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Growth Inhibitory Effects of Sesamolin from Sesame Seeds on Human Leukemia HL-60 Cells

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Abstract – This study was carried out to test the growth inhibitory effects of sesamolin obtained from sesame seeds. Sesamolin inhibited the growth of human leukemia HL-60 cells in cultures and the synthesis of macromolecules in dose- and time-dependent manners. Sesamolin in the 60~100 μg/ml range was cytostatic. At concentrations greater than 200 μg/ml sesamolin was cytocidal to HL-60 cells and at 60 μg/ml inhibited the synthesis of DNA, RNA and protein in HL-60 cells by 35.1, 6.1, and 5.3%, whereas at 200 μg/ml these inhibitions were 86.8, 81.5 and 96.7%, respectively. The inhibitory effect of sesamolin on DNA synthesis was irreversible.

Key words – Sesame (*Sesamum indicum*), sesamolin, growth inhibitory effects, human leukemia HL-60 cells

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops known to human, not only for its high oil content, but also its resistance to oxidative deterioration and medicinal effects. The seed contains minor components, including lignan compounds, such as sesamin and sesamolin, and the seed oil also exhibits other unusual chemical and physiological properties than any other edible oil. Its remarkable oxidative stability has been suggested to be due to the presence of the endogenous antioxidants sesamol, sesaminol and sesamolinol, together with tocopherol.[1,2] It has also been reported that sesamin reduces cholesterol levels in the liver and serum by inhibiting the absorption and synthesis of cholesterol.[4-6] It has shown inhibition of 5 desaturase, which catalyzes arachidonic acid from dihomo-γ-linoleic acid in microbes and in rat liver microsomes due to the sesamin and other lignans content.[7-9] There are numerous reports that have demonstrated the effectiveness of antioxidants against chemically induced cancers.[4,10-14] The anticarcinogenic activity of lignans has been extensively examined in podophyllum toxin and related lignans.[15] These reports prompted us to study the favorable physiological function of sesamolin. Although, sesamin, a major lignan in sesame seeds, is expected to serve as an anticarcinogenic agent, but the effect of sesamolin remains to be studied. The sesaminol content of sesame oil was found to be dramatically increased during the manufacturing process, in particular, from the bleaching process.[1]

Sesaminol was also found in high concentration in unroasted sesame oil, due to the high conversion yield of sesamolin to sesaminol, due to the intermolecular group transfer catalyzed by the acid clay used in the decolorization process.[16] Four sesaminol stereoisomers exist, all of which show quite strong antioxidative activities.[1] Sesaminol has shown a remarkable synergism with α-tocopherol.[17] When the levels of sesaminol in commercially available sesame oils were quantified by HPLC, the total level of sesaminol isomers in the most commercially available sesame seed oils was about four times more than that of α-tocopherol.[2] In connection with our systematic isolation and structural elucidation of the biologically active compounds from crude drugs, our results on the antitumor activity of sesamolin from sesame oil are described.

Materials and Methods

Materials – The [^{3}H]thymidine (50 μCi/μmol), [^{3}H]uridine (55 μCi/μmol) and [^{3}H]leucine (150 μCi/μmol) were purchased from NEN Life Science Products, Inc (Boston, MA). The HL-60 cells were purchased from the American Type Culture Collection (ATCC : Rockland, MD). The RPMI medium, fetal calf serum, trypsin, penicillin and streptomycin were purchased from GIBCO BRL (Grand Island, NY). The glass microfibre filters (2.5 cm in diameter), Scienti Verse, trichloroacetic acid, and HPLC grade dimethyl sulfoxide

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(DMSO), ethyl acetate, methanol and *n*-butanol, were purchased from Fisher Scientific (Springfield, NJ). The Diaion HP20 was obtained from Mitsubishi Chemical Corporation (Tokyo, Japan).

Preparation of sesamolin – The sesame seeds (250 g) were ground and defatted with hexane and extracted with methanol. The methanol extract was subjected to reversed phase chromatography, with a Develosil ODS column and eluted with an hexane-ethyl acetate (4:1) isocratic system, as described in a previous report.[18] The sesamolin was purified as whitish needles, by recrystallization from methanol. The structure of sesamolin, with 98% purity, was deduced from the spectroscopic data.[19,20]

Culture of HL-60 cells – The HL-60 cells (5×10^5 cells/ml) were grown in 100 mm diameter culture dishes, in RPMI medium, supplemented with 10% fetal calf serum and 1% penicillin-streptomycin, in a humidified atmosphere containing 5% CO_2 at 37°C. In order to maintain normal cell growth, the cells were usually passed at every 2~3 days. To measure the effect of sesamolin on the growth of the HL-60 cells, sesamolin, at different concentrations, was added to the HL-60 cells (5×10^5 cells per ml) in a dish containing 20 ml of RPMI medium supplemented as before. The cultures were incubated at 37°C for 4 days. With the cell numbers counted daily, using a hemacytometer, by microscopy at 10×magnification, and the results recorded.

Measurement of DNA, RNA, and protein synthesis in HL-60 cells – The HL-60 cells were harvested by centrifugation for 5 min at 1000×g. The cells were resuspended in RPMI, without fetal calf serum, and placed in a series of 13 ×100 mm test tubes (5×10^5 cells/ml per tube). To measure the DNA, RNA or protein synthesis, 3 μl of [^{3}H]thymidine (50 μCi/mol), 5 μl of [^{3}H]uridine (50 μCi/mol) or 10 μl of [^{3}H]leucine (50 μCi/μmol) was added to each individual tube followed by 2 μl of DMSO or an inhibitor in DMSO. The tubes were incubated at 37°C for 120 min. The reaction was terminated by the addition of 2 ml of ice-cold phosphate buffer saline (PBS) solution and kept in an ice bath. The tubes were then centrifuged for 5 min at 1000×g. The supernatant was discarded, and the cells washed twice with PBS. Finally, the cells were resuspended in 2 ml of ice-cold deionized water and 2 ml of 10% trichloroacetic acid (TCA) solution. The precipitates were collected on a glass fibre filter and washed three times with 5% cold TCA-solution. The dry glass fibre filters were placed in scintillation vials, with 10 ml of Scienti Verse fluid, and the radioactivity was determined in a Beckman LS 1701 scintillation counter. Each treatment was replicated 4 times, with a completely randomized design.

Results and Discussion

The yield of sesamolin was 0.1~0.4% of the original sesame seeds. The sesamolin was used to studies its effects on the growth of HL-60 cells and the synthesis of macromolecules as follows:

The sesamolin inhibited the growth of the HL-60 cells in a dose-dependent fashion (Fig. 1). The addition of sesamolin, at 50, 200 or 400 μg/ml, to the HL-60 cells inhibited the growth by 50.3, 85.1 or 93.3%, respectively. The inhibitory effect of sesamolin on the growth of HL-60 cells was also incubation time-dependent. Incubation of the HL-60 cells with 50 μg/ml of sesamolin at 37°C, for 1, 2, 3 or 4 days, inhibited the growth of HL-60 cells by 50.4, 73.2, 84.5 or 89.3%, respectively. The cytostatic concentration was found between 60 and 100 μg/ml. Sesamolin concentrations greater than 200 μg/ml were cytocidal (Fig. 1).

The inhibitory effects of sesamolin on the synthesis of DNA, RNA and protein in the HL-60 cells are shown in Fig. 2. The initial rates of incorporation of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]leucine into trichloroacetic acid (TCA) insoluble materials were utilized to estimate the rates of DNA, RNA and protein synthesis in the HL-60 cells, respectively. The presence of sesamolin, at 6, 12.5, 25, 50, 100, 200 or 400 μg/ml,

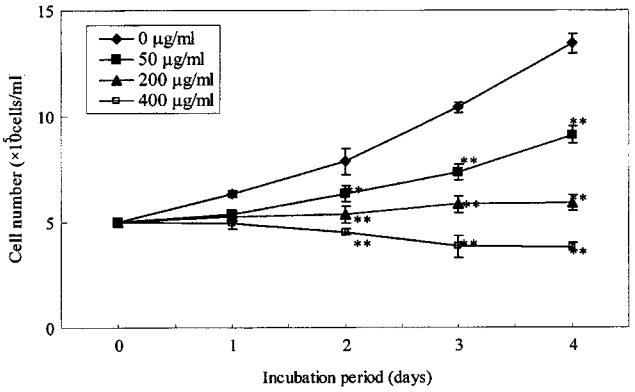

Fig. 1. The inhibitory effect of various concentrations of sesamolin on the growth of HL-60 cells. The HL-60 cells (5×10^5 cells/ml), suspended in RPMI medium supplemented with 10% calf serum and 1% penicilin and streptomycin, were incubated with various concentrations of sesamolin, or a control (vehicle only), at 37°C for 4 days. Every 24 hr, the number of HL-60 cells was counted under a microscope. Significantly different from the control group.
*P<0.05, **P<0.01 vs. control.

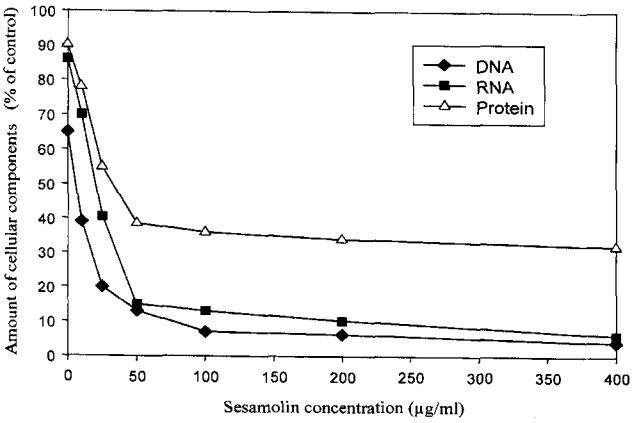

Fig. 2. The inhibitory effect of various concentrations of sesamolin on the synthesis of DNA, RNA, and protein in HL-60 cells, suspended in RPMI medium without calf serum, at a concentration of 5×10^5 cells/ml. [^{3}H]thymidine (50 μCi/μmol: 3 μl), [^{3}H]uridine (55 μCi/μmol: 5 μl), or [^{3}H]leucine (200 μCi/μmol: 10 μl) were added. The cells were incubated at 37°C for 120 min, and the reactions were terminated by the addition of 2 ml of cold PBS. The rate of DNA, RNA and protein synthesis was determined as described in the materials and methods. The percentage of incorporation is expressed relative to cultures without an inhibitor.

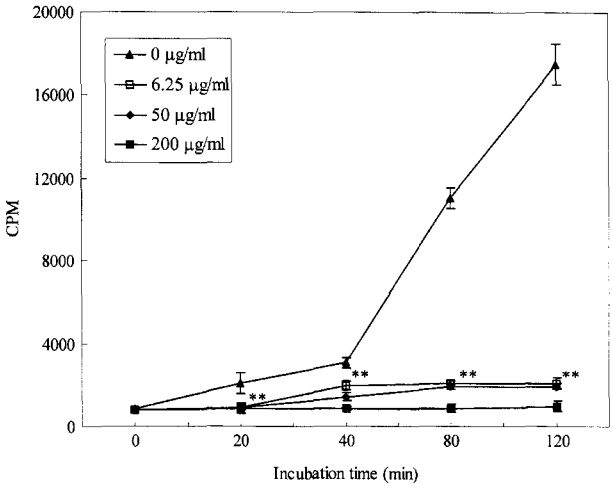

Fig. 3. The effect of sesamolin on the synthesis of DNA in HL-60 cells at various times following exposure to the inhibitor. The HL-60 cells (5×10^5 cells/ml), suspended in RPMI medium, were divided into 4 portions. [^{3}H]thymidine (50 μCi/μmol: 3 μl) was added and cells were further incubated at 37°C. Using 1 ml of sample in 2 ml of cold PBS, the rated of DNA synthesis were determined as described in the materials and methods. Significantly different from the control group, $^{**}P<0.01$ vs. control.

in the cultured HL-60 cells, incubated for 120 min, inhibited the incorporation of [^{3}H]thymidine into DNA by 35.1, 59.2, 81.2, 83.2, 85.4, 86.8 or 98.3%, respectively (Fig. 2), the incorporation of [^{3}H]uridine into RNA by 6.1, 31.5, 57.4, 76.2, 78.3, 81.5 or 96.7%, respectively, and the incorporation of [^{3}H]leucine into protein by 5.3, 15.6, 22.3, 60.4, 69.3, 78.7 or 81.6%, respectively. The results indicate that sesamolin is a potent inhibitor of DNA synthesis, but is somewhat less effective at inhibiting the RNA and protein synthesis in HL-60 cells.

The inhibitory effect of sesamolin on the DNA synthesis in HL-60 cells occurred rapidly. The inhibition caused by 200 μg/ml of sesamolin was essentially complete after 10 min. The inhibitory effect of sesamolin on the DNA synthesis was incubation timedependent (Fig. 3). On incubation of the HL-60 cells (5×10^5 cells/ml) with sesamolin, at 6.25, 50 or 200 μg/ml, at 37°C for 10 min, the DNA synthesis was inhibited by 20.4, 38.5 or 55.2%, respectively whereas incubation under the same condition for 120 min, caused 58.1, 80.1 or 95.2% inhibition, respectively. The inhibitory effect of sesamolin on the DNA synthesis was irreversible (Table I). The HL-60 cells were pre-incubated with sesamolin, at 12.5, 50 and 200 μg/ml

Table I. Irreversible inhibitory effect of sesamolin on the synthesis of DNA in HL-60 cells

Concentration of sesamolin (μg/ml)	[^{3}H]thymidine incorporation into TCA insoluble materials (cpm)		% of control		IC_{50} (μg/ml)
	A	B	A	B	
0	14077±130.6	15555±113.4	100	100	–
12.5	7320±43.6	7778±54.6	52	50	10.3
50	2112±23.5	933±20.6	15	6	7.8
200	563±10.6	466±19.8	4	3	4.6

A, The HL-60 cells (5×10^5 cells/ml) were pre-incubated with 2 μl DMSO, or sesamolin in DMSO at 37°C for 120 min, then washed 3 times with PBS. The cells were resuspended in fresh RPMI medium, 2 μl DMSO and [^{3}H]thymidine were added and incubated at 37°C for 120 min. The radioactivity incorporated into the TCA insoluble materials was determined as described in the text. **B**, The HL-60 cells (5×10^5 cells/ml) were incubated with 2 μl DMSO at 37°C for 120 min, then washed 3 times with PBS. The cells were resuspended in fresh medium, 2 μl DMSO, or sesamolin in DMSO, and [^{3}H]thymidine were added and incubated at 37°C for 120 min. The radioactivity incorporated into the TCA insoluble materials was determined as described in the text. The IC_{50} values were calculated by extrapolation.

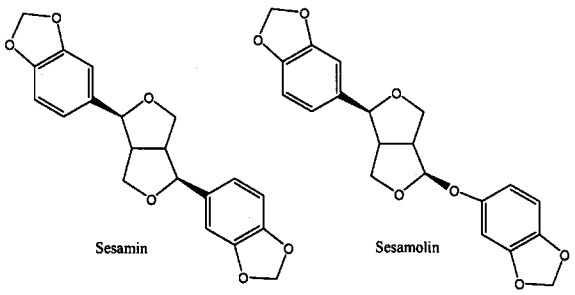

Fig. 4. Main components of sesame seeds.

for 120 min at 37°C, and then washed three times with PBS to remove the sesamolin. The cells were resuspended in RPMI medium, [^{3}H]thymidine added and the DNA synthesis was determined. The result in column A of Table I shows that the inhibitory effect on the DNA synthesis was dependent on the pre-incubation of the HL-60 cells with various concentrations of sesamolin.

The present investigation has shown that sesamolin inhibits the growth of human leukemia and the synthesis of macromolecules in HL-60 cells. The inhibitory effect of sesamolin on the DNA synthesis was irreversible.

These results suggest that components in the sesame seeds have antitumor activities. Very recently, sesamolin was reported to show growth inhibitory effects toward human lymphoid leukemia Molt 4B cells due to the induction of apoptosis in the cells.[21] Additional studies, including the determination of the inhibitory effects of sesamolin on the growth of HL-60 cells, and the synthesis of macromolecules, and studies on the possible inhibitory mechanism need to be explored.

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참깨에서 분리된 세사몰린의 백혈병 세포주 HL-60 생장억제 효과

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요 약 – 본 연구는 참깨 종자로부터 추출, 분리된 세사몰린의 백혈병 HL-60 세포의 생장억제에 미치는 영향을 조사하였다. 세사몰린은 농도와 시간 의존적으로 HL-60 세포의 생합성을 억제하였다. 60~100 μg/ml 농도의 세사몰린 범위에서 세포증식이 억제적이었다. 200 μg/ml 이상의 세사몰린 농도에서 HL-60 세포에 세포파괴성으로 나타났다. 그리고 60 μg/ml에서 HL-60 세포의 DNA, RNA, 단백질의 합성억제 정도는 35.1%, 6.1%, 5.3%였다. 반면에 200 μg/ml에서의 억제 정도는 각각 86.8%, 81.5%, 96.7%였다. DNA 합성에 대한 세사몰린의 억제효과는 비가역적이었다.