Effects of Sesamin and Related Lignan Compounds on Fatty Acid Metabolism in Hep G2 Cell

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Synopsis

The effects of sesamin and episesamin, lignan compounds which are present in sesame seeds, on fatty acid biosynthesis, and on the docosahexaenoic acid (DHA) biosynthetic pathway in particular, were investigated in the human hepatoma cell line, Hep G2.

Eicosapentaenoic acid (EPA), sesamin and episesamin were incorporated into the cell depending upon their concentrations, and EPA was metabolised into docosapentaenoic acid (DPA) and DHA. However, no significant changes in fatty acid composition were observed. This result indicates that sesamin and episesamin do not inhibit the biosynthesis of DHA from EPA.

Introduction

Sesame has long been used extensively as a traditional health food in the Orient for various purpose. The effective components, especially lignan compounds, in the health food have recently been investigated. Sesame oil contains relatively large quantities of sesamin and related lignan compounds, and they have an antioxidant action in vitro [1-3].

Akimoto *et al.* reported that sesamin and related lignan compounds present in sesame seeds or their oil are specific inhibitors of Δ5-desaturase, which catalyzes the desaturation of dihomo-γ-linolenic acid to arachidonic acid in PUFA biosynthesis in both microorganisms and animals [4].

In this report, we investigated the effect of sesamin and episesamin on docosahexaenoic acid (DHA) biosynthesis from eicosapentaenoic acid (EPA) in human hepatoma cell line, Hep G2.

Materials and Methods

Chemicals. 8, 11, 14, 17-Eicosatetraenoic acid (20:4 n-3) was purified from mycelial lipid of *Mortierella alpina* M226-9 grown with linseed oil [5]. 7, 10, 13, 16, 19-Docosapentaenoic acid (22:5 n-3) was purified from cellular lipid of *Schizochytrium* sp. SR21 [6]. All other authentic fatty acids were obtained from Sigma (St. Louis, MO). DME medium was from NISSUI Pharmaceutical Co. (Tokyo, Japan) and fetal bovine serum (FBS) from GIBCO BRL (Rockville, USA). All other reagents were of analytical grade.

Cell culture. Hep G2 cells derived from human hepatoma were from American Type Culture Collection (Bethesda, USA) and subcultured at the ratio of 1:4. Confluent monolayer of Hep G2 cells was treated with 0.25% trypsin. Isolated cells were seeded into 60 mm dishes with 4 ml of DME containing 10% FBS at a concentration of 1.5 x 10^6 cells/dish. The Hep G2 cells were incubated at 37℃ in atomosphare of 5% CO_2-95% air. After 3 days of incubation, cells reached subconfluent and were used in the experiment.

Incorporation and metabolism of eicosapentaenoic acid (EPA), sesamin and episesamin in Hep G2 cell. The solution of EPA methyl ester, sesamin and episesamin were added to the culture medium as ethanol solution. The cells were incubated for 24 or 48 h at 37℃ in the 5% CO_2-95% air. After incubation, the medium was removed and cells were harvested by a rubber policeman.

Lipid analysis. Cellular lipids were extracted by

the method of Folch et al.[7] An internal standard of fatty acid, 20 μl of margaric acid (17:0, 400 mM solution), was added to the cellular lipid extracts. The resultant lipid extract was evaporated to dryness under reduced pressure at 35℃ and then used for experimint.

For analysis of the fatty acid composition, each extarct was transmethylated with 10% methanolic HCl at 80℃ for 2h. The resultant fatty acid methyl esters were extracted with 4 ml of *n*-hexane which was washed out with 2% $KHCO_3$, concentrated in a centrifugal evaporator at 70℃, and then dissolved in acetonitrile for analysis by capillary gas-liquid chromatography (GLC). The analytical conditions were as follows: apparatus, GC-14B (Shimadzu, Japan) equipped with a flame ionization detector (FID) with a split injector; column, ULBON HR-SS-10 (0.25 mm x 50m; Shinwa Chemical Industries, Japan); column temperature, 200℃; injection port temperature, 250℃; carrier gas, He (inlet pressure, 200 kPa); make up gas, N_2 (60 ml/min); air and H_2, 60 kPa; and split ratio, 25:1.

For analysis of the cellular level of lignan compounds in Hep G2 cell, each extarct was dissolved in chloroform for analysis by high performance liquid chromatography (HPLC). The analytical conditions were as follows: HPLC: pump, LC-5A (Shimadzu); column, Inertsil ODS-2 (4.6 x 250 mm; GL Science, Japan); detector, SPD-2A (Shimadzu); wavelength, 290 nm; mobile phase, methanol/water (80:20, vol/vol); flow rate, 1 mL/min; and column temperature, 30℃.

Other methods. Protein content of cells was determined by the method of Lowry et al.[8] and each fatty acid concentration in the cells was expressed as nmol acid/mg protein.

Results and Discussion

Effect of EPA concentration on growth of Hep G2 cell. Hep G2 cells were incubated with various concentrations of EPA at 37℃ for 24 or 48 h. As shown in Fig. 1, the cell growth was not inhibited at EPA concentration less than 100 μM, but further increases in the EPA concentration caused drastical decreases in the cell growth.

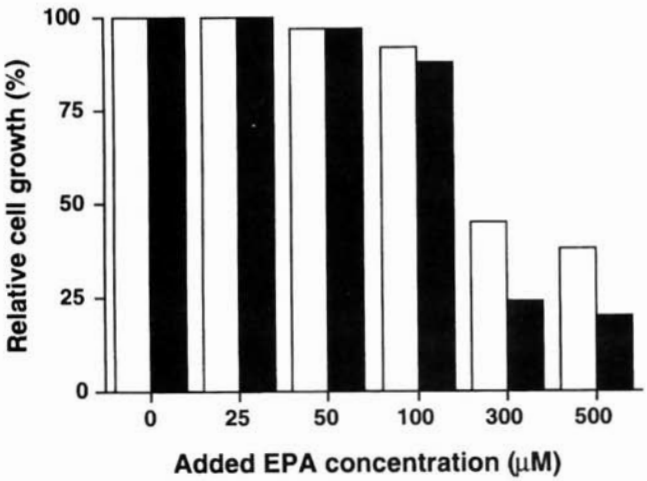

Fig. 1. Effects of EPA concentration on the growth of Hep G2 cells. The cells were incubated for 24 h (open bars) or 48 h (meshed bars) in a DME medium containing the indicated concentrations of EPA. All values are means of four determinations, and the standard deviation is less than 7%.

Incorporation and metabolism of EPA in Hep G2 cell. There was no significant difference between fatty acid levels of incorporation at 48 h and 24 h (Table 1). EPA was incorporated into cells depending upon the concentration of EPA concentration ($<40\mu$M) and metabolized to DHA. Further increases in the EPA concentration ($>40\mu$M) resulted in increases in the EPA and DPA levels in the cell, but the cellular level of DHA was not increased. This result indicates that EPA was easily metabolized to docosapentaenoic acid (DPA), but cellular activity of conversion of DPA to DHA was weak.

Table 1. Effects of EPA concentration and incubation period on the incorporation and metabolism of n-3 polyunsaturated fatty acids in Hep G2

1) 24 hour

	Added EPA (μM)			
fatty acids	0	20	30	40
16:0	143.3± 9.9	153.4±20.9	163.4±16.3	154.3±11.9
18:0	33.9± 1.1	36.6± 3.9	38.5± 4.3	35.6± 4.1
18:1	125.7± 3.4	108.2±10.2	106.5± 1.3	99.3±11.8
18:2	8.7± 3.1	8.3± 2.9	7.3± 1.1	6.0± 0.9
18:3 n-6	nd	nd	nd	nd
18:3 n-3	1.0± 0.3	1.3± 0.1	1.5± 1.3	0.8± 0.2
18:4 n-3	5.9± 0.5	5.7± 1.3	4.9± 0.1	4.3± 0.6
20:3 n-6	2.7± 0.2	2.6± 0.1	2.8± 0.2	3.0± 1.3
20:4 n-6	13.4± 0.5	13.0± 0.8	13.6± 0.9	11.9± 1.5
20:4 n-3	nd	nd	nd	nd
20:5 n-3 (EPA)	1.0± 0.1	17.0± 1.2	24.7± 0.8	27.9± 2.7
22:5 n-3 (DPA)	2.3± 0.1	7.2± 1.3	11.5± 1.7	15.0± 2.4
22:6 n-3 (DHA)	7.5± 0.5	12.5± 2.7	14.6± 2.4	14.0± 2.4

(nmol/mg protein)

2) 48 hour

	Added EPA (μM)			
fatty acids	0	20	30	40
16:0	134.0±22.7	145.9±29.7	145.6±17.9	155.4±15.6
18:0	36.5± 5.9	41.8± 8.8	42.2± 6.4	45.8± 7.5
18:1	130.5±22.0	122.3±28.1	119.6±19.3	114.1±19.7
18:2	7.6± 1.6	7.9± 1.4	9.6± 2.4	9.5± 3.0
18:3 n-6	nd	nd	nd	nd
18:3 n-3	1.2± 0.0	1.5± 0.3	1.5± 0.1	1.5± 0.6
18:4 n-3	5.7± 1.2	5.4± 1.7	4.9± 0.8	5.2± 0.1
20:3 n-6	3.0± 0.4	2.8± 0.8	2.9± 0.3	3.0± 0.1
20:4 n-6	14.7± 2.2	14.9± 2.8	14.2± 1.7	14.8± 0.8
20:4 n-3	nd	nd	nd	nd
20:5 n-3 (EPA)	1.2± 0.0	15.7± 1.6	20.8± 1.3	28.0± 1.0
22:5 n-3 (DPA)	2.3± 0.2	6.9± 1.2	10.1± 1.1	14.9± 0.9
22:6 n-3 (DHA)	8.1± 1.1	15.1± 4.3	16.7± 3.7	18.7± 3.1

Values indicate mean±S.D. (n=3). (nmol/mg protein)

Abbreviation used: nd, not detected; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

Effects of lignan compounds on PUFA metabolism in Hep G2 cell. The cellular fatty acid level in the presence of sesamin and episesamin without exogenous EPA supplement was showed in Table 2. About 40 % of sesamin and episesamin added to medium were found in the cells depending upon the concentration added (less than 20 μg/ml), and no inhibitory effects on the cell growth was observed under the condition (data not shown). The addition of sesamin and episesamin caused a slight increase in DGLA/AA ratio and DPA/DHA ratio, but no significant changes in the fatty acid levels were ob-

served. The fatty acid compositions of the cell without exogenous fatty acid supplement were similar to those of serum which was added to the culture medium, and the fatty acid levels in the cells were so low that fatty acid metabolism in the cell was unclear.

Table 2. Effects of sesamin (a) and episesamin (b) on the metabolism of n-6 and n-3 polyunsaturated fatty acids by Hep G2 cell

(a)

	concentrations of sesamin added (μg/ml)			
fatty acids	0	5	10	20
20:3 n-6 (DGLA)	2.5± 0.4	2.6± 0.4	2.9± 0.5	2.8± 0.4
20:4 n-6 (AA)	14.4± 0.8	12.6± 2.0	13.6± 2.4	13.4± 2.5
20:4 n-3	0.7± 0.1	0.9± 0.1	1.0± 0.2	1.0± 0.2
20:5 n-3 (EPA)	1.1± 0.3	1.1± 0.2	1.2± 0.2	1.1± 0.2
22:5 n-3 (DPA)	2.4± 0.5	2.1± 0.3	2.2± 0.4	2.2± 0.2
22:6 n-3 (DHA)	7.1± 0.7	6.3± 1.1	6.6± 1.2	6.4± 1.0
20:3 n-6/ 20:4 n-6	0.17	0.21	0.21	0.21
22:5 n-3/ 22:6 n-3	0.34	0.33	0.33	0.34

(nmol/mg protein)

(b)

	concentrations of episesamin added (μg/ml)			
fatty acids	0	5	10	20
20:3 n-6 (DGLA)	2.5± 0.4	3.3± 0.8	2.7± 0.3	2.9± 0.4
20:4 n-6 (AA)	14.4± 0.8	15.5± 1.9	16.6± 4.7	12.8± 2.0
20:4 n-3	0.7± 0.1	0.9± 0.2	0.8± 0.2	0.9± 0.2
20:5 n-3 (EPA)	1.1± 0.3	1.3± 0.2	0.9± 0.3	1.1± 0.4
22:5 n-3 (DPA)	2.4± 0.5	2.5± 0.5	2.5± 0.7	2.5± 0.7
22:6 n-3 (DHA)	7.1± 0.7	6.3± 1.8	6.3± 1.3	6.2± 1.8
20:3 n-6/ 20:4 n-6	0.17	0.20	0.21	0.23
22:5 n-3/ 22:6 n-3	0.34	0.40	0.39	0.40

Values indicate means±S.D. (n=4) (nmol/mg protein)

Abbreviation used: DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid. For other abbreviations, see legend to Table 1.

The cellular levels of EPA, DPA and DHA and DPA/DHA ratio in the presence of sesamin and episesamin with exogenous EPA (30 μM) were showed in Table 3. The cellular levels of EPA, DPA and DHA were slightly increased by addition of both sesamin and episesamin, but were independent of the concentrations of sesamin and episesamin added with no signifcant difference.

In the previous report, sesamin exhibited no inhibitory effects on Δ6 desaturases, which catalyzes the desaturation of linoleic acid (18:2n-6) to γ-linolenic acid (18:3n-6) in microorganism and animal [4]. On the other hand, the recent report demonstrated that the pathway of DHA synthesis in

animal cells involves the microsomal chain elongation of DPA (22:5n-3) to 24:5n-3, followed by its desaturation to 24:6n-3. This microsomal product is then metabolized to DHA (22:6n-3) via peroxisomal β-oxaidation. In this pathway, it is thought that desaturation of 24:5n-3 to 24:6n-3 was catalyzed by Δ6-desaturase[9]. The result in the present study well agreed with these facts. But the effect of sesamin and episesamin on DHA biosynthesis in Hep G2 was not completely clear because of a low activity in the fatty acid metabolism in Hep G2 cells. It is necessary that additional studies with radio isotope labeled compounds are required to clarify these problems.

Table 3. Effects of sesamin (a) and episesamin (b) on DHA formation from EPA

(a)

	concentrations of sesamin added (μg/ml)			
fatty acids	0	5	10	20
20:5 n-3 (EPA)	19.5± 0.8	24.8± 0.9	25.7± 1.2	24.1± 0.8
22:5 n-3 (DPA)	8.2± 1.7	9.5± 1.2	10.1± 0.9	9.4± 1.3
22:6 n-3 (DHA)	12.3± 2.4	14.2± 1.8	14.8± 1.2	13.5± 1.0
22:5 n-3/ 22:6 n-3	0.67	0.67	0.68	0.69

(nmol/mg protein)

(b)

	concentrations of episesamin added (μg/ml)			
fatty acids	0	5	10	20
20:5 n-3 (EPA)	19.5± 0.8	23.8± 1.0	25.9± 1.5	25.4± 1.2
22:5 n-3 (DPA)	8.2± 1.7	9.4± 0.5	11.2± 0.7	11.3± 0.9
22:6 n-3 (DHA)	12.3± 2.4	13.8± 1.1	15.8± 1.3	15.6± 1.8
22:5 n-3/ 22:6 n-3	0.67	0.68	0.71	0.72

Values indicate means±S.D. (n=4) (nmol/mg protein)

Abbreviation used: see legend to Table 1.

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ヒト肝癌由来培養細胞株 Hep G2 の高度不飽和脂肪酸生合成系へのゴマリグナン類の影響

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要　　約

ヒト肝癌由来培養細胞株 Hep G2 は，培地に添加したエイコサペンタエン酸（EPA）を，濃度依存的に細胞内に取り込むことが確認された。また，微弱ではあるが低濃度（添加量40 μM以下）においては濃度依存的にドコサヘキサエン酸（DHA）へと変換する事が確認され，Hep G2 細胞には EPA から DHA を生合成する経路が存在することが確認された。

また，胡麻に含まれるリグナン類であるセサミンおよびその鏡像異性体であるエピセサミンは添加濃度に依存して細胞内への取り込まれることが確認されたが，添加による脂肪酸組成の顕著な変化は認められず，Hep G2 細胞における EPA からの DHA の生成を阻害するという結果は得られなかった。