Research Article

Tissue distribution and elimination of sesaminol triglucoside and its metabolites in rat

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Sesame exhibits many beneficial physiological effects, which are mostly related to its lignan compounds, such as sesaminol glucosides. This investigation studies the distribution and elimination of sesaminol triglucoside from sesame in Sprague Dawley (SD) rats. In order to investigate the distribution of sesaminol triglucoside (p.o. 500 mg/kg) in SD rats, the changes in concentration of sesaminol triglucoside and its metabolites were determined in tissues and plasma within 24 h period after tube-feeding to SD rats. Results showed that sesaminol triglucoside may be deglycosylated to form sesaminol first by intestinal microflora and then incorporated *via* lymphatic absorption into the cardiovascular system, transported to other tissues. The concentrations of sesaminol triglucoside and its metabolites in rectum, caecum, colon, and small intestines are higher than those in liver, lung, kidney, and heart. Its concentration in brain is low but detectable. Glucuronidation and sulfation was the main metabolic pathway for sesaminol in urine, and fecal elimination was a major route of elimination. From LC/MS/MS analysis of rat organs, sesaminol triglucoside can be converted to mammalian lignans, enterodiol (END), and enterolactone (ENL), by rat intestinal microflora. In the plasma, concentrations of END and ENL were 5.9 ± 0.2 and 5.5 ± 0.2 μmol/mL, respectively.

Keywords: Enterodiol / Enterolactone / Sesaminol / Sesaminol conjugated metabolites / Sesaminol triglucoside

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1 Introduction

Sesame has long been categorized as one of the traditional health foods in Japan, China, and other East Asian countries. It has preventive effects against various disordered conditions including hypertension, hypercholesterolemia, or atherosclerosis [1–3]. Sesame seeds contain linoleic acid-rich oil and significant quantities of lignans that consist of sesamin, sesaminol, sesamolinol, and sesamolin. Several precursors to the lignans including sesaminol mono-, di-, or tri-glucosides have also been isolated from the sesame [4]. Di- or tri-glucosides of sesaminol have been isolated from water–alcohol extract of defatted meal of sesame seeds [5]. It was reported that the deglucosidation of the sesame lignan glucosides could not be done with β-glucosidase alone, but it could be accomplished by the combination of β-glucosidase and cellulase [6].

Sesaminol glucosides are weak active oxygen scavengers in *in vitro* tests but are expected to act as potential antioxidants through deglucosidation by the action of β-glucosidase or intestinal microbes [7]. Production of demethylenated catechol type metabolites was also observed on sesamin and sesaminol triglucoside by culturing with some *Aspergillus fungi*, and these products, especially sesaminol-6 catechol, showed strong radical scavenging activity in the DPPH test [8]. However, as described previously, a considerable amount of water-soluble sesaminol glucosides found in the defatted sesame meal may act as radical scavengers [9]. Moreover, they are assumed to play an important role as potential antioxidants *in vivo* through deglucosidation by intestinal microorganisms. In Alzheimer's disease experiment on the protective effect in Aβ-induced cell death in cultured rat pheochromocytoma (PC12) cells, sesaminol glucosides completely suppressed Aβ-induced generation

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Abbreviations: AUC, area under the concentration–time curve; **END,** enterodiol; **ENL,** enterolactone; **Hb,** hemoglobin; **SRM,** selected reaction monitoring

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of active oxygen species, the formation of 8OdG from DNA and the elevation of calcium level concomitant with prevention of cell death by the modulation of the expression of proapoptotic and antiapoptotic genes [10]. Sesaminol glucosides also have an anti-inflammatory effect through inhibition of NF-κB, and may be a useful agent for prevention of inflammatory disease like Alzheimer's disease. Its presence at the cellular level could compensate the oxidative vulnerability of neuronal cells in aging or neurodegenerative diseases [11].

There were no comprehensive and in-depth investigations on the pharmacokinetics, absorption, and distribution of sesaminol triglucoside from sesame *in vivo*. This study aimed at investigating the time-dependent appearance and disappearance of sesaminol triglucoside in circulation, and to study the distribution of sesaminol triglucoside and its main metabolites among various organs including liver, lung, heart, kidney, brain, plasma, and intestines. In addition, enterolignan type metabolites of sesaminol triglucoside present in organs were measured by LC/MS/MS.

2 Materials and methods

2.1 Chemicals

Hesperetin, sulfatase (type H-1, from *Helix pomatia*, containing of sulfatase and β-glucuronidase) and enterodiol (END) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade. Liquid chromatographic grade solvents and reagents were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA). Triply deionized water (Millipore, Bedford, MA, USA) was used for all preparations.

2.2 Extraction and isolation of sesaminol triglucoside and sesaminol

For the isolation of sesaminol triglucoside, sesame seeds were defatted with *n*-hexane and extracted with 80% MeOH. The 80% MeOH extract was charged into an Amberlite XAD-2 column and eluted with H_2O, 20% MeOH, 40% MeOH, and 60% MeOH. The 60% MeOH fraction was then purified by preparative HPLC under the following condition: column Cosmosil ODS (250×20 mm^2 i.d.), solvent MeOH, flow rate 4 mL/min [4]. For the isolation of sesaminol, the 60% MeOH extract (1 g) was incubated at 50°C for 48 h with β-glucosidase (0.6 g) and cellulase (0.7 g) in 50 mM acetate buffer (pH 4.5). The reaction mixture was extracted with EtOAc. The EtOAc extract was fractionated into sesaminol using preparative HPLC under the following conditions: column Cosmosil ODS (250×20 mm^2 i.d.), solvent MeOH, flow rate 4 mL/min. Sesaminol triglucoside and sesaminol prepared had purity of 99 and 98%, respectively.

2.3 Animals and diets

The experimental protocol was approved by the National Laboratory Animal Center (Taipei, Taiwan). Inbred male Sprague Dawley rats (body weight 275 ± 25 g, mean ± SD) were housed in pairs in cages in a room with controlled temperature (20–22°C), controlled relative humidity (50–70%), and a 12 h light/dark cycle (lights on at 0700 h). The rat diet was AIN 93M diet (Purina Mills, St. Louis, MO, USA). Rats consumed their food *ad libitum* and had unlimited access to water; their weight and food consumption were recorded weekly.

2.4 Distribution experiment

Five groups (1, 3, 6, 9, and 24 h) of animals ($n = 6$) were administered *via* gastric gavage, 500 mg/kg body weight sesaminol triglucoside dissolved in normal saline for four days to three daily doses (1500 mg/kg/day), for this dosing schedule as follow.

After consuming the sesaminol triglucoside diet for 4 days (total dose 5000 mg/kg), rats were anesthetized in the morning at fourth day (500 mg/kg), without overnight fasting, using CO_2 as a carrier. The liver, lung, brain, plasma, small intestines, caecum, colon, and rectum were collected at 1, 3, 6, 9, and 24 h after the administration. The intestines of rats were rinsed free of its contents, which would normally be a standard procedure for estimating the concentrations of samples in intestine tissue. Rats were fully bled *via* the abdominal aorta. Blood (8–12 mL) was collected in heparin tubes and plasma was subsequently prepared in centrifuge tubes by centrifuging for 20 min at $1000 \times g$ and 4°C. After blood collection, the tissues were dissected, weighed, and immediately frozen in liquid nitrogen.

2.5 Extraction

All tissues were lyophilized before further processing. Rat tissues were pooled *per* intake group (6 rats/group) and ground and homogenized (Polytron). Liver, kidney, lung, brain, small intestines, caecum, colon, and rectum required additional homogenization in the mill. Samples were stored in airtight containers at –20°C.

Whole rat tissues were weighed in 50 mL tubes and homogenized (Polytron) in 10 mL of 0.5 mol/L sodium acetate buffer (NaOAc, pH 5.0, with 200 mg/mL ascorbic acid)/g tissue with a vortex. The samples were deproteinized with ACN. The extract was transferred to a 25 mL tube and centrifuged for 10 min at $10\,000 \times g$ and 4°C. The supernatant was then transferred to a clean tube and the residue was extracted two more times. The solvent from the supernatant was evaporated at 50°C. Tubes were weighed before and after evaporation of extraction solvent. Subsequently, the residues were dissolved with 1 mL of 0.1 N NaOAc buffer (pH 5.0, with 200 mg/mL ascorbic acid). Each sample (100 μL) was

either hydrolyzed with 50 μL enzyme (1000 U sulfatase) in 0.1 N NaOAc buffer (pH 5.0, with 200 mg/mL ascorbic acid)/g tissue for incubator at 37°C, or not hydrolyzed but processed immediately with the addition of the same volume of NaOAc buffer without enzyme mix [12–14]. Subsequently, all samples were deproteinized with 250 μL of internal standard (hesperetin, 5 μg/mL in ACN) solution and centrifuged for 10 min at 10000 × *g* and 4°C. After centrifugation, 20 μL supernatant was injected into the HPLC system. Plasma and tissue samples were analyzed by a similar method as described previously [12].

2.6 Determination of sesaminol triglucoside distributed in tissues

The HPLC system consisted of Hitachi L7100 pumps (Hitachi, Tokyo, Japan) and a Hitachi L7420 UV–Vis detector (Hitachi). Separation was achieved by injecting 20 μL sample onto a Luna C18 column (250 × 4.6 mm^2, 5 μm, Phenomenex, Torrance, CA, USA), protected by an RP18 guard column (15.0 mm × 3.2 mm, 5 μm, Phenomenex). Columns were housed in a column heater set at 30°C. The solvents for elution were 100% methanol (solvent A) and 1% acetic acid, pH 6 (solvent B). The elution program for rat tissue at a flow rate of 1 mL/min was as follows: 0–5 min, linear gradient from 50 to 80% A; 5–10 min, linear gradient from 80 to 100% A. The elution program for rat plasma extract at a flow rate of 1 mL/min was as follows: 0–15 min, linear gradient from 40 to 70% A; 15–16 min, linear gradient from 70 to 100% A. Peaks were detected with a UV–Vis detector at 290 nm.

2.7 Excretion experiment

Rats (n = 6) were administered *via* gastric gavage, 500 mg/kg body weight sesaminol triglucoside in normal saline for 4 days. Urine and feces samples were collected at the following time periods: 0–4, 4–8, 8–12, and 12–24 h after administration of sesaminol triglucoside. Urine and feces from rats housed in metabolic cages were collected for the 24 h experiment. After consuming the sesaminol triglucoside diet for 4 days, rats were anesthetized in the morning, without overnight fasting, using CO_2 as a carrier. Ascorbic acid (200 mg/mL) was added to urine samples, which were then stored at −80°C until analysis.

2.8 Extraction of feces and urine samples

The feces were lyophilized before further processing. The feces were pooled *per* intake group and ground and homogenized (Polytron and mill). Samples were stored in airtight containers at −80°C.

Feces of rat were weighed in 50 mL tubes and then homogenized (Polytron) in 10 mL of ACN with a vortex. The extract was transferred to a 25 mL tube and centrifuged for 10 min at 10000 × *g* and 4°C. The supernatant was transferred to a clean tube and the residue was extracted two more times. The organic solvent from the supernatant was evaporated at 50°C. Tubes were weighed before and after evaporation of extraction solvent. Subsequently, the residues were dissolved with 1 mL of 0.1 N NaOAc (pH 5.0, with 200 mg/mL ascorbic acid) buffer. Each sample (100 μL) was either hydrolyzed with 50 μL enzyme [1000 U sulfatase activity in 0.1 N NaOAc buffer (pH 5.0, with 200 mg/mL ascorbic acid)]/g excreta for incubation at 37°C, or not hydrolyzed but processed immediately with the addition of the same volume of NaOAc buffer without enzyme mix [12–14].

Urinary sesaminol triglucoside and sesaminol conjugated metabolites analysis was performed using a previously described method with slight modifications in rat excreta [15]. Briefly, 2 mL of 0.1 mol/L sodium acetate buffer (pH 4.5) and 50 μL of 1000 U sulfatase activity in 0.1 mol/L sodium acetate buffer were added to 1 mL urine sample, and the mixture was incubated in a 37°C water bath overnight to hydrolyze the sesaminol conjugates.

Subsequently, all samples were deproteinized by centrifuging for 10 min at 10000 × *g* and 4°C. A 250 μL of internal standard (hesperetin, 2.0 μg/mL in ACN) solution was added to each sample before deproteinization. After centrifugation, 20 μL supernatant was subjected to HPLC analysis.

2.9 Determination of sesaminol triglucoside distributed in tissues, urine, and feces

The HPLC system consisted of Hitachi L7100 pumps (Hitachi) and a Hitachi L7420 UV–Vis detector (Hitachi) was used. Separation was achieved by injecting 20 μL sample onto a Luna C18 column (250 × 4.6 mm^2, 5 μm, Phenomenex), protected by an RP18 guard column (15.0 mm × 3.2 mm^2, 5 μm, Phenomenex). Columns were housed in a column heater set at 30°C. The solvents for elution were 100% methanol (solvent A) and 1% acetic acid, pH 6 (solvent B). The elution program of rat feces extracts at a flow rate of 1 mL/min was as follows: 0–15 min, linear gradient from 40 to 70% A; 15–16 min, linear gradient from 70 to 100% A. Peaks were detected with a UV–Vis detector at 290 nm. The elution program of rat urine extracts at a flow rate of 1 mL/min was as follows: 0–15 min, linear gradient from 30 to 40% A; 15–19 min, linear gradient from 40 to 100% A. The elution program of rat tissue at a flow rate of 1 mL/min was as follows: 0–5 min, linear gradient from 50 to 80% A; 5–10 min, linear gradient from 80 to 100% A. Peaks were detected with a UV–Vis detector at the wavelength of 290 nm. The elution program for rat plasma extract at a flow rate of 1 mL/min was as follows: 0–15 min, linear gradient from 40 to 70% A; 15–16 min, linear gradient from 70 to 100% A. Peaks were detected with a UV–Vis detector at 290 nm.

2.10 Determination and identification of sesaminol triglucoside, sesaminol conjugated metabolites and enterolignans in tissues and excreta

Identification of sesaminol triglucoside, sesaminol conjugated metabolites, and enterolignans was carried out by LC-MS-MS analysis. These analyses were performed on a Thermo HPLC system equipped with ESI IT mass spectrometer (ThermoFinnigan LXQ Advantage, San Jose, CA, USA). The separation was achieved using YMC Hydrosphere C18 column (2.0×150 mm^2 i.d.; 5 μm, YMC, Tokyo, Japan). For the operation in MS/MS mode, a mass spectrometer with an ESI was used. During the analyses, the ESI parameters were set as follows: capillary voltage, 49 V for negative mode; source voltage, 4.5 kV; source current, 100 μA; sheath gas flow rate, 35 a.u.; capillary temp, 350°C; tube lens voltage, −110 V. The collision energy of *m/z* 301 $[M-H]^-$ was adjusted to maximize the intensity of the deprotonated molecular ion (precursor) as 30% and the collision energy was also adjusted to optimize the product ion signals as 30% for END analysis. The cone energy of *m/z* 297 $[M-H]^-$ was 30% and the collision energy was 30% for enterolactone (ENL) analysis. The selected reaction monitoring (SRM) was used to monitor the transition of the molecule to the product ion for enterolignan analysis. All LC-MS/MS data were processed by the Xcalibur version 2.0 data acquisition software.

2.11 Method validation

Method validation was examined for linearity, accuracy, and precision. A standard stock solution of ENL, END, sesaminol, and sesaminol triglucoside (1 mg/mL) was prepared with methanol. To examine linearity, standard ENL, END, sesaminol, and sesaminol triglucoside solutions of 0.02–25.0 μg/mL were prepared by serial dilutions from the stock solution. The standard curve was constructed using a linear least-square regression equation derived from the peak area. The inter- and intraday variabilities of the method were validated at the seven concentrations on the same day (six replications) and six consecutive days. The precision evaluation by the RSD was calculated from the observed concentration (C_{obs}) as follows: %RSD = [SD/(C_{obs})] × 100. The accuracy (% bias) was evaluated by calculating from the nominal concentration (C_{nom}) and the mean value of the observed concentration as the following equation: bias (%) = [($C_{obs} - C_{nom}$)/C_{nom}] × 100.

2.12 Correction for residual blood

The tissues of rats had not been perfused after excision, they contained residual blood containing sesaminol triglucoside and its metabolites. Correction for residual blood was done by comparing the hemoglobin (Hb) content of tissues to the Hb content of whole blood determined with a spectrophotometric method [14, 16]. Rat whole blood was taken from a Sprague Dawley rat. The tissues, 0.1 g for pooled rat tissues, were homogenized in 14 mL Tris-HCl pH 7.4, with 50 mmol/L EDTA/g tissue and centrifuged at $10\,000 \times g$ for 10 min at 10°C. Then, 2 μL of the supernatant was used for spectrophotometric determination of the Hb content with a Hitachi (U-3210 UV–Vis Spectrophotometer). An absorbance spectrum was taken from 220 to 750 nm and all samples were analyzed in duplicate. The fraction of residual blood (f_{bl}) in each tissue was calculated by dividing the peak maximum at 540 nm of each tissue extract (Hb_{tissue}) with the peak maximum at 540 nm of a whole blood extract (Hb_{bl}) (Eq. 1)

$$F_{bl} = Hb_{tissue}/Hb_{bl} \quad (1)$$

The sesaminol triglucoside and its metabolites concentrations that were found in each tissue extract (C_{tot}) were corrected for blood contamination by subtracting the sesaminol triglucoside and its metabolites concentrations that were found in plasma samples (C_{pl}) multiplied by the fraction of residual blood in that tissue (f_{bl}) (Eq. 2)

$$C_{corr} = C_{tot} - f_{bl} \times Cl_{pl} \quad (2)$$

where C_{corr} is the corrected concentration for sesaminol triglucoside and its metabolites. Plasma concentrations of sesaminol triglucoside and its metabolites were assumed to be equal to whole-blood concentrations.

2.13 Statistical analysis

All samples were extracted in triplicate. Sesaminol triglucoside, sesaminol glucuronide, and sesaminol sulfate concentrations were expressed in nmol/g tissue or nmol/mL plasma. Tissues of six rats were pooled before analysis. Data were analyzed by *t*-test analysis of variance, and differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Distribution study

The analytical methods of sesaminol triglucoside, sesaminol, and its conjugated metabolites (sesaminol glucuronide/sulfate) in tissues were developed and validated in this study. The CV of intra- and interday assays were less than 5% in the concentration range of 0.02–25.0 μg/mL. For the analysis of sesaminol triglucoside and sesaminol, the lower limits of quantitation were 0.2 and 0.4 μg/mL and the limits of detection were 0.05 and 0.02 μg/mL, respectively. The correlation coefficient (r^2) of the standard curve was examined for linearity. The linear equations are $y = 37\,487x + 21.4$ for sesaminol ($r^2 = 0.9999$), $y = 9967.9x + 54.6$ for

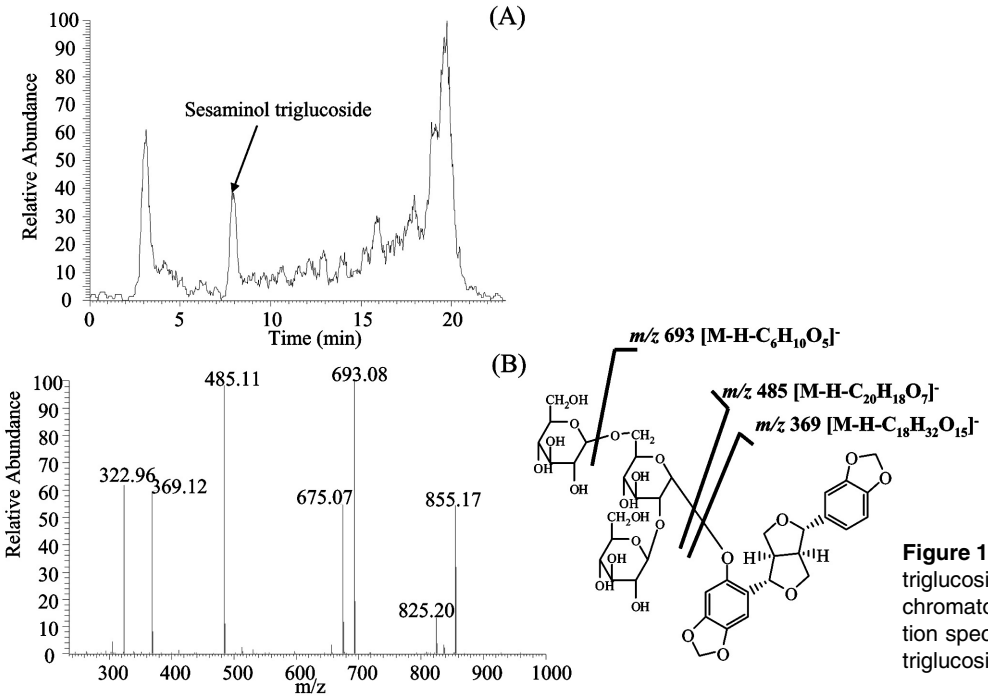

Figure 1. Mass spectra of sesaminol triglucoside in the plasma. (A) Total ion chromatogram. (B) Mass fragmentation spectrum of MS/MS for sesaminol triglucoside (*m/z* 855 $[M-H]^-$).

sesaminol triglucoside ($r^2 = 0.9997$), $y = 865\,060x + 6.6$ for ENL ($r^2 = 0.9987$), and $y = 858\,324x + 44\,332$ for END ($r^2 = 0.9972$). The assay precision, defined by the RSD, ranged from 0.1 to 4.5% for enterolignans and from 0.1 to 2.9% for sesaminol and sesaminol triglucoside. The assay accuracy, expressed as the percentage bias, varied from −2.8 to 3.7% for enterolignans and from −4.8 to 3.9% for sesaminol and sesaminol triglucoside.

For the analytical condition of sesaminol triglucoside, the full scan in negative ion mode (scan range from *m/z* 50 to 1000) was used to identify the analyte. With full-scan mass spectra for the determination of sesaminol triglucoside (precursor ion is 855 $[M-H]^-$) a tube lens offset voltage −40 eV was applied. Then, collision energies for CID adjusted to 27% was optimized to produce the main product ion at *m/z* 693 and 485, as shown in Fig. 1. Panels A and B of Fig. 1 show the MS/MS spectrum, with mass transitions of *m/z* 855 $[M-H]^- \rightarrow$ *m/z* 693, 485, and 369 for sesaminol triglucoside in collected rat plasma after sesaminol triglucoside administration (500 mg/kg, p.o.).

To investigate the distribution of enterolignans in rats, an analytical method was developed. Due to the complexity of the LC/MS/MS profile of enterolignans (Fig. 2), the more sensitive SRM MS was used for their analyses. Panels B and C of Fig. 2 show the SRM MS/MS chromatograms of collected rat urine after sesaminol triglucoside administration, with mass transitions of *m/z* 301 $[M-H]^- \rightarrow 253$ $[M-H-CO_2]^-$ and 297 $[M-H]^- \rightarrow 253$ $[M-H-CO_2]^-$ for ENL and END, respectively. Their response to the specific END transition (*m/z* values: 301/253) and ENL transition (*m/z* values: 297/253), respectively, confirmed their structures.

To investigate the distribution of sesaminol triglucoside in rats, the concentrations of sesaminol triglucoside were determined in tissues and plasma within 9 h after administration to rats. Sesaminol conjugated metabolites (sesaminol glucuronide/sulfate) were widely distributed in rat tissues, with the highest concentrations in heart and kidney and the lowest in plasma. Sesaminol triglucoside may be, at first, incorporated into the liver and then transported to the other tissues (lung, kidney, and brain) (Table 1 and Fig. 3).

In the distribution of sesaminol triglucoside and sesaminol conjugated metabolites, we administered sesaminol triglucoside to rats and determined their concentration in tissues within 24 h after administration. In the tissues (Fig. 3), the concentrations of sesaminol triglucoside and sesaminol conjugated metabolites had reached maxima at 60–360 min after administration, and were rarely found after 9 h. However, sesaminol triglucoside and sesaminol conjugated metabolites concentrations were significantly greater than sesaminol in the tissues. In the heart, sesaminol triglucoside and sesaminol conjugated metabolites were first detected at 60 min after administration comparatively early than the other tissues. In the front of intestines (small intestine), sesaminol triglucoside concentration was significantly greater than sesaminol and its conjugated metabolites. The highest sesaminol and sesaminol conjugated metabolites concentrations were found in rectum and caecum (Fig. 4). Hence, sesaminol triglucoside could be hydrolyzed by β-glucosidases, thus releasing the aglycone form by rat fecal microbiota.

Table 1 summarizes the time of observed maximum concentration (T_{max}), maximum concentration (C_{max}), elimina-

Table 1. Pharmacokinetic parameters of sesaminol triglucoside, free form, and conjugated sesaminol in rat tissues and intestines

AUC (min mmol/mL or min mmol/g)	C_{max} (nmol/mL)				T_{max} (min)				$t_{1/2}$ (min)			
	Sesaminol triglucoside	Sesaminol	Sesaminol glucuronide/sulfate	Sesaminol triglucoside	Sesaminol	Sesaminol glucuronide/sulfate	Sesaminol triglucoside	Sesaminol	Sesaminol glucuronide/sulfate	Sesaminol triglucoside	Sesaminol	Sesaminol glucuronide/sulfate
Liver	13.7 ± 6.1	5.81 ± 0.8	29.4 ± 5.8	39.3 ± 14.4[a)]	4.5 ± 1.8	26.4 ± 10.9	310.0 ± 122. 5	324.0 ± 80.5	324.0 ± 80.5	217.9 ± 73.6[b)]	548.7 ± 394.7	461.2 ± 199.1
Lung	16.2 ± 6.0[a)]	8.6 ± 4.4	37.3 ± 24.6	49.2 ± 23.3[a)]	7.8 ± 9.5	31.6 ± 22.5	60.0 ± 0.0[a,b)]	420.0 ± 207.8	270.0 ± 103.9	218.6 ± 97.4[a)]	653.8 ± 116.7	351.6 ± 67.0
Kidney	46.4 ± 9.9[a,b)]	5.4 ± 2.3	23.6 ± 8.5	83.0 ± 25.8[a,b)]	2.2 ± 0.9	36.1 ± 12.5	156.0 ± 53.7	216.0 ± 222.9	180.0 ± 0.0	5077.1 ± 2121.0[b)]	1334.4 ± 672.7	287.8 ± 72.7
Heart	36.7 ± 17.4[a)]	18.0 ± 10.8	71.0 ± 17.0	14.8 ± 8.2[a)]	11.08 ± 31.0	163.6 ± 58.8	60.0 ± 0.0	180.0 ± 147.0	60.0 ± 0.0	150.2 ± 10.0[a)]	757.9 ± 591.2	272.6 ± 89.4
Plasma	25.8 ± 14.9[b)]	3.8 ± 1.8	4.3 ± 2.2	20.9 ± 12.0[a,b)]	2.6 ± 0.9	3.4 ± 1.7	180.0 ± 207.8[b)]	135.0 ± 150.0	468.0 ± 98.6	3888.9 ± 3251.6[b)]	1103.9 ± 984.1	2506.6 ± 1427.6
Brain	2.8 ± 1.2[b)]	8.0 ± 3.6	25.9 ± 20.9	6.9 ± 3.4	6.3 ± 2.0	16.1 ± 12.4	230.0 ± 149.0	396.0 ± 80.5	360.0 ± 197.2	348.4 ± 205.6[b)]	485.3 ± 51.1	828.7 ± 285.1
Rectum	1570.6 ± 1201.8	3290.1 ± 2291.1	4270.9 ± 1415.0	5280.7 ± 5137.9	3093.5 ± 1980.9	7897.7 ± 1467.8	432.0 ± 98.6	360.0 ± 0.0	360.0 ± 0.0	548.1 ± 293.9	244.4 ± 99.0	460.5 ± 310.2
Caecum	583.9 ± 440.8	1443.7 ± 1618.6	5284.2 ± 6114.0	2189.7 ± 2015.9	856.9 ± 1018.4	3388.4 ± 4155.5	192.0 ± 107.3[b,a)]	468.0 ± 98.6	432.0 ± 161.0	306.7 ± 108.4	171.9 ± 31.8	1780.1 ± 1240.5
Colon	2416.8 ± 1304.7[b)]	2660.5 ± 1167.2	1501.4 ± 713.3	8596.5 ± 2391.7[a,b)]	1904.204 ± 969.9	2963.0 ± 1536.6	270.0 ± 103.9[b)]	480.0 ± 93.0	288.0 ± 98.6	1882.7 ± 616.2[a,b)]	149.6 ± 40.0	304.2 ± 107.0
Small intestines	1235.2 ± 509.6[b)]	38. 8 ± 29.9	73.8 ± 32.7	3632.5 ± 1306.0[a,b)]	24.6 ± 17.0	69.5 ± 40.5	80.0 ± 49.0	270.0 ± 239.2	210.0 ± 199.9	265.8 ± 48.9[b)]	252.7 ± 81.1	471.0 ± 100.3

Data are expressed as mean ± SD.
a) $p < 0.05$ compared with group sesaminol.
b) $p < 0.05$ compared with group sesaminol glucuronides/sulfate.

Figure 2. HPLC and SRM chromatograms of the analyzed enterolignans in rat urine. (a) A280, (b) ENL, (c) END.

tion half-life ($t_{1/2}$), and total area under the concentration–time curve (AUC) of sesaminol triglucoside and its metabolites in tissues and plasma. An important parameter for pharmacokinetic analyses of a drug is the AUC which represents the total drug exposure integrated over time. It is the best estimate of drug delivery and an indicator of response. AUCs after the administration of sesaminol triglucoside was in the following order: kidney > heart > plasma > lung > liver > brain. It was important to note that AUC of sesaminol conjugated metabolites in liver, heart, and lung increased remarkably. In the intestines, sesaminol triglucoside metabolites concentrations were also significantly greater than other tissues (Table 1). Of all the tissues examined, C_{max} of sesaminol triglucoside and its metabolites were highest in intestines. These values (C_{max}) were considerably higher than that measured in liver, lung, kidney, brain, and heart. The heart and lung had T_{max} values of 60–180 and 60–420 min, respectively. These values were lower than in intestines. Most sesaminol triglucoside and sesaminol conjugated metabolites rapidly appeared in the heart and reached their maximum level at 60 min except sesaminol that reached their maximum at 180 min (Table 1). The $t_{1/2}$ of sesaminol triglucoside and its metabolites in tissues ranged between 150 and 3889 min. The $t_{1/2}$ values were in the following order: sesaminol triglucoside > sesaminol > sesaminol conjugated metabolites. After administration, sesaminol triglucoside was first deglycosylated to sesaminol, and then converted to END and ENL by intestinal microflora. The mammalian lignans END and ENL after 24 h were found in the intestines (caecum, colon, and rectum) higher than tissues in rat (Tables 2). In the plasma,

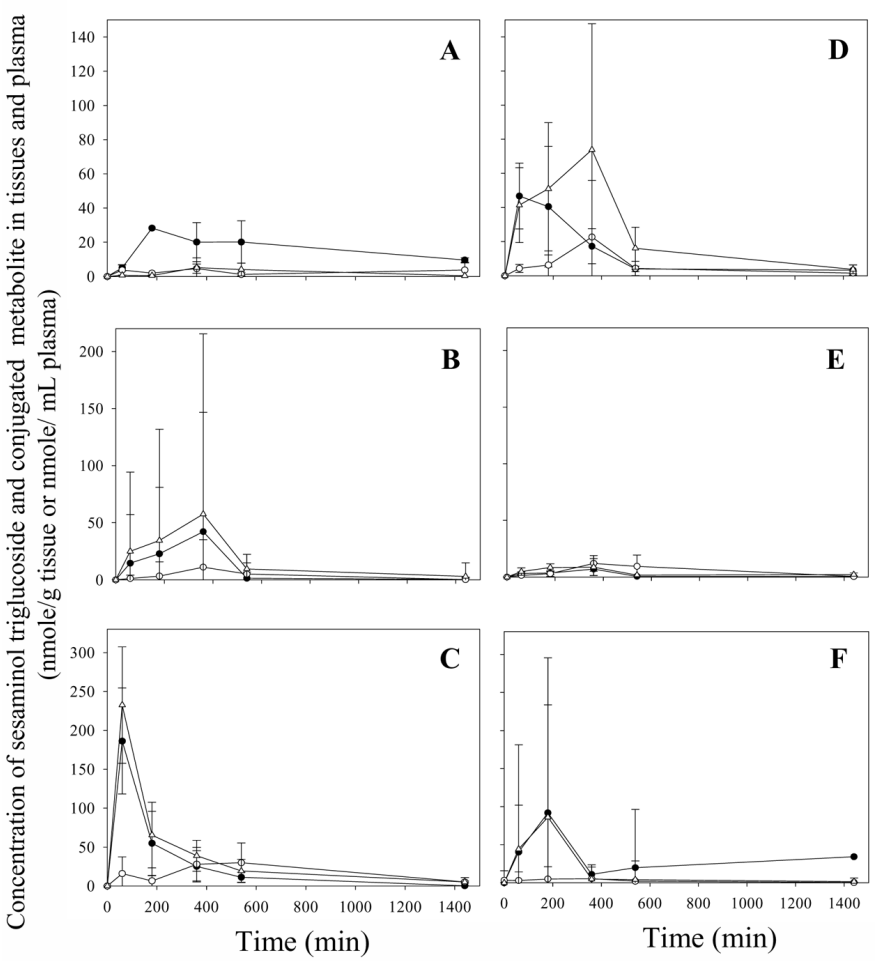

Figure 3. Time-dependent changes of sesaminol triglucoside (●), sesaminol (○), and sesaminol glucuronide/sulfate (△) concentration in rat tissues after oral administration. Sesaminol metabolites (glucuronide and sulfate) were extracted from the tissues collected at 60, 180, 360, 540, and 1440 min after their administration and analyzed by HPLC. A, plasma; B, liver; C, heart; D, lung; E, brain; F, kidney.

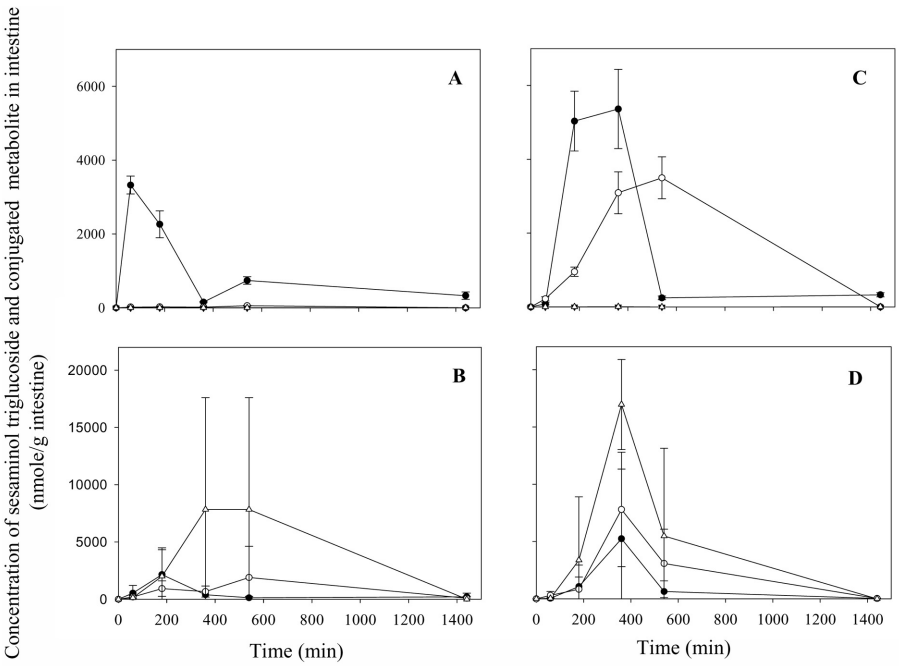

Figure 4. Time-dependent changes of sesaminol triglucoside (●), sesaminol (○), and sesaminol glucuronide/sulfate (△) concentration in rat tissues after oral administration. Sesaminol metabolites (glucuronide and sulfate) were extracted from the tissues collected at 60, 180, 360, 540, and 1440 min after their administration and analyzed by HPLC. A, small intestine; B, caecum; C, colon; D, rectum.

Table 2. Tissue and intestine concentrations of END and ENL in rat at 24 h

Concentration (μmol/mL or μmol/g)	Liver	Lung	Kidney	Heart	Plasma	Brain	Rectum	Caecum	Colon	Small intestines
END	2.5 ± 0.2	0.5 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	5.9 ± 0.2	0.4 ± 0.0	4.7 ± 0.2	7.1 ± 0.1	6.7 ± 1.0	0.6 ± 0.1
ENL	4.9 ± 0.2[a)]	0.1 ± 0.0[a)]	0.3 ± 0.0[a)]	0.1 ± 0.0[a)]	5.5 ± 0.2[a)]	0.1 ± 0.0[a)]	4.3 ± 0.0[a)]	3.0 ± 0.1[a)]	15.3 ± 2.1[a)]	0.1 ± 0.1[a)]

Data are expressed as mean ± SD.
a) $p < 0.05$ compared with group END.

Table 3. Urinary and fecal excretions of sesaminol triglucoside and its metabolites during each time interval after oral administration of sesaminol triglucoside (500 mg/kg)

Concentration (nmol/mL) (time (h))	Urine			Feces		
	Sesaminol triglucoside	Sesaminol	Sesaminol glucuronide/sulfate	Sesaminol triglucoside	Sesaminol	Sesaminol glucuronide/sulfate
0–4 (4 h)	187.3 ± 112.2	1.5 ± 1.3	282.0 ± 148.8[a)]	12.3 ± 0.3	93.5 ± 3.5	43.2 ± 0.8[b,a)]
4–8 (4 h)	146.7 ± 73.4	0.8 ± 0.2	168.6 ± 69.2[a)]	38.0 ± 1.1	166.4 ± 9.7	80.5 ± 4.9[a)]
8–12 (4 h)	207.8 ± 154.4	1.5 ± 1.2	2266.7 ± 471.6[a,b)]	3422.2 ± 434.0	11303.8 ± 772.9	10467.4 ± 1382.7[b)]
12–16 (4 h)	108.8 ± 65.7	2.7 ± 1.5	28.1 ± 15.1	423.5 ± 50.9	31827.4 ± 1885.2	37608.6 ± 4766.9
16–24 (8 h)	18.0 ± 14.5	1.9 ± 1.8	3.4 ± 3. 5	76.8 ± 7.1	1867.0 ± 119.1	862. 6 ± 53.8[b,a)]
% of dosing	0.1 ± 0.0	0.002 ± 0.00	0.5 ± 0.1	0.8 ± 0.1	8.6 ± 0.5	9.3 ± 1.2[b)]

Data are expressed as mean ± SD.
a) $p < 0.05$ compared with group sesaminol.
b) $p < 0.05$ compared with group sesaminol glucuronides/sulfate.

concentrations of END and ENL were 5.9 ± 0.2 and 5.5 ± 0.2 μmol/mL, respectively.

3.2 Elimination study

To investigate the exclusion of sesaminol triglucoside in rats, the concentrations of sesaminol triglucoside were determined in urine and feces within 24 h after administration to rats. Sesaminol triglucoside may be, at first, incorporated into the liver and then transported to the kidney, and then excreted in bile undergoes enterohepatic circulation to be eventually excreted in urine.

In urinary excretion (Table 3), the concentrations of sesaminol conjugated metabolites were significantly higher than the concentrations of sesaminol triglucoside and sesaminol. The highest concentrations of sesaminol conjugated metabolites were found at 8–12 h after administration. The elimination of sesaminol triglucoside was rapid at 0–4 h from urine. The majority of sesaminol conjugated metabolites were excreted in the urine. Thus, sesaminol triglucoside and sesaminol conjugated metabolites are primarily eliminated from the plasma *via* the kidney by active tubular secretion. Glucuronidation and sulfation were the main metabolic pathways for sesaminol triglucoside in urine. The percentage of conjugated metabolites excreted in urine in rats during 0–24 h accounted for 0.5 ± 0.1% of the total dose for sesaminol glucuronide/sulfate. Urinary amounts of sesaminol triglucoside and sesaminol conjugated metabolites increased in response to sesaminol triglucoside ingestion, reaching a peak at 8–12 h. The measured amounts of sesaminol triglucoside were 207.8 ± 154.4 nmol/mL for the free form, and 2267.7 ± 471.6 nmol/mL for sesaminol conjugated metabolites. The unchanged sesaminol was present in very low concentrations and represented only 0.002% up to 24 h after administration.

Table 4. Excreta concentration of END and ENL in rat at 24 h

Concentration (μmol/mL or μmol/g)	Urine	Feces
END	0.3 ± 0.0	14.7 ± 0.6
ENL	1.2 ± 0.1[a)]	25.3 ± 1.3[a)]

Data are expressed as mean ± SD.
a) $p < 0.05$ compared with group enterodiol.

In the feces (Table 3), the concentrations of sesaminol triglucoside, sesaminol, and its conjugated metabolites reached maxima at 8–12 and 12–16 h, respectively. The concentrations of free sesaminol and its conjugated metabolites were significantly greater than sesaminol triglucoside in the feces. The percentage of sesaminol and sesaminol conjugated metabolites excreted in feces (Table 4) in rats were highest in the first 24 h, accounting for 8.6 ± 0.5 and 9.3 ± 1.2% of the total dose for sesaminol and sesaminol conjugated metabolites, respectively. From HPLC-ESI-MS analysis of rat organs and excreta, sesaminol triglucoside

was found to convert to mammalian lignans by rat intestinal microflora. The mammalian lignans END and ENL after 24 h were found in the excreta (feces and urine) higher than tissues in rat (Table 4). In the feces, the concentrations of END and ENL were 14.7 ± 0.6 and 25.3 ± 1.3 μmol/mL, respectively.

4 Discussion

The most interesting phenomenon we observed in our current study is the direct absorption and distribution of sesaminol triglucoside to the blood in rats. The absorption of other glycosides such as anthocyanins has been under intense study in recent years [17]. Animal studies have shown that anthocyanins are absorbed mainly in their intact glycosidic form, and rapidly reach the circulatory system within 0.25 – 2 h. In rats, after a single oral administration of *Vaccinium myrtillus* anthocyanins the plasma concentrations of anthocyanins reached peak level after only 15 min and then rapidly declined within 2 h [18]. The fast appearance of intact anthocyanin in plasma (at 15 min) after oral administration of red fruit anthocyanin (cyanidin-3-glucoside) *via* stomach intubation into rats was also confirmed [19]. Besides anthocyanins, the intact form of C-glucoside of isoflavone, puerarin, has been shown to be the principal form in the blood for the period of 4 – 72 h after oral administration suggesting that puerarin is rapidly absorbed from the intestine [20]. On the other hand, bilitranslocase has been suggested to be involved in gastric anthocyanin absorption [21]. Our study also suggested that sesaminol triglucoside could enter the body by means of a carrier similar to bilitranslocase. Sesaminol triglucoside was the main component accumulated in plasma for the period of 1 h after administration. Comparison of the time course of changes of sesaminol triglucoside in plasma concentrations in our study to those reported for puerarin and cyanidin-3-glucoside, sesaminol triglucose showed the peak concentration at 1 h in blood and those for puerarin and cyanidin-3-glucoside were 1 – 2 h. These findings imply that sesaminol triglucoside is fast absorbed before it reaches the intestines, and the stomach and the jejunum are sites of sesaminol triglucoside absorption in rats.

In our tissue distribution study, we have detected sesaminol triglucoside and its metabolites in the brain tissue of rats. This is the first report that a lignan glycoside and its metabolites are able to target the brain of rats. It has been noted that puerarin was detected in the extract of brain tissue of puerarin-treated rats. It is assumed that blood – brain barrier-specific influx transporters may be involved for the brain delivery of puerarin because drug penetration into the brain is restricted under normal conditions [20]. The fact that sesaminol triglucoside and its metabolites are able to reach brain tissue may explain a previous report that sesaminol glucosides could exert protective activities against the oxidative damages responsible for numerous neurological disorders [11]. Certainly it remains to be seen which transporters may be involved in transporting sesaminol triglucoside into the brain.

The absorption phenomena of lignans have been reported, lignans and their metabolites were efficiently conjugated either with glucuronic acid or, to a lesser extent, sulfate. Conjugation takes place in the intestinal epithelium and liver by UDP-glucuronosyltransferases and sulfotransferases, and the conjugates were excreted in urine and bile. Those that are re-excreted through the bile duct were deconjugated by bacterial β-glucuronidase and can undergo enterohepatic recycling [22]. When ^{3}H-secoisolariciresinol diglycoside was orally administered to rats, Rickard and Thomson [23] observed that the tissue radioactivity was highest in the caecum, and the level in the liver was higher than in other nongastrointestinal tissues. In our study, two possible absorption pathways of sesaminol triglucoside were observed in rats. First, sesaminol triglucoside administered *via* stomach tube may diffuse directly through gastric and small intestinal walls because the sesaminol triglucoside concentrations were extremely high not only in the plasma, but also in the heart and liver. Another pathway, sesaminol triglucoside may be deglycosylated to sesaminol first by intestinal microflora, all the sesaminol conjugated metabolites that appear in plasma are the result of conversions occurring in the end of the small intestine. Sesaminol conjugated metabolites are produced in the small intestine, or pass into the portal vein and are further converted in liver and then incorporated *via* lymphatic absorption into the cardiovascular system, transported to other tissues. However, sesaminol triglucoside and its metabolites were still present in the intestines, and as enterohepatic cycling is highly probable in rat, this could partly explain the apparent slower elimination of sesaminol conjugated metabolites as compared to sesaminol triglucoside. Thus, organs of digestive area are suggested to be the metabolic pathway with enzymatic conversions (sulfation and/or glucurono-conjugation) for sesaminol.

The biotransformation of lignans has been reported by microflora. Lignans such as sesamin, secoisolariciresinol diglycoside, and matairesinol could be converted to mammalian lignans by intestinal microflora [24, 25]. The "mammalian" lignan metabolites (END and ENL) were first discovered in human urine and plasma [26]; they have shown estrogenic effects in cultured cells and can modulate the response to endogenous estrogens [27, 28]. Transit time of material through the large intestine is an important factor affecting the availability of dietary components to the host, primarily because colonic bacterial fermentation can influence circulating concentrations of compounds produced by colonic bacteria [29, 30]. Peñalvo [31] reported that sesamin could be metabolized to the mammalian lignans by fecal microbiota. Our studies suggested that lignans can be effectively absorbed, and the parent compounds and their

metabolites were transported to peripheral tissues through the blood circulation. Sesaminol triglucoside was converted to mammalian lignans *in vitro* by fecal microbiota in rats. These tissues metabolites (sesaminol conjugated metabolites) of sesaminol triglucoside could have undergone enterohepatic circulation, reached the colon, and been metabolized further by the colonic microbiota to other metabolites including END and ENL. The concentrations of ENL in both liver and colon were remarkably higher than those of END. The concentrations of enterolignans in tissues were in the order of intestines ≈ liver ≈ plasma ≈ brain ≈ lung ≈ heart ≈ kidney.

The major metabolites of sesaminol triglucoside in rats were identified as enterolignans (ENL and END). These results demonstrate that sesame seed is one of the richest dietary sources of mammalian lignan precursors and sesaminol triglucoside is one of them. The results of this study have provided, for the first time, detailed quantitative concentrations of conjugated metabolites of sesaminol triglucoside, sesaminol, and enterolignans in the plasma and tissues after oral administration of sesaminol triglucoside. The pharmacokinetic data presented should allow better and more relevant studies of the bioactivity and role of dietary lignans in disease prevention.

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