STIMULATION OF MAMMALIAN CELL PROLIFERATION BY LAVANDUCYANIN

Sir:

Lavanducyanin (Fig. 1) produced by Streptomyces aeriusifer was isolated as an antitumor substance against P388 and L1210 cells. It was found later that this compound stimulated cell growth of some mammalian cells 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 [3H]thymidine incorporation into TCA-precipitable materials.

HeLa cells were seeded at the density of 2.5 x 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 [3H]thymidine. As shown in Fig. 2, lavanducyanin stimulated strongly cell proliferation and DNA synthesis at the concentrations between 0.1 ~ 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 [3H]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. BALB/c 3T3 cells were grown in DMEM containing 0.15% 

![Fig. 1. Structure of lavanducyanin.](image1)

![Fig. 2. Effects of lavanducyanin on DNA synthesis and cell proliferation in HeLa cells.](image2)
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.

![Graph showing effect of lavanducyanin on \[^{3}H\]thymidine incorporation in HeLa cells.](image)

HeLa cells were seeded at 1 x 10⁴/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.

![Graph showing effect of lavanducyanin on \[^{3}H\]thymidine incorporation in BALB/c 3T3 cells.](image)

Open bars: Control, closed bars: lavanducyanin.

![Graph showing effect of lavanducyanin on \[^{3}H\]thymidine incorporation at various time in BALB/c 3T3 cells.](image)

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.

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. 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. It is assumed that lavanducyanin acts at a
point in signal transduction different from that of
TPA. The detailed mechanism of action of lavan-
ducyanin on cell proliferation is now under

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