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Biochemical Pharmacology 65 (2003) 259–267

Biochemical Pharmacology

Effects of glycolipids from spinach on mammalian DNA polymerases

Chikako Murakami[a], Taeko Kumagai[b], Takahiko Hada[b], Ui Kanekazu[a], Satoshi Nakazawa[c], Shinji Kamisuki[c], Naoki Maeda[a], Xianai Xu[a], Hiromi Yoshida[a,d], Fumio Sugawara[c], Kengo Sakaguchi[c], Yoshiyuki Mizushina[a,d,*]

[a]*Laboratory of Food and Nutritional Sciences, Department of Nutritional Science, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan*
[b]*Research and Development Department, Bizen Chemical Co., Ltd., Kumayama-cho, Akaiwa-gun, Okayama 709-0716, Japan*
[c]*Department of Applied Biological Science, Frontier Research Center for Genomic Drug Discovery, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*
[d]*High Technology Research Center, Kobe-Gakuin University, Nishi-ku, Kobe, Hyogo 651-2180, Japan*

Received 23 August 2002; accepted 14 October 2002

Abstract

We purified the major glycolipids in the class of monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG) from a green vegetable, spinach (*Spinacia oleracea* L.). MGDG was an inhibitor of the growth of NUGC-3 human gastric cancer cells, but DGDG and SQDG had no such cytotoxic effect. Therefore, we studied MGDG and its monoacyglycerol-form, monogalactosyl monoacylglycerol (MGMG), in detail. MGMG with one fatty acid molecule was obtained from MGDG with two fatty acid molecules by hydrolyzing with a pancreatic lipase. MGMG was also found to prevent the cancer cell growth. MGDG was a potent inhibitor of replicative DNA polymerases such as α, δ and ε. MGMG inhibited the activities of all mammalian DNA polymerases including repair-related DNA polymerase β with IC_{50} values of 8.5–36 μg/mL, and the inhibition by MGMG was stronger than that by MGDG. Both MGDG and MGMG could halt the cell cycle at the G1 phase, and subsequently induced severe apoptosis. The relationship between the DNA polymerase inhibition and the cell growth effect by these glycolipids is discussed.

Keywords: Glycolipid; MGDG (monogalactosyl diacylglycerol); MGMG (monogalactosyl monoacylglycerol); Spinach; DNA polymerase; Enzyme inhibitor

1. Introduction

Multiple organisms are known to contain at least 12 types of DNA polymerase [1,2]. To clarify the unique roles of each of them in DNA replication and repair, we should establish an organism in which each DNA polymerase gene is knocked out, or find an inhibitor specific to each DNA polymerase. Based on this idea, we have searched for and found many new DNA polymerase inhibitors over the past 7 years, e.g. long-chain fatty acids and their derivatives [3–7], a bile acid such as lithocholic acid [8], terpenoids [9–11], flavonoids [12,13], sulfate-containing glycolipids [14–22], Vitamin A-related compounds [23] and nucleotide analogs [24,25] from natural resources. Of these, in particular, sulfo-glycolipids in the class of SQDG and sulfoquinovosyl monoacylglycerol (SQMG) from a fern [18] and an alga [19,20] were potent inhibitors and we concentrated our efforts to investigate their *in vivo* anti-cancer effects. SQDG/SQMG were not only potent inhibitors of the DNA polymerases *in vitro*, but also of human lung cancer *in vivo* [26]. SQDG/SQMG must be very promising as human cancer chemotherapy agents. The data suggest that other glycolipids could also be human cancer chemotherapy agents. In this connection, therefore, we have widely screened for other types of natural glycolipids which show such inhibition activities. Some of the glycolipids from a green vegetable, spinach (*Spinacia oleracea* L.), showed these activities. The purpose of this

* Corresponding author. Tel.: +81-78-974-1551x3232; fax: +81-78-974-5689.
E-mail address: mizushin@nutr.kobegakuin.ac.jp (Y. Mizushina).

Abbreviations: MGDG, monogalactosyl diacylglycerol; MGMG, monogalactosyl monoacylglycerol; DGDG, digalactosyl diacylglycerol; SQDG, sulfoquinovosyl diacylglycerol; pol., DNA-directed DNA polymerase (EC 2.7.7.7); dTTP, [^{3}H]-2′-deoxythymidine 5′-triphosphate.

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PII: S0006-2952(02)01483-1

report is to show that glycolipids from a green vegetable could inhibit the DNA polymerase activities and human cancer cell growth.

2. Materials and methods

2.1. Materials

Dried spinach (*S. oleracea* L.) was purchased from Kodama Foods Co. Nucleotides and chemically-synthesized DNA template-primers such as [^{3}H]-2′-deoxythymidine 5′-triphosphate (dTTP, 43 Ci/mmol) and poly(dA), oligo(dT)$_{12–18}$ were purchased from Amersham Biosciences Inc. Porcine pancreatic lipase was purchased from Sigma Chemical Co. Acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartic acid (Ac-DEVD-MAC, a caspase substrate) and acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspart-1-al (Ac-DEVD-CHO, a caspase-3 inhibitor) were purchased from Peptide Institute Inc. All other reagents were of analytical grade and were purchased from Wako Chemical Co. Ltd.

2.2. Isolation of glycolipids from spinach

The glycolipids were extracted from the spinach with chloroform/methanol (1:1, v/v). The extract was concentrated and was applied to silica gel column chromatography and eluted using increasing concentrations of ethyl acetate in chloroform. The fraction containing MGDG was obtained in chloroform/ethyl acetate (5:45, v/v). The column was then successively eluted using increasing concentrations of methanol in ethyl acetate. The fractions A and B were eluted with ethyl acetate/methanol (5:1, v/v) and ethyl acetate/methanol (2:1, v/v), respectively. The fraction A was concentrated and dissolved in a small volume of ethyl acetate. It was then applied to the same silica gel column, and was eluted using increasing concentrations of acetone in ethyl acetate. The fraction C was obtained in ethyl acetate/acetone (1:4, v/v). The fraction C was concentrated and dissolved in a small volume of methanol/water (4:1, v/v), and applied to a Sep-Pak C18 column, and eluted using increasing concentrations of methanol in water. The fraction containing DGDG was obtained in methanol. The fraction B was concentrated and dissolved in a small volume of ethyl acetate. It was then applied to the same silica gel column. The fraction D was eluted in ethyl acetate/methanol/water (100:20:1.5, v/v/v). The fraction D was concentrated and dissolved in a small volume of methanol/water (4:1, v/v), and applied to a Sep-Pak C18 column, and eluted using increasing concentrations of methanol in water. The fraction containing SQDG was obtained in methanol/water (9:1, v/v). All the glycolipid compounds were determined by ^{1}H, ^{13}C and Distortionless Enhancement by Polarization Transfer (DEPT) NMR spectroscopic analyses. The chemical structure of three glycolipids (i.e. MGDG, DGDG and SQDG) are shown in Fig. 1.

2.3. Hydrolysis of MGDG by pancreatic lipase

Enzymatic hydrolysis of MGDG was mediated by pancreatic lipase [27]. Five milligrams of chemically-synthesized MGDG was suspended in 0.5 mL of 0.2 M Tris–HCl buffer (pH 7.6) containing 5 mg of pancreatic lipase and 0.25 M $CaCl_2$. The reaction mixture was incubated at 37° for 20 min, and then 0.1 mL of 6 N HCl was added to the reaction mixture. Thereafter, the hydrolysates were extracted with diethyl ether. The reaction products were separated by thin layer chromatography (TLC, chloroform/methanol (2:1, v/v)).

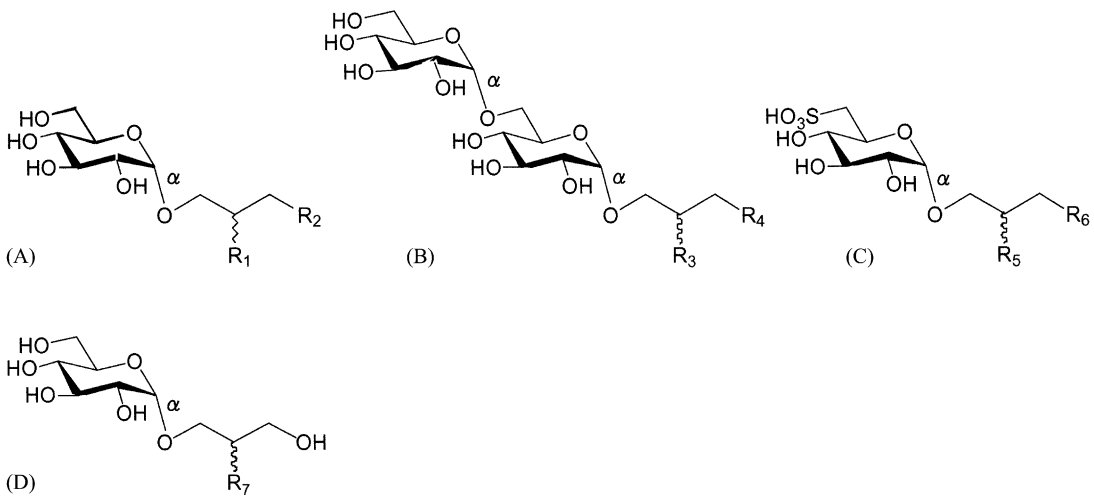

Fig. 1. Chemical structures of the glycolipids from spinach. (A) MGDG (monogalactosyl diacylglycerol). (B) DGDG (digalactosyl diacylglycerol). (C) SQDG (sulfoquinovosyl diacylglycerol). (D) MGMG (monogalactosyl monoacylglycerol). R1–R7 in these structures are fatty acids.

2.4. Fatty acid analysis of glycolipids

The methyl ester of the purified glycolipids from spinach was analyzed by gas chromatography (GC, Shimadzu GC-14A) equipped with an integrator (Shimadzu C-R4A) for quantitation of the peak areas. The column was 200 cm × 3.0 mm (i.d.) and packed with 15% EGSS-X on 100–200 mesh Gaschrom Q (Nishio Co., Inc. Ltd.). The column oven temperature was run isothermally at 190°, and the injection port and detector temperatures were both 250°. The helium carrier gas flow rate was 40 mL/min. The sample was dissolved in *n*-hexane for injection. Identification of the peaks was accomplished by comparing the relative retention times with standard fatty acid mixtures (F & OR mixtures No. 3, Applied Science).

2.5. Cell culture and measurement of cell viability

A human gastric cancer cell line, NUGC-3, was obtained from Health Science Research Bank. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37° in a humid atmosphere of 5% CO_2/95% air. For the cell viability assay, NUGC-3 cells were plated at 5×10^3 cells into each well of 96-well microplates with various concentrations of the purified glycolipids. These compounds were dissolved in phosphate-buffered saline (PBS) at a concentration of 10 mM as a stock solution. The stock solutions were diluted to the appropriate final concentrations with growth medium just before use. The cell viability was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [28].

2.6. DNA polymerase and other DNA metabolic enzyme assays

DNA polymerase α (pol. α) was purified from calf thymus by immunoaffinity column chromatography as described previously [29]. Pol. β was purified from a recombinant plasmid expressing rat pol. β [30]. Pol. δ was purified from calf thymus, and pol. ε was purified from HeLa cells as described previously [31,32]. The activities of the DNA polymerases were measured by the methods described previously [3,4]. For the DNA polymerases, poly(dA)/oligo(dT)$_{12-18}$ (A/T = 2/1) and dTTP were used as the DNA template-primer and nucleotide substrate, respectively. These compounds were dissolved in dimethyl sulfoxide at various concentrations and sonicated for 30 s. Four microliters of each sonicated sample was mixed with 16 μL of each enzyme (final 0.05 U) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM EDTA, and kept at 0° for 10 min. These inhibitor–enzyme mixtures (8 μL) were added to 16 μL of each of the enzyme standard reaction mixtures, and incubation was carried out at 37° for 60 min. One unit of each DNA polymerase activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of deoxyribonucleotide triphosphates into synthetic DNA template-primers at 37° for 60 min.

Other DNA metabolic enzyme assays were performed as described previously [33].

2.7. Cell cycle analysis

The cells (3×10^5 cells in a 35-mm dish) were collected by trypsinization and washed with ice-cold PBS by centrifugation. The NUGC-3 cells were suspended in PBS, fixed with 70% ethanol (v/v), and stored at −20°. The cells were collected by centrifugation and stained with 4′,6-diamidino-2-phenylindole (DAPI, 2 μg/mL) for at least 20 min at room temperature in the dark. The DNA content of the 8000 stained cells was analyzed using a cell counter analyzer (Partec, CCA model) with Muticycle 3.11 software (Phoenix Flow Systems). The cell debris and fixation artifacts were gated out.

2.8. Analysis of DNA fragmentation

DNA fragmentation was determined by electrophoresis in 1.5% agarose gels. The NUGC-3 cells (6×10^5 cells in a 60-mm dish) were lysed with 10 mM Tris–HCl (pH 7.4) containing 10 mM EDTA, 0.5% Triton X-100 and RNase A (0.2 mg/mL) and incubated at 37° for 1 hr followed by digestion with proteinase K (0.5 mg/mL) at 50° for 30 min. After the addition of 1/2 volume 10 M ammonium acetate, the DNA was precipitated with 2.5 volume ethanol, dissolved in gel loading buffer (40 mM Tris—5 mM sodium acetate—1 mM EDTA (pH 7.8)) and separated by electrophoresis in a 1.5% agarose gel. The gel was stained with ethidium bromide, and then the DNA bands were visualized under UV light.

3. Results and discussion

3.1. Purification of glycolipids from spinach

The glycolipids were extracted with chloroform/methanol (1:1, v/v) from dried spinach (*S. oleracea* L.). Three major glycolipids were purified by silica gel column chromatography, and their chemical structures were determined. These compounds were MGDG (Fig. 1A), DGDG (Fig. 1B) and SQDG (Fig. 1C). These compounds could theoretically have stereoisomers of two configurations, α- or β-type, between the sugar and the glyceride. All of the natural glycolipids had α-type (Fig. 1). The weight-percents of MGDG, DGDG and SQDG in the dried spinach were 5.89, 2.06, and 0.23%, respectively, and no other glycolipids were detected. The purified MGDG, DGDG and SQDG were used in this study.

The fatty acid contents of these compounds are shown in Table 1. The major fatty acid in DGDG and SQDG was

Table 1
Fatty acid composition of glycolipids

Fatty acid	Total fatty acids (mol%) in the presence of			
	MGDG	DGDG	SQDG	MGMG
14:0	1.64	8.24	1.57	0.13
14:1	0.58	11.48	4.51	0.20
16:0	9.41	46.69	47.78	13.17
16:1	2.44	6.51	0.31	1.52
16:3	0.76	5.16	0.35	1.87
18:0	27.90	3.03	0.52	26.13
18:1	27.49	11.32	1.89	1.23
18:2	3.48	1.52	4.78	3.10
18:3	26.30	6.05	38.29	52.65

palmitic acid (16:0), and MGDG mostly consisted of stearic acid (18:0), oleic acid (18:1) and linolenic acid (18:3).

3.2. Effect of the purified glycolipids on cell growth

To clarify the *in vivo* effects of the purified glycolipids from spinach, we tested their influence on human stomach cancer cell (NUGC-3) survival. The cells were incubated with these compounds for 48 hr. As shown in Fig. 2, neither DGDG nor SQDG inhibited the cell growth. On the other hand, MGDG efficiently inhibited the cell growth in a dose-dependent manner, and the LD_{50} value was approximately 49 μg/mL. It is of interest that the compound from a healthy green vegetable is cytotoxic. These results suggested that DGDG and SQDG were hardly able to penetrate into cells, while MGDG was cell permeable. The palmitic acid (16:0) content of MGDG was lower than that of DGDG and SQDG (Table 1), therefore, the saturation and content of fatty acids in glycolipid might be related to penetrate into cells. As described previously, SQMG was more permeable into cells, and stronger inhibitor of the cell growth than SQDG.

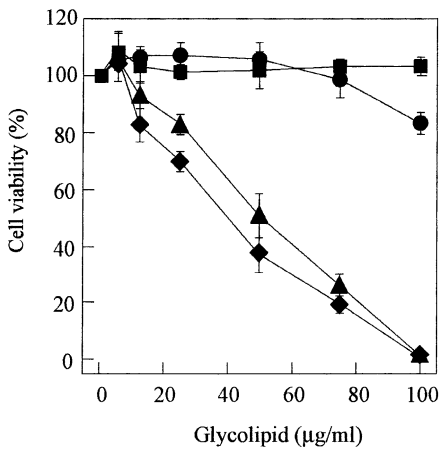

Fig. 2. Effect of the glycolipids from spinach on the proliferation of NUGC-3 cancer cells. Dose–response curves of growth inhibition of NUGC-3 cells incubated with the purified glycolipids (MGDG (▲), DGDG (■) and SQDG (●), and the enzymatically-synthesized MGMG (◆) for 48 hr. Cell proliferation was determined by MTT assay [28]. Data are shown as means ± SEM of four independent experiments.

SQMG has, to date, been developed pharmaceutically as a much more promising anti-cancer chemotherapy agent [17,22,26]. If one of the fatty acids in MGDG could be removed, MGMG would be a stronger cell growth inhibitor. Therefore, we also obtained MGMG as follows, and MGMG was used in comparison with the MGDG effect.

3.3. Enzymatic hydrolysis of MGDG by pancreatic lipase

The cytotoxic glycolipid, MGDG, has two molecules of fatty acid located at the 1 and 2 positions in the glycerol moiety. We tried enzymatic digestion of the purified MGDG from spinach (lane 3 in Fig. 3). After a 20-min incubation with the pancreatic lipase, MGDG was hydrolyzed to MGMG (lane 4 in Fig. 3). MGMG has one molecule of fatty acid at the 2 position in the glycerol moiety (Fig. 1D). The hydrolyzed MGMG was purified through silica-gel column chromatography using chloroform:methanol (4:1, v/v). The enzymatically-synthesized MGMG was routinely used in the later experiments.

The fatty acid composition at the 2 position in the glycerol moiety of MGMG was mainly linolenic acid (52.65%), therefore, the two fatty acid constitutions at the 1 and 2 position in the glycerol moiety of MGDG were mainly oleic acid and linolenic acid, respectively (Table 1). MGMG also suppressed the cancer cell growth in a dose-dependent manner, and the LD_{50} value was

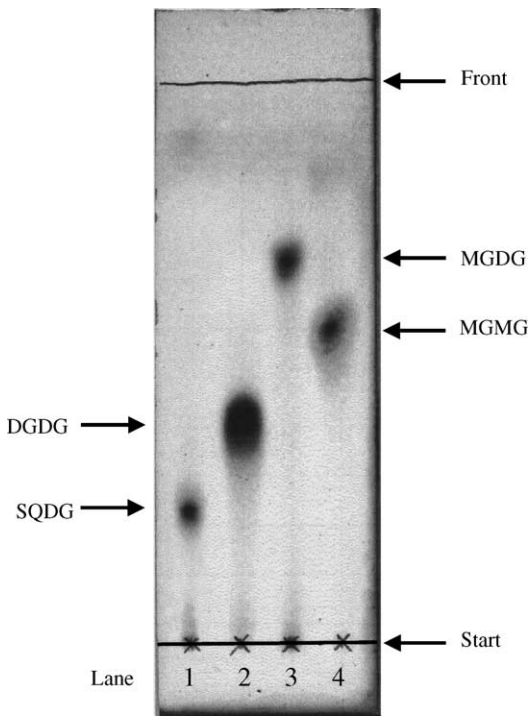

Fig. 3. Enzymatic hydrolysis of MGDG by pancreatic lipase. Lanes 1–4 are purified SQDG, purified DGDG, purified MGDG, and purified MGMG which was hydrolyzed from MGDG by pancreatic lipase, respectively. The enzyme reaction and thin layer chromatography (TLC, chloroform:methanol = 3:1) were described in Section 2. A photograph of TLC detected by 50% sulfuric acid spray is shown.

approximately 40 μg/mL (Fig. 2). The cell growth effect by MGMG was slightly stronger than that by MGDG.

3.4. Effects of MGDG/MGMG on the activities of mammalian DNA polymerases and other DNA metabolic enzymes

As briefly described in Section 1, we screened for and found many DNA polymerase inhibitors from natural resources [3–13,18–25], and some of the glycolipids, which inhibit the polymerase activities, might be suitable human anti-cancer chemotherapy agents [14–22]. This is the reason we widely screened for other glycolipids including the spinach compounds. In this section, therefore, we investigated the effects of the spinach glycolipids (i.e. MGDG, DGDG, SQDG and MGMG) on mammalian DNA polymerases and other DNA metabolic enzymes.

The effects of the spinach glycolipids including MGMG on various DNA polymerases and other DNA metabolic enzymes are depicted in Fig. 4. MGDG, SQDG and MGMG inhibited the activities of mammalian DNA polymerases, but DGDG had no such inhibitory effect. Interestingly, MGDG has no effect on pol. β, although MGMG could inhibit its activity. These four glycolipids had no inhibitory effect on plant pol. α and β, prokaryotic DNA polymerases and other DNA-metabolic enzymes such as calf terminal deoxynucleotidyl transferase, human immunodeficiency virus type-1 (HIV-1) reverse transcriptase, T7 RNA polymerase, and calf deoxyribonuclease I (Fig. 4). MGDG, SQDG and MGMG were selective mammalian DNA polymerase inhibitors.

Fig. 5A shows the dose–response curves of the inhibition of MGDG and MGMG against mammalian DNA polymerases. MGDG could clearly inhibit the activities of pol. α, δ and ε, which are replicative polymerases, in a dose-dependent manner, and their IC_{50} values were 24, 21 and 11 μg/mL, respectively (Fig. 5A). However, MGDG could not influence the pol. β activity (Fig. 5A). MGMG lipase hydrolyzed from MGDG inhibited not only the activities of all the replicative DNA polymerases, but also of pol. β (Fig. 5B). The inhibition of pol. ε activity by MGMG was the strongest, and the IC_{50} value was 8.5 μg/mL. The inhibitory effects of MGMG were stronger than those of MGDG (Fig. 5). The dose–response curves in Fig. 5 did not change when activated DNA was used as the DNA template-primer (data not shown). The cell growth inhibition by MGMG was stronger than that by MGDG (Fig. 2), and the inhibitory effect by MGDG or MGMG must be led by the inhibition of the polymerase activities. MGDG/MGMG may also be promising tumoricidal agents like SQMG

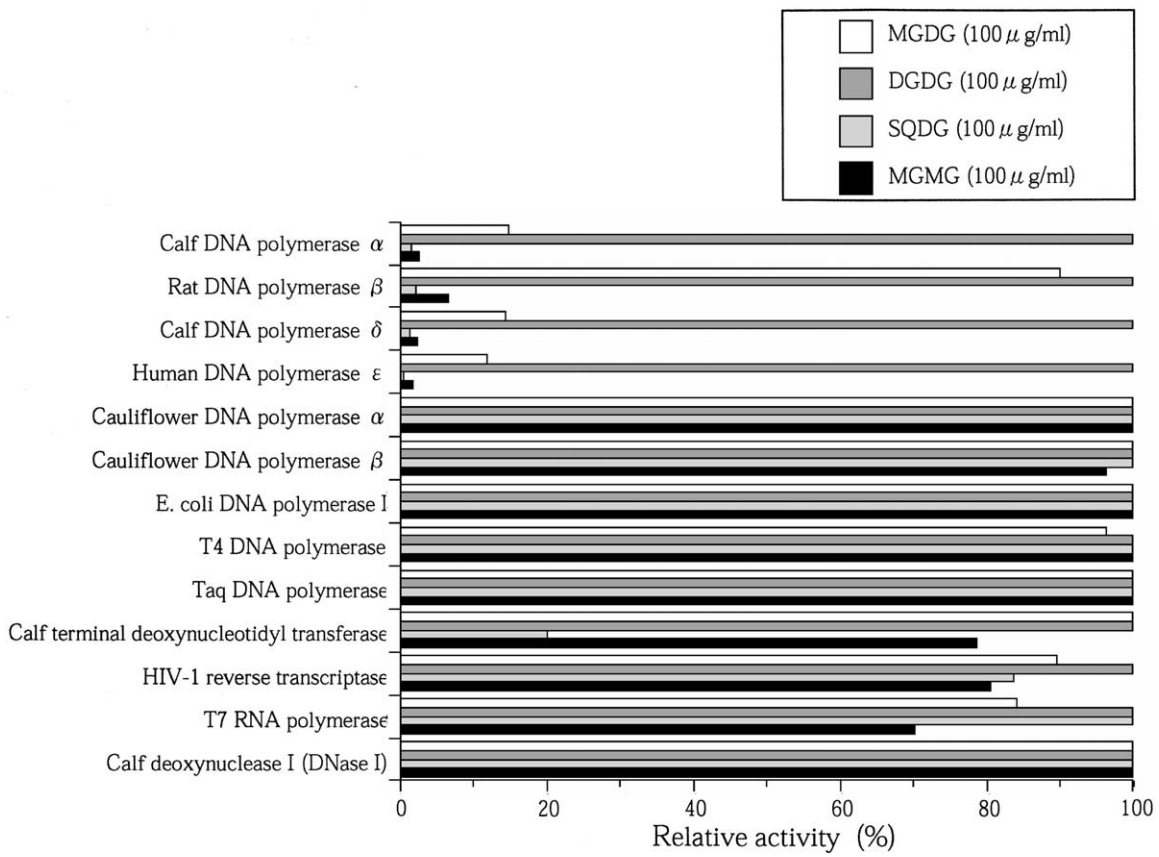

Fig. 4. Effects of the purified glycolipids from spinach on the activities of various DNA polymerases and other DNA metabolic enzymes. MGDG, DGDG, SQDG and MGMG (100 μg/mL each) were incubated with each enzyme (0.05 U). The enzymatic activity was measured as described in Section 2. Enzyme activity (5000 cpm) in the absence of the compounds was taken as 100%.

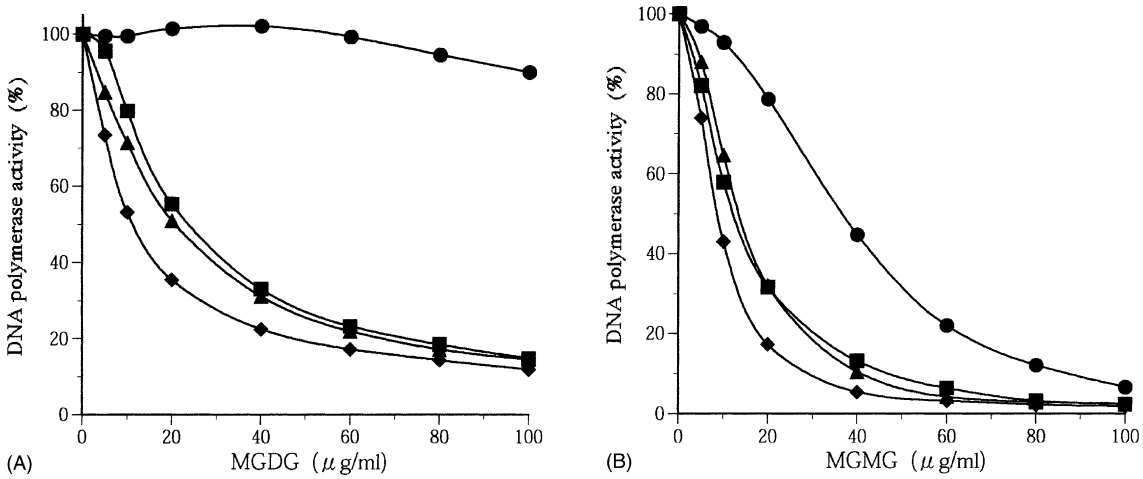

Fig. 5. Inhibition of activities of mammalian DNA polymerases by MGDG (A) and MGMG (B). Each mixture contained 0.05 U of enzyme. DNA polymerase activity (5000 cpm) in the absence of the compounds was taken as 100%. The mammalian DNA polymerases tested and symbols used are as follows: calf DNA polymerase α (■); rat DNA polymerase β (●); calf DNA polymerase δ (▲); human DNA polymerase ε (◆).

reported previously [17,22,26]. If so, MGDG/MGMG would not be mere cell growth inhibitors, but cytocidal agents. The remainder of this report, therefore, focuses on the MGDG/MGMG effects in cells.

3.5. Cell cycle regulation by MGDG/MGMG

To further investigate the effects of each of MGDG/MGMG in detail, the cell cycle of NUGC-3 cells with or

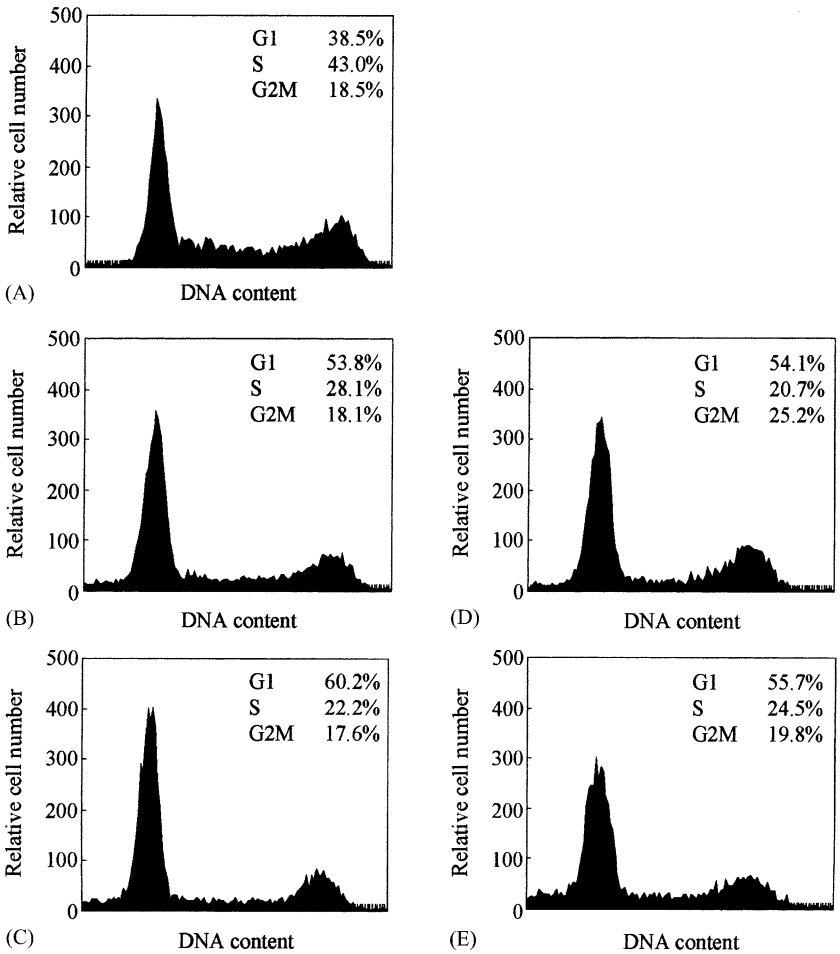

Fig. 6. Effect of cell cycle distribution by MGDG and MGMG. NUGC-3 cells were incubated with 49 μg/mL MGDG for 0 (A, control), 24 (B), and 48 hr (C). NUGC-3 cells were incubated with 40 μg/mL MGMG for 0 (A, control), 24 (D), and 48 hr (E). The cell cycle distribution was calculated as the percentage of cells that contained G1, S, G2/M DNA analyzed by flow cytometry with DAPI staining.

without the agent was studied by flow cytometry. The ratio of the three phases (i.e. G1, S and G2/M) in the cell cycle is shown in Fig. 6. When the cells were incubated with 49 μg/mL MGDG for 48 hr, the percentage of cells in the S phase decreased, and those in the G1 phase increased time-dependently (Fig. 6A–C). The percentage of cells in the G2/M phase was constant throughout the incubation. On the other hand, when the cells were incubated with 40 μg/mL of MGMG for 48 hr, the percentage of cells in the G1 phase increased time-dependently, but the percentages of cells in the other phases did not change in a time-dependent manner (Fig. 6A, D and E). The percentage of cells in the S phase decreased significantly after 24 hr, but it slightly recovered after 48 hr. The percentage of cells in the G2/M phase was enriched after 24 hr, and then it recovered to the initial level after 48 hr. Both MGDG and MGMG could arrest the cells at the G1 phase. Since aphidicolin and ara-C, both well-known replicative DNA polymerase inhibitors, arrested the cells in the G1 phase [34,35], the action of MGDG/MGMG probably resulted from the inhibition of the replicative polymerases. MGDG/MGMG must inhibit the cell growth by blocking the primary step of DNA replication, which occurs by influencing the replicative DNA polymerases. The G1 phase-arrest occurred more effectively by MGDG than by MGMG. This may be explainable by the fact that MGDG was more specialized to the replicative polymerases, and MGMG could influence not only the replicative polymerases but also a β-type repair-related polymerase. The inhibition of the repair-related polymerase can lead to destruction of the cells irrespective of the cell cycle regulation system.

3.6. Induction of apoptosis by MGDG/MGMG

To examine if the cell growth inhibition by MGDG/MGMG induces apoptosis, the cells were incubated with 49 μg/mL MGDG or 40 μg/mL MGMG for 48 hr. Apoptosis was detected by the formation of oligonucleosomal DNA fragments, which is a late event in apoptosis. The formation of fragmented DNA was observed in the presence of either MGDG or MGMG (lanes 3 and 5 in Fig. 7). During apoptosis, the activation of a group of caspases such as caspase-3 and the subsequent cleavage of cellular substrates such as PARP are crucial components of the cell death pathway [36], and the inhibition of caspases prevents apoptosis [37]. In particular, caspase-3 is a key executioner of apoptosis mediated by various anti-tumor agents [38]. The formation of fragmented DNA induced by MGDG or MGMG was completely blocked when the cells were preincubated with a caspase-3 inhibitor, Ac-DEVD-CHO (lanes 2, 4 and 6 in Fig. 7). This result suggested that the apoptosis induced by MGDG/MGMG was mediated through caspase-3 activation. The cell growth inhibition by MGDG/MGMG must lead to the apoptotic cell death. Although a number of well-known antitumor DNA-damaging agents, e.g. etoposide, camptothecin, doxorubicin and

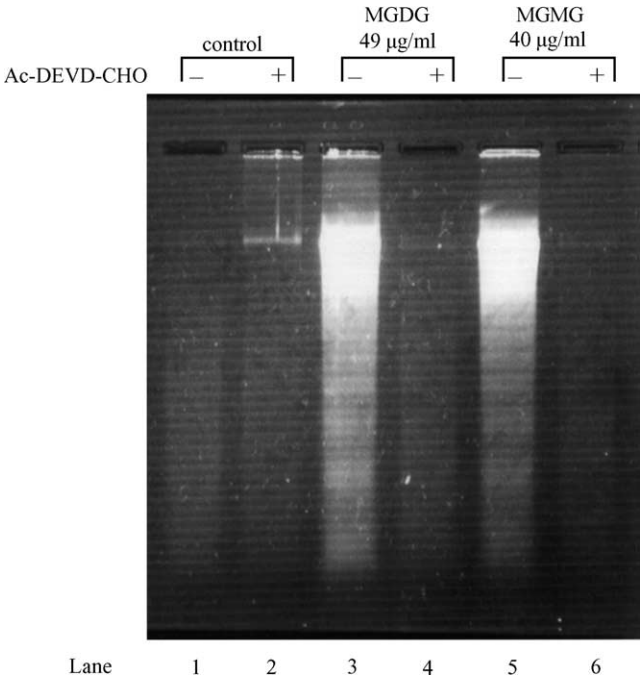

Fig. 7. Induction of apoptosis by MGDG and MGMG. Agarose gel electrophoresis of DNA fragments. NUGC-3 cells were preincubated with or without 100 μM Ac-DEVD-CHO for 3 hr followed by incubation with or without 49 μg/mL MGDG (lanes 3 and 4) or 40 μg/mL MGMG (lanes 5 and 6) for 48 hr. DNA fragments were analyzed by 1.5% agarose gel electrophoresis.

antimetabolites, which influence DNA metabolic enzymes, induce apoptosis via caspase-3 activation, their mechanism of cytotoxic damage is different and the cytotoxic concentrations are substantially lower than those of MGDG/MGMG.

Pol. α is the only enzyme capable of initiation of DNA synthesis *de novo* by first synthesizing a RNA primer and then extending the primer by polymerization to produce a short DNA extension. Then, chromosomal DNA, after being initiated and having entered into the S phase, is elongated by pol. δ and ε replaced from pol. α. Therefore, the replicative DNA polymerases are highly conserved in eukaryotes, and the inhibition of replicative DNA polymerases could induce the cell cycle arrest at the G1 and S phases, and subsequently, apoptosis. Nevertheless, spinach MGDG/MGMG did not inhibit the activity of plant pol. α. The results of the present study indicate that glycolipids including spinach MGDG/MGMG are useful as mammalian DNA polymerase-specific inhibitors both *in vivo* and *in vitro*. In addition, we also investigated whether the agents could be useful key drugs to develop a design strategy for cancer chemotherapy agents. Subsequently, as shown in this paper, MGDG/MGMG could also be considered as the lead compound of a group of potentially useful cancer chemotherapy agents.

Another question remains. MGDG/MGMG were obtained from a green vegetable, spinach, which is considered a healthy vegetable. To our knowledge, there is no information indicating that ingestion of plenty of spinach is

either cancer-preventive or oncogenic. MGDG/MGMG would be rapidly destroyed in the human body. Similar glycolipids, SQDG/SQMG, are present in a large variety of vegetables and edible algae, and SQDG/SQMG are very stable in rats and mice, when injected intravenously [18–22,26]. Therefore, if MGDG/MGMG were injected intravenously, they could be either cancer-preventive or oncogenic.

Acknowledgments

We are grateful to Dr. M. Takemura of Nagoya University, Dr. A. Matsukage of Japan Women's University and Dr. S. Linn of the University of California, Berkeley, for preparing calf DNA polymerase α, rat DNA polymerase β and human DNA polymerase ε, respectively, and for their valuable discussions concerning inhibitors. This work was partly supported by the Grant-in-Aid from Kobe Gakuin University Joint Research (B) (CM, HY and YM) and by the Sasakawa Scientific Research Grant (to CM) from the Japan Science Society. YM acknowledges the Grant-in-Aid 14780466 for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

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