisting of 95% air and 5% CO_2, lavanducyanin at various concentrations were added to the medium. After 68 hours incubation, the cells were cultured for additional 4 hours in MEM containing 1 μCi/ml [^{3}H]thymidine. On the other plate, after 72 hours incubation, the cells were not stained by tripan blue were counted. [^{3}H]Thymidine incorporation into trichloracetic acid-precipitable materials was measured by the method of McNeil *et al.*[7].

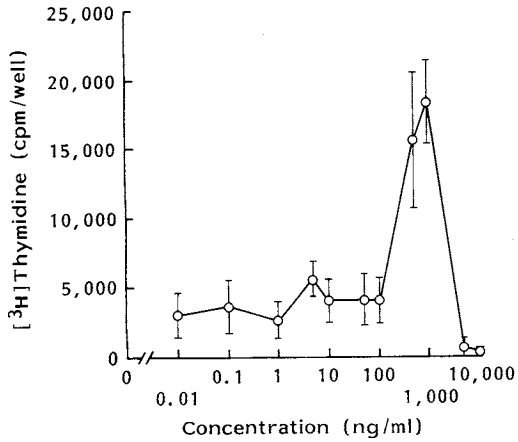
Fig. 3. Effect of lavanducyanin on [^{3}H]thymidine incorporation at various time in HeLa cells.

○, In the presence of lavanducyanin; ●, in the absence of lavanducyanin.



HeLa cells were seeded at 1×10^4/ml in 96-wells plates in MEM containing 1% fetal calf serum. After 3 hours incubation at 37°C, lavanducyanin at 10 ng/ml were added to the medium. Then, after 2, 8, 14 and 20 hours incubation, each sample were cultured for 4 hours in MEM containing 1 μCi/ml [^{3}H]thymidine. Values are the mean ± SE for four determinations.

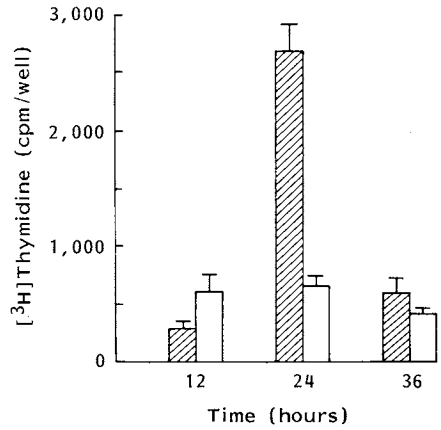
Fig. 4. Effect of lavanducyanin on [^{3}H]thymidine incorporation in BALB/c 3T3 cells.



Lavanducyanin was added at various concentrations to quiescent cells in 96-wells plates and the cells were cultured for 24 hours in DMEM containing 1 μCi/ml [^{3}H]thymidine. [^{3}H]Thymidine incorporation was measured as described under legend to Fig. 2. Values are the mean ± SE for four determinations.

Fig. 5. Effect of lavanducyanin on [^{3}H]thymidine at various time in BALB/c 3T3 cells.

Open bars: Control, closed bars: lavanducyanin.



Quiescent cells were prepared in 96-wells plates. Lavanducyanin was added at 1,000 ng/ml to the medium. Incubation of the cells with 1 μCi/ml [^{3}H]thymidine were carried out for 8 hours at the time of 4~12 hours, 16~24 hours and 28~36 hours. [^{3}H]Thymidine incorporation was measured as described under legend to Fig. 2. Values are the mean ± SE for four determinations.

$NaHCO_3$ supplemented with 10% fetal calf serum of selected lot and 100 units/ml benzylpenicillin in a humidified atmosphere consisting of 95% air and 5% CO_2 at 37°C. The cells were cultured for 3 days without renewing the medium to give density-arrested monolayer of the cells. The cells were further incubated for 24 hours with the same medium to give quiescent cells and then lavanducyanin was added at various concentrations to the medium. As shown in Fig. 4, lavanducyanin stimulated the growth of the quiescent cells at higher concentrations than in the case of HeLa cells.

G_0/G_1-arrested BALB/c 3T3 cells are known to be induced to the S stage by growth factors such as FGF for 15~16 hours after their addition. In the case of BALB/c 3T3 cells that were synchronized to the G_0/G_1 stage more strictly than HeLa cells, lavanducyanin induced them to the S stage after the similar lag time as did growth factors (Fig. 5). The results suggest that the action of growth stimulation of lavanducyanin depends on the cell cycle.

Doxorubicin (adriamycin) was reported to stimulate the cell growth[3]. The activity of lavanducyanin, however, is an order of magnitude stronger than that of doxorubicin. When applied under the appropriate conditions to HeLa cells and quiescent BALB/c 3T3 cells, some low molecular compounds such as phorbol ester, ionophore and prostaglandins stimulate cell growth, and these

compounds are known to act important points of signal transduction of cell growth[4,5]. The results reported in this paper suggest that the action mechanism of lavanducyanin is related to signal transduction. IMAI *et al.* reported that 12-*O*-tetradecanoylphorbol 13-acetate (TPA) did not show any effects to stimulate cell proliferation of rat normal cell line RLN-8, while lavanducyanin did[6]. It is assumed that lavanducyanin acts at a point in signal transduction different from that of TPA. The detailed mechanism of action of lavanducyanin on cell proliferation is now under study.

Acknowledgment

This work was supported by a Grant-in-Aid for Scientific Research, The Ministry of Education, Science and Culture, Japan (03453141 to H.S.).

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(Received August 10, 1991)

References

1) IMAI, S.; K. FURIHATA, Y. HAYAKAWA, T. NOGUCHI & H. SETO: Lavanducyanin, a new antitumor substance produced by *Streptomyces* sp. J. Antibiotics 42: 1196~1198, 1989
2) PLEDGER, W. J.; C. D. STILES, H. N. ANTONIADES & C. D. SCHER: Induction of DNA synthesis in BALB/c 3T3 cells by serum components: reevaluation of the commitment process. Proc. Natl. Acad. Sci. U.S.A. 74: 4481~4485, 1977
3) VICHI, P. & T. R. TRITTON: Stimulation of growth in human and murine cells by adriamycin. Cancer Res. 49: 2679~2682, 1989
4) NISHIZUKA, Y.: The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature 308: 693~698, 1984
5) FUKAMI, K. & T. TAKENAWA: Quantitative change in polyphosphoinositides 1,2-diacylglycerol and inositol 1,4,5-trisphosphate by platelet-derived growth factor and prostaglandin $F_{2\alpha}$. J. Biol. Chem. 264: 14985~14989, 1989
6) IMAI, S.; M. MATSUMOTO, Y. HAYAKAWA, T. NOGUCHI & H. SETO: Exfoliazone and lavanducyanin, potent growth promoting substances of rat liver cell line, RLN-8, produced by *Streptomyces exfoliatus* and *Streptomyces aeriouvifer*. J. Antibiotics, in preparation
7) MCNEIL, P. L.; M. P. MCKENNA & D. L. TAYLOR: A transient rise in cytosolic calcium follows stimulation of quiescent cells with growth factors and is inhibitable with phorbol myristate acetate. J. Cell Biol. 101: 372~379, 1985