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Antitumor Activity of Crude Sesaminol in Sesame Seed

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ABSTRACT

Sesaminol in sesame seed was postulated to have antitumor activity. The present study was performed to characterize the role of crude sesaminol extracted from sesame seed (Sesame Crude Sesaminol; SCS) on inhibiting the *in vitro* growth of human leukemia HL-60 cells. SCS inhibited the growth of human leukemia HL 60 cells in culture and macromolecular synthesis in a dose and time dependent manner. The cytostatic range of SCS concentration was found to be 60 to 100 μg/ml. SCS concentration greater than 200 μg/ml were cytocidal to HL-60 cells. When SCS concentraction was 6 μg/ml and 50 μg/ml, the synthesis of HL-60 cells was inhibited by 35% for DNA, 6% for RNA and 5% for protein and 83% for DNA, 76% for RNA and 60% for protein. Of specific interest was the irreversible effect of SCS in inhibiting DNA synthesis of HL-60 cells. This was evidenced from the fact that, even after washed with PBS three times, preincubated HL-60 cells still showed the inhibited DNA synthesis.

Key words : sesame, antitumor, sesaminol.

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops known to man, not only of its high oil content but also because of its resistance to oxidative deterioration and its medicinal effects. The seed also contains unusual minor components, including lignan compounds such as sesamin and sesamolin, and the seed oil exhibits more 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 (Fukuda et al., 1986 ; Osawa et al., 1992).

High concentrations of lignans with characteristic structures, sesamin and sesamolin, are found in sesame seeds (Fukuda et al., 1985, 1986). Sugano et al. (1990) and Hirose et al. (1991, 1992) also reported lower blood chloesterol concentrations in rats fed with sesamin when compared with those fed 20% casein purified diets in the presence or absence of cholesterol. Shimizu et al. (1991) and Fujiyama-Fujiwara et al. (1992, 1995) have shown that 5 desaturase, catalyzing arachidonic acid from dihomo-γ-linoleic acid, was inhibited by sesamin and other ligans in microbes and in rat liver microsomes. There were numerous reports which demonstrated the effectiveness of antioxidants against chemically induced cancers (Akimoto et al., 1993 ; Asami et al., 1993 ; Harman et al., 1969 ; Hirose et al., 1992 ; King & Mc Cay, 1983 ; Tricker & Shklar, 1987). The anticarcinogenic activity of ligans has been extensively examined in podophyllum to toxin and related lignan (Weiss et al., 1975). Thus, sesamin, a major lignan in sesame, is expected to serve as an anticarcinogenic agent, but sesaminol effects have not yet been studied. Sesaminol content of sesame oil was found to be dramatically increased during the manufacturing process, in particular bleaching process (Fukuda et al., 1986).

Sesaminol was also found in high concentration in unroasted sesame oil, due to the high yield conversion of sesamolin to sesaminol by intermolecular group transfer catalyzed by the acid clay used for decolorization (Fukuda et al., 1994). Four sesaminol stereoisomers exist and all of these isomers have quite strong antioxidative activity (Fukuda et al., 1986). Sesaminol showed a remarkable synergism with α-tocopherol (Yamashita et al., 1992). When the amounts of sesaminol were quantified by HPLC in commercially available sesame oils, the total amounts of sesaminol isomers was about four times that of α-tocopherol in the most commercially available sesame seed oils (Osawa, 1992).

The objective of this study was to test the antitumor activity of the SCS. The technique involved the inhibition of human leukemia HL-60 cells as affected by the concentration of SCS and incubation time.

MATERIALS AND METHODS

Materials

[^{3}H]Thymidine (50μCi/μmol) and [^{3}H]leucine (150μCi/μmol) were purchased from Du Pont Chemical Company, NEN Reassert Products (Boston, MA). HL-60 cells were purchased from the American Type Cell Cultures (ATCC : Rockland, MD). RPMI medium, fetal calf serum, trypsin, penicillin, and streptomycin were purchased from GIBCO BRL (Grand Island, NY). Glass microfibre filter (2.5 cm in diameter), Scienti Verse, trichloroacetic acid and HPLC grade dimethyl sulfoxide (DMSO), ethyl acetate, methanol, and n-butanol were purchased from Fisher Scientific (Springfield, NJ). Diaion HP20 was obtained from Mitsubishi Chemical Corporation (Tokyo, Japan).

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Preparation of crude sesaminols

Sesame seed (250 g) was ground and defatted with hexane and extracted with 80% ethanol. The ethanol extract was dissolved in 50 mM acetate buffer (pH 5.4) and hydrolysed overnight with β-glucosidase. The reaction mixture was extracted with ethyl acetate and the extract purified by preparative HPLC to give six compounds (compounds 1~6). Compound 7 (sesaminol triglucoside) was isolated from the 80% ethanol extract of sesame seed using an XAD-2 column and preparative HPLC (Ryu et al., 1998). Their purities were confirmed by mass spectromety and proton nuclear magnetic resonance (1H-NMR) as reported previously (Katsuzaki et al., 1994).

Culture of HL-60 cells

Human leukemia HL-60 cells were used to test autitumor activity of SCS. HL-60 cells (5×10^5 cells per 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℃. Under these conditions, HL-60 cells had a doubling time of about 24~30 h depending on the medium and calf serum being used. In order to maintain normal cell growth, cells were usually passed at every 2~3 days.

To measure the effect of the SCS on the growth of HL-60 cells, SCS was added to HL-60 cells (5×10^5 cells per ml) in dish containing 20 ml of RPMI medium supplemented with 10% fetal calf serum and 1% penicillin-streptomycin. Cultures were incubated at 37℃ for 4 days. Cell number was counted daily using a hemacytometer under a microscope with 10×magnification every 24 h, and recorded.

Measurement of DNA, RNA, and Protein synthesis in HL-60 cells

HL-60 cells were harvested by centrifugation for 5 min at 1000×g. 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). For measurement of DNA, RNA or protein synthesis, 3 μl of [^{3}H]hymidine (50 μCi/ μmol), 5 μl of [^{3}H]uridine (50 μCi/ μmol) or 10 μl of [^{3}H]uridine (50 μCi/ μmol) or 10 μl of [^{3}H]leucine (50 μCi/ μmol) were added, respectively, to each individual tube followed by 2 μl of DMSO or inhibitor in DMSO. Tubes were incubated at 37℃ for 120min. The reaction was terminated by adding 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 cells were washed twice with PBS. Finally, cells were in 2 ml of ice-cold deionized water and 2 ml of 10% trichloroacetate acid (TCA) solution. The precipitates were collected on glassfibre filter and washed three times with 5% cold TCA solution. The dry glassfibre filter was placed in scintillation vials with 10 ml of Scienti Verse fluid and radioactivity was determined in a Beckman LS 1701 scintillation counter. Each treatment was replicated 4 times with completely randomized design.

RESULTS AND DISCUSSION

The yield of SCS was 0.06~0.1% from original sesame seeds. This preparation was used for studies of its effects on the growth of HL-60 cells and macromolecular synthesis as described in the following paragraphs.

The SCS inhibited the growth of HL-60 cells in a dose-dependent fashion (Fig. 1). Addition of 50, 200, or 400 μg/ml of SCS inhibited the growth of HL-60 cells by 50, 85, or 93%, respectively. The inhibitory effect of SCS on the growth of HL-60 cells also depended on the time of incubation. Incubation of HL-60 cells with SCS at 50 μg/ml at 37℃ for 1, 2, 3 or 4 days inhibited the growth of HL-60 cells by 50, 73, 84 or 89%, respectively. Cytostatic concentration was found to be in the range of about 60~100 μg/ml. Concentration of SCS greater than 200 μg/ml were cytocidal (Fig 1).

The inhibitory effects of SCS on the synthesis of DNA, RNA, and protein in 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, respectively, in HL-60 cells. The presence of 6, 12.5, 25, 50, 100, 200, or 400 μg/ml of SCS in cultured HL-60 cells incubated for 120 min inhibited the incorporation of [^{3}H]thymidine into

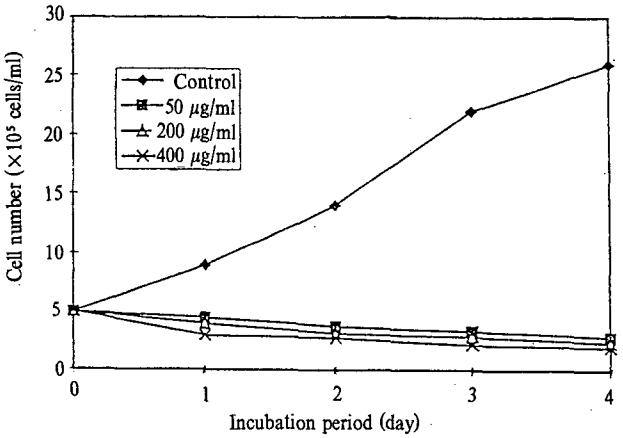

Fig. 1. Inhibitory effect of various concentrations of SCS on the growth of HL-60 cells. HL-60 cells (5×10^5 cells/ml), suspended in RPMI medium supplemented with 10% calf serum and 1% penicillin and streptomycin were incubated with various concentration of SCS and control (vehicle only) at 37℃ for 4 days. Every 24h, the number of HL-60 cells was counted under a microscope.

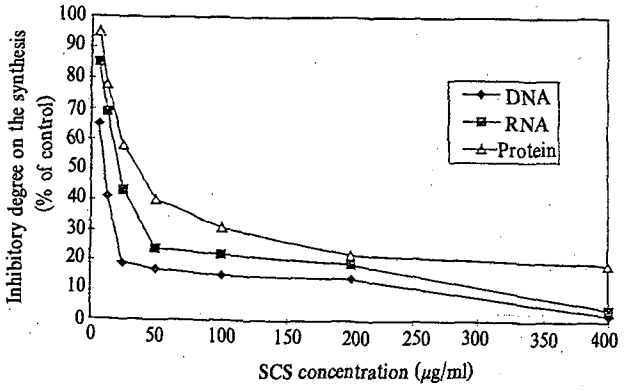

Fig. 2. Inhibitory effect of various concentration of SCS on the synthesis of DNA, RNA, and protein in HL-60 cells, suspended in RPMI medium without calf serum at concentration of 5×10^5 cells/ml. [^{3}H]thymidine (50 μCi/μmol : 3 μl), [^{3}H]uridine(55 μCi/μmol : 5 μl), or [^{3}H]leucine (200 μCi /μmol : 10 μl) were added. The cells were incubated at 37℃ for 120 min, and the reactions were terminated by addition 2 ml of cold PBS. The rate of DNA, RNA, and protein synthesis were determined as described in the text. The percentage of incorporation shown is expressed relative to cell cultures to which no inhibitor was added.

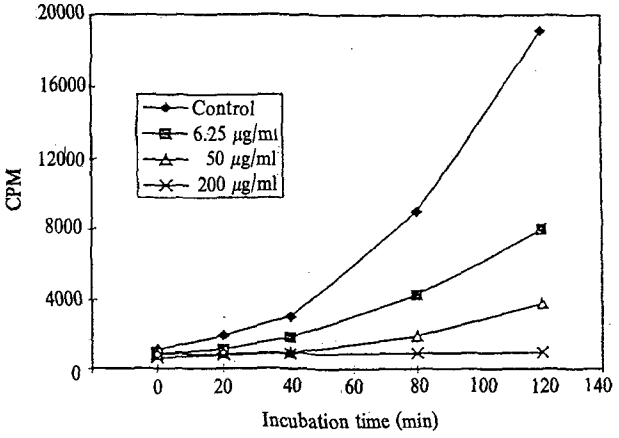

Fig. 3. Effect of SCS on the synthesis of DNA in HL-60 cells at various times following exposure to inhibitor. HL-60 cells (5×10^5 cells/ml), suspended in RPMI medium, were divided into 4 portions. SCS was added to each of 3 portions of cultures at a final concentrations of 6.25, 50 or 200 μg/ml. DMSO was added to one of the 4 portions of cultures and served as positive control. [^{3}H] thymidine (50 μCi / μmol : 3 μl) was added at to each protion. The cultures were further incubated at 37℃, and at the indicated times 1 ml of sample was pipetted into 2 ml of cold PBS and the rate of DNA synthesis were determined as described in the text.

DNA by 35, 59, 81, 83, 85, 86 or 98%, respectively (Fig. 2), the incorporation of [^{3}H]uridine into RNA by 6, 31, 57, 76, 78, 81 or 96%, respectively, and the incorporation of [^{3}H]leucine into protein by 5, 15, 22, 60, 69, 78 or 81%, respectively. The results indicate that SCS is a potent inhibitor of DNA synthesis, but is somewhat less effective on RNA and protein synthesis in HL-60 cells.

DNA synthesis was inhibited rapidly by adding SCS (Fig 3). Especially, when 200 μg/ml SCS was added to HL-60 cells, DNA synthesis was inhibited completely within 10 min. Also, inhibitory effect of SCS on the DNA synthesis was dependent on the incubation time (Fig 3). For incubation of HL-60 cells (5×10^5 cells/ml) with SCS at 6.25, 50 or 200 μg/ml at 37℃ for 10 min, DNA synthesis was inhibited by 20.4, 38.5 or 55.2%, respectively, and incubation for 120 min.

DNA synthesis was inhibited by 58.1, 80.1 or 95.2%, respectively.

The inhibitory effect of SCS on DNA synthesis was ir-

Table 1. Irreversible inhibitory effect of the SCS on the DNA in HL-60 cells.

SCS concentration (μg/ml)	[^{3}H]Thymidine incorporation into TCA insoluble materials		% of control	
	A (cpm)	B (cpm)	A (%)	B (%)
0	14,077	15,555	100	100
12.5	7,320	7,778	52	50
50	2,112	933	15	6
200	563	466	4	3

A, HL-60 cells (5×10^5 cells/ml) were preincubated with 2 μl DMSO, or SCS in DMSO at 37℃ for 120 min, then cells were washed with PBS 3 times, each time with 2 ml of PBS to remove the SCS. Cells were resuspended in fresh RPMI medium and 2 μl DMSO and [^{3}H]thymidine was added, incubated at 37℃ for 120 min. The radioactivity incorporation into TCA insoluble materials was determined as described in the text. B, HL-60 cells (5×10^5 cells/ml) were incubated with 2 μl DMSO at 37℃ for 120 min, then cells were washed with PBS 3 times, each time with 2 ml of PBS to remove the DMSO, cells were resuspended in fresh medium and 2 μl DMSO or SCS in DMSO and [^{3}H]thymidine was added, incubated at 37℃ for 120 min. The radioactivity incorporation into TCA insoluble materials was determined as described in the text.

reversible (Table 1) HL-60 cells were preincubated with SCS at 12.5, 50, and 200 μg/ml for 120 min at 37℃ and then washed with PBS three times to remove the SCS. The cells were re-suspended in RPMI medium and [^{3}H] thymidine was added and the DNA synthesis was determined. It is surmised that the inhibitory effect on DNA synthesis was dependent upon preincubation of HL-60 cells with various concentrations of SCS. It is not still clear whether SCS can be removed by washing with PBS or whether SCS may bind tightly to cells. Further studies are needed to help explain the irreversible inhibitory effect of preincubated SCS, even when washed by PBS, on the DNA synthesis of HL-60 cells.

The present investigation showed that SCS inhibited growth of HL-60 cells and the synthesis of macromolecules in HL-60 cells. The inhibitory effect of SCS on DNA synthesis was irreversible, indicating that some active components in the SCS have antitumor activity. Apparently, sesaminol glycosides in sesame seed may play the role of inhibiting the growth of HL-60 cells and the synthesis of macromolecules. Additional studies including (a) isolation, purification, and identification of the sesaminols, (b) determination of the inhibitory effects of pure sesaminol on the growth of HL-60 cells and on the synthesis of macromolecules, and (c) determination of the possible inhibitory mechanism of sesaminols need to be undertaken.

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