Anti-Proliferative Effect of Long-Chain Monoglyceride Derivatives on Human Cervical Carcinoma Cell Line

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Objective: *We investigated the effect of long-chain monoglyceride derivatives on cervical cancer (HeLa) cells proliferation and compared those with normal cells (Vero).*
Material and Method: *The long-chain monoglycerides (MGs) used in this study were monomyristin, monopalmitin, monostearin, monoolein and monolinolein. The anti-proliferative effect of MGs was conducted to assess the living cell metabolic activity of two different cell types; HeLa and Vero cell lines by using MTT assay. The cell percentage of viabilities and IC50 values were determined after the cells treated with different concentrations of MGs for 24 h. The apoptosis cell death induced by MGs was evaluated by using caspase-3 activity-ELISA immunoassay and DNA ladder assay.*
Results: *The dose dependent effect of MGs on HeLa cell growth inhibition was observed. The IC50 of MGs treated HeLa cells were significantly different from those treated with Vero cells ($p<0.001$). Interestingly, no cytotoxicity on normal cells (Vero) treated with high concentrations of MGs (1,000 μg/mL) was observed. Among saturated and unsaturated long-chain MGs used, monomyristin (C14: 0) and monolinolein (C18: 2) showed the lowest anti-proliferative activity. The inhibitory activity of MGs was higher on HeLa cells treated with the saturated fatty acid moiety than the unsaturated fatty acid of the same carbon chain length MG (C18). The degree of growth inhibition was not depending on the carbon chain length of fatty acid moiety of long-chain MGs used. The caspase-3 activity, a hallmark of apoptosis cell death were increased in HeLa cells-treated group and was significantly different from the untreated control group ($p<0.001$). In addition, DNA laddering pattern was demonstrated in MGs treated HeLa cells.*
Conclusion: *We firstly demonstrated that long-chain MGs derivatives inhibited cervical cancer cell growth and induced apoptosis cell death.*

Keywords: *Monoglycerides, Monoacylglycerols, Caspase-3, Apoptosis, Crvical cancer, HeLa*

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In 2012, CDC reported that the most common causes of cancer death among females were breast, lung, colorectal, cervical, and stomach cancers[1] and cervical cancer ranks as the fourth most frequent cancer among women in the world[2]. In Thailand, cervical cancer ranked as the second most frequent cancer among women and especially women between 15 and 44 years of age[3]. There are factors required for development of the cervical cancer, which is associated with HPV infection. All cases of cervical cancer are caused by HPV, and HPV type 16 and 18 are responsible for about 70% of all cases[4,5].

Monoacylglycerols or monoglycerides (MGs), an amphipathic molecule, consisting of a single fatty acid attached to a glycerol backbone, are found in mammalian tissues[6]. Their structures comprise hydrophilic and hydrophobic moieties in the same molecule, which makes them a good mediator in emulsification process in various applications such as bioremediation, cosmetic, food and pharmaceutics[7]. During the last decade, there were reports on the biological activity of MGs on antimicrobial activity[8,9] and anti-proliferative activity against cancer cells[6,10-12]. Some MGs have been isolated from microorganisms, algae and shown to have pharmaceutical applications[13-17]. Long-chain MGs but not medium-chain MGs had been shown to induce apoptosis in murine thymocytes and human leukemic cells[10,11]. Their further work showed the cellular specificity of MGs on cell death mediation which biased towards T and B lymphocytes while epithelial cells, fibroblasts, natural killer cells, macrophages, and

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erythroid cells lineages are unaffected by MGs treatment[12]. They also suggested that MGs may be the candidate in treatment for specific lymphoid leukemia. These findings led us to investigate the effect of long-chain MGs on the apoptosis cell death induction against cervical cancer cell lines which is an important cancer among women. Although, there were studies on the anti-proliferation monoglyceride derivatives in leukemia[10-12], however there is no report on cervical cancer cell growth.

Material and Method

Cell culture

Human cervical carcinoma cell line (HeLa) and monkey kidney cell line (Vero) obtained from the American Type Culture Collection (ATTC) were used in this study. They were maintained in Dulbecco's modified Eagle's medium (DMEM; GIBCO, USA) containing 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 µg/mL). Two types of culture cells used were incubated at 37°C in a humidified atmosphere with 5% CO_2. The old medium was replaced with fresh medium twice a week, and sub-cultured every 2 to 3 days to maintain cell growth in the log phase.

Preparation of MGs

MGs (purity ≥99%) used in this study were 1-myristoyl-rac-glycerol (monomyristin, MM, C14:0), 1-monopalmitoleoyl-rac-glycerol (monopalmitin, MP, C16:0), 1-stearoyl-rac-glycerol (1-monostearin C18:0), 1-monooleoyl-rac-glycerol (monoolein, MO,C18:1), 1-linoleoyl-rac-glycerol (monolinolein, C18:2) which were purchased from Sigma-Aldrich chemical Co, (St Louis, MO, USA). MGs were dissolved in absolute ethanol. A stock solution was prepared by diluting with the culture medium and sonicated before diluting further to various concentrations used for treatment with HeLa cell lines and Vero cell lines.

Cell proliferation assay

The effects of MGs on cell proliferation was examined using the MTT (3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium-bromide, Sigma-Aldrich, St Louis, MO) assay[18]. Briefly, the cells were trypsinized with 0.25% trypsin-EDTA. The viable cells were estimated by the trypan blue dye and counted by using a hemocytometer. The cells were seeded into 96-well plates ($1x10^4$ cells per well) and incubated for 24h. Then, the medium was removed and replaced with 100 µL culture medium containing MGs with varying concentrations and incubated for another 24 h. The vehicle control group was replaced with cultured medium contained 0.01% ethanol. The next day, 100 µL of 5 mg/mL of MTT was added into each well, incubated for 4 h and 100 µL of dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis) was added to dissolve the formazan crystals. The optical density was measured at 570 nm with a spectrophotometer (Multiskan, Finland). The MTT assay was done in triplicate wells and at least 3 independent experiments. The inhibitory effects of MGs on cell proliferations were calculated from the percentage of viable cells as the following formula. The percentage of viable cells = (absorbance of treated sample/ absorbance of untreated sample) x 100. Dose-response curve was plotted with percentage of growth inhibition versus the MGs concentrations. The IC50 of MGs in each experiment was determined using the nonlinear regression equation (curve fit, graph Pad Prism).

Caspase-3 activity assay

Caspase-3 protein was measured by using human active caspase-3 immunoassay kit (R&D Systems Inc., USA. Cat. No. KM300). Briefly, HeLa cells ($5x10^5$cells/mL) were incubated with MGs at 50 µg/mL and 80 µg/mL for 24 h. Then, cells were harvested and incubated with the biotin-ZVKD-fmk for 2 h at room temperature. Cells were lysed with extraction buffer containing protease inhibitors. The 100 µl of cell lysate were placed into ELISA-well plate that pre-coated with caspase-3 specific monoclonal antibody. The wells were washed and HRP-streptavidin were added and incubated for 1 h. Then the substrate solution were added and incubated for 30 min, finally the stop solution were added and mixed. The OD of the solution was determined with a spectrophotometer (Multiskan, Finland) at 450 nm. Each sample was tested in duplicate.

DNA Ladder assay

The HeLa cell at $3x10^6$ cells were used to treat with MGs, at the concentrations which found to mediate 70% cytotoxicity, and incubated at 37°C, 5% CO_2 for 24 h. At the end of incubation, the chromosomal DNA of HeLa cell lines were prepared with an apoptotic DNA ladder kit (Roche, Cat. No.1835246001). Briefly, the treated cells was harvested and centrifuged at 8,000 x rpm for 5 min. The lysis buffer was added into the pellets, and incubated for 10 min. Then, the isopropanol was added, mixed before passing through a filter tube and washed. The DNA obtained was treated with RNase at 37°C for 30 min. The DNA fragments were observed by loading onto 2% agarose gel electrophoresis, run at 50

V/cm for 3 h. The non-treated cancer cell was done in parallel.

Statistical analysis

All experiments were repeated at least three times. Data were described as the mean ± standard error of mean. The dose-response effects were analyzed by one-way analysis of variance (ANOVA) with Tukey's HSD (honest significant difference) test, and statistical analysis was carried out using Student's t-test. The difference was considered statistically significant when the *p*-value was less than 0.05.

Results

Effects of MGs on cell proliferation

The antiproliferative activity of MGs against HeLa cells was shown in dose dependent manner. No inhibitory effect on Vero cells was observed, even at high concentration of MGs used (1,000 µg/mL) (Fig. 1A and Fig. 1B). Monolinolein (C18:2) demonstrated the lowest anti-proliferative activity among all MGs tested.

The inhibitory effect of MGs was presented as IC50 (the concentration that causes 50% reduction in proliferation of cancer cells). The IC50 of MGs against Vero cells were ranged from 1,489 to 2,056 µg/mL suggested very low toxicity or no toxic to normal cells (Vero). The IC50 of MGs against HeLa cells was summarized in Table 1. The IC50 of monomyristin (C14: 0), monopalmitin (C16: 0), monosterain (C18: 0), monoolein (C18: 1) and monolinolein (C18: 2) were 48.62±0.84 µg/mL, 26.05±0.545 µg/mL, 28.36±0.91 µg/mL, 34.96±0.185 µg/mL and 91.31±1.28 µg/mL, respectively. All MGs tested showed significantly different in anti-proliferative activity against HeLa cell compared to Vero cell (Table 1).

Derivatives of MGs on anti-proliferation activity

We observed the variation in anti-proliferation activity against HeLa cells of long-chain MGs derivatives used (Table 1). The IC50 values were not related to degree of chain length of fatty acid of MGs (Fig.2A). Comparing IC50 of long-chain MGs used in this study, monomyristin (C14:0) displayed the lowest anti-proliferative activity among saturation long-chain MGs used with significant difference ($p<0.001$) from

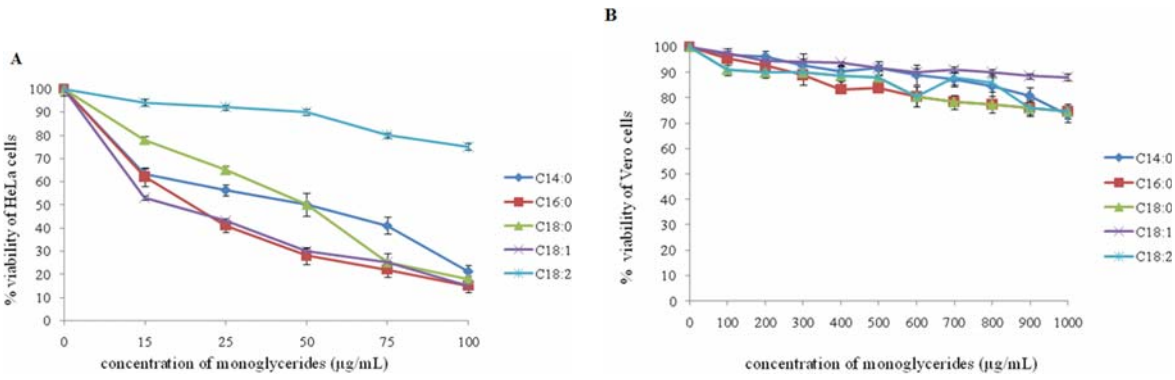

Fig. 1 Effect of MGs against human cervical carcinoma cell lines (A) and Vero cells (B), after 24 h treatment with different concentrations of MGs: monomyristin (C14: 0), monopalmitin (C16: 0), monostearin (C18: 0), monoolein (C18: 1) and monolinolein (C18: 2).

Table 1. The IC50 values of MGs in HeLa cells after 24 h treated

Cell types	IC50 (µg/mL)				
	Monomyristin C14:0	Monoplamitin C16:0	Monostearin C18:0	Monooleoin C18:1	Monolinolein C18:2
HeLa cells	48.62±0.84***	26.05±0.545***	28.36±0.91***	34.96±0.185***	91.31±1.28***

Data were expressed as the mean±standard error of mean
*** indicates significantly different compared between HeLa cell and Vero cell lines at $p<0.001$

monopalmitin (C16: 0) and monostearin (C18: 0) (Fig. 2A). However, monopalmitin (C16: 0) and monostearin (C18: 0) showed about the same level of IC50. This result implies the anti-proliferative activity of saturated MGs was not correlated to the chain length of fatty acid. Regarding to the degree of unsaturation, monolinolein (C18: 2), the unsaturated fatty acid of the longest chain MG used in this study showed the lowest anti-proliferative activity among the C18 group (p<0.001) (Fig. 2B).

MGs induces apoptosis in HeLa cells

Caspase-3 is the hallmark and indispensable downstream protein of cell apoptosis process which lead to DNA condensation and fragmentation[18]. We therefore quantified the caspase-3 protein in MGs-treated HeLa cells by using ELISA assay. We used two concentrations of MG at 50 µg/mL and 80 µg/mL which showed strong inhibitory effects of MGs. The result showed that MGs significantly activated caspase-3 protein in HeLa cells after 24 h of treatment compared with untreated control group. The caspase-3 expression response was related to MGs treated doses (Fig. 3). The caspase-3 activity levels of HeLa cell treated with monomyristin (C14:0) at 50 µg/mL and 80 µg/mL were 3.925 ng/mL and 7.85 ng/mL, respectively. The similar results were observed with HeLa cell treated with monopalmitin (C16: 0) and monoolein (C18: 1), by which at 50 µg/mL and 80 µg/mL, the caspase-3 activities were 11.95 ng/mL, 33.3 ng/mL and 10.95 ng/mL and 27.475 ng/mL, respectively. The caspase-3 activities were high in MG-treated HeLa cells and significantly different from untreated cultured HeLa cells. The different types of fatty acid moiety of MGs induce significantly difference of caspase-3 expression (Fig. 3). These results indicated that MGs mediated HeLa cell apoptosis in different level. This caspase-3 activity result was correlated to the %cell viability of MGs-treated HeLa cells shown in Fig. 1A.

The DNA fragmentation

We next investigated the DNA fragmentation to confirm the late phase of apoptosis by using DNA ladder assay. This is a key feature of programmed cell death by the activation of caspase enzymes. The DNA ladder patterns in HeLa cells treated with MGs were observed but not in the untreated HeLa cells (Fig. 4). This result confirmed the apoptosis cell death induction of MGs on HeLa cell.

Discussion

The monoglyceride derivatives vary by the structure of the carbon chain length or the unsaturation (double bond) of fatty acid moiety conjugated to the glycerol core. MGs are commonly found in mammalian tissue and usually used as food emulsifier in bakery products[6,19]. There is no evidence that the presence of monoglycerides or diglycerides of food fats has any deleterious effect on cells or tissue so they are recognized as Generally Regards as Safe (GRAS)[19]. The potency of monoglyceride derivatives on growth inhibition of murine thymocytes proved to be depended on the fatty acid moiety, whereas glycerol backbone or corresponding fatty acids alone were

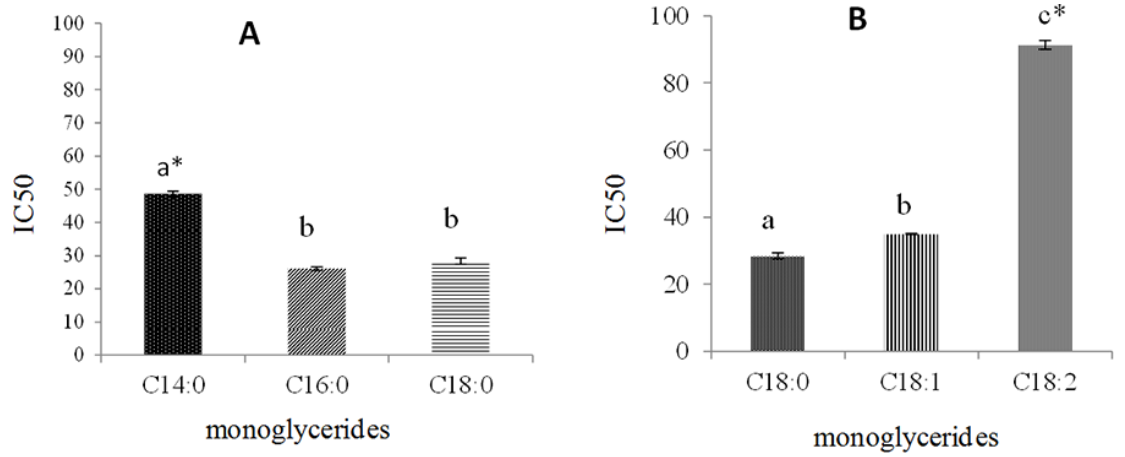

Fig. 2 IC50 of MGs on HeLa cell proliferation incubated with different chain length of fatty acids (A) and saturation of fatty acids moiety (B). The values not sharing the same superscript letters (a, b, c, d) are significantly different (p<0.01).

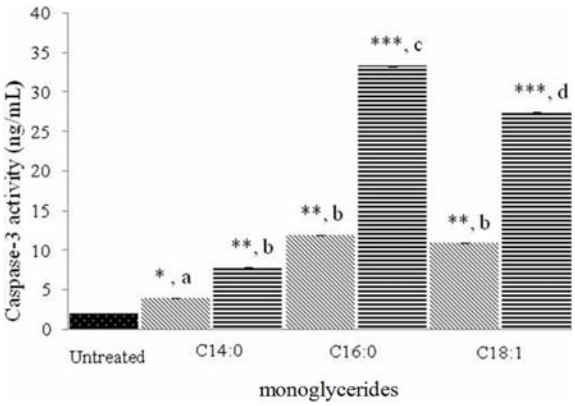

Fig. 3 Expression of caspase-3 in human cervical carcinoma cell lines exposed with the indicated MGs at 50 µg/mL, ▨ and 80 µg/mL, ▤ for 24 h. *,**,*** Indicates significant difference compared with control group at $p<0.1$, $p<0.01$and $p<0.001$, respectively. The values not sharing the same superscript letters (a, b, c, d) are significantly different at $p<0.01$.

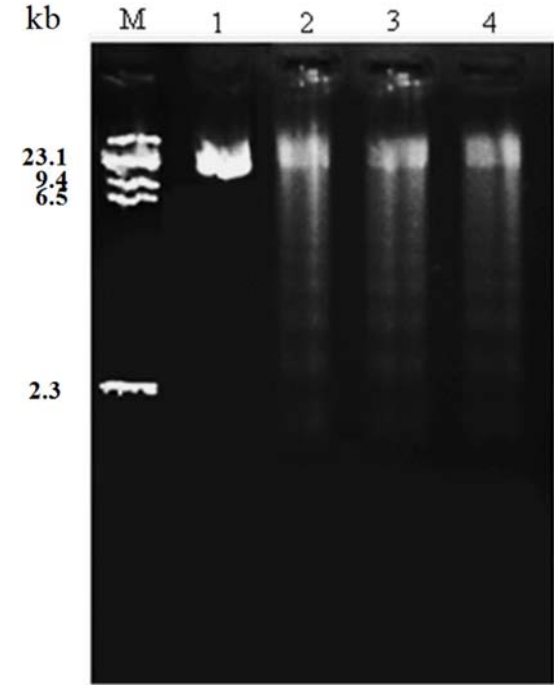

Fig. 4 Agarose gel electrophoresis of chromosomal DNA of HeLa cells treated with MGs for 24 h. M = Molecular weight marker, lane 1: HeLa-untreated control, lane 2, HeLa-monomyristin (C14: 0) lane 3, HeLa-monopalmitin (C16: 0) lane 4, HeLa-monoolein (C18: 1).

ineffective, in addition the medium chain MGs (C6-C12) were low effective compared to long-chain MGs[10]. In this study, we focused on the anti-proliferative effect of long-chain MGs (C14-C18) which exhibited cytotoxic against HeLa cancer cells in dose dependent manner (Fig. 1) and no toxicity on Vero cells which is similar to the previous studies[10,11,15]. Interestingly, Philippousis et al revealed the MGs cytotoxicity was specific to cancer cell type by which adenocarcinomas; mammary, prostate, and endometrial tumors were quite resistant to MGs treatments[12]. Additionally, MGs-induced cell death was clearly shown to be cellular specificity, i.e. cells' differentiation and activation status[12]. Our study is the first report on the potency of MG derivatives on cytotoxicity against HeLa cell.

Regarding the structure of fatty acid moiety of MGs, there were other reports on the effect of fatty acid moiety on biological activity[10,20]. We observed the cytotoxicity of long-chain MGs was not depending on the carbon chain length of fatty acid moiety of MGs (C14-C18) (Fig. 2A) which is correlated to the previous report[10]. However, in contrast to Philippoussis et al in 2001, we observed that the degree of unsaturation of the fatty acid moiety decreased the cytotoxicity (Fig. 2B, Table 1). The variation of the chain length and the degree of unsaturation of fatty acid moiety of MGs was also studied on the inhibition effect on P-glycoprotein (P-gp) activity[20]. P-gp, the product of MDR1 gene in Caco-2 cells is highly expressed in the tumor cells membrane to excrete the hydrophobic drugs from the cells in an ATP-dependent manner. Monopalmitin (C16: 0) only altered the pharmaco-kinetics of drugs by inhibiting the P-gp mediated efflux but not cause damage to Caco-2 cells[20]. Contrary to our study and Philipoussis et al[10], the effect of MG derivatives on the P-gp activity was not dependent on the degree of unsaturation but on the chain length. Although we studied the unsaturation only in the group of C18-fatty acid MG, nevertheless our result may add on the information in this interesting aspect. The discrepancy of the biological activity on the structure of MGs derivatives and on cancer cell types infer the specificity of the biological activity of MGs structure and on the cancer cell types[10-12,20].

Apoptosis, or programmed cell death is a popular target of many treatment strategies. Process of apoptosis initiates through two pathways, extrinsic or intrinsic which finally leads to the caspase enzymes activation. Caspase-3 playing essential roles in degrading the nuclear material and cytoskeletal proteins[21]. The cytotoxicity tested by MTT assay indicated the potency of MGs; therefore, we investigated the caspase-3 activity which is an

important marker for the detection of apoptotic events. We selected C14: 0, C16: 0 and C18: 1 as the representative of short-, medium-, long-, saturated- and unsaturated-chain fatty acid moieties of long-chain MGs to observe the HeLa cell apoptosis. The concentration at 50 μg/mL and 80 μg/mL of MGs which showed high activity in the proliferative activity experiment (Fig. 1A) were used in order to compare the effect of each MG on apoptosis cell death between low and high dose of the MGs. The results of caspase-3 activity shown in Fig. 3 were in dose response to the MGs used. Consequently, the MGs mediated HeLa cell death by activating caspase-3 protein acting as an endogenous endonuclease cleaving chromosomal DNA into fragments which appears as the separation of DNA bands look like a ladder on an agarose gel (Fig. 4). These results confirm that MGs mediated HeLa apoptosis cell death.

Monoglycerides are a kind of glycolipid which is believed to inactivate pathogenic bacteria by disrupting the membrane lipid fluidity leading to events of cell lysis[22]. But there were evidences suggested that glycolipid biosurfactant or MGs have growth inhibition specificity to some type of cancer cell and not on normal cells[11,15]. Each type of cancer has specific characteristics and different cause of becoming cancerous so the particular compound which shown to be potential to inhibit cell growth must trigger some signals to mediate cancer cell apoptosis. Monopalmitin (10 to 300 μM, MP) incubated with colon cancer (Caco-2) changed the P-gp activity but had no effect on the LDH release suggested that the MP was not cytotoxic to Caco-2 cells, i.e. MP did not cause cell damage or leakage[20]. These results suggested that MGs do not cause cell death through a simple 'detergent effect'. In addition, the signal transduction events of growth arrest, induce calcium flux and apoptosis were observed in MGs-treated cancer cells[10,23] suggesting that MG-induced cell death requires de novo mRNA and protein synthesis in the specific cancer cells[10]. The mechanism of MGs on mediation HeLa cells apoptosis will be further investigated.

Conclusion

We report here the anti-proliferative activity of the monoglycerides derivatives on cervical carcinoma cell line (HeLa). The cytotoxicity of MGs was in dose dependent manner and not toxic to normal (Vero) cells. The MGs mediated HeLa cell death by apoptosis process as demonstrated by increased in caspase-3 expression and chromosomal DNA fragmentation.

Acknowledgements

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What is already known on this topic?

Monoglycerides (MGs) is a kind of lipid which is widely used as food emulsifier and regarded as safe to human. There were reports on the biological activity of MGs on induction of murine thymocytes and human leukemic cells but not on normal cells. However, the cytotoxicity of MGs was specific to type and status of cancer cells. The fatty acid moiety plays important role on the MGs biological activity,

What this study adds?

The long chain fatty acid (C14-C18) MGs used in this study showed dose dependent anti-proliferative effect on cervical cancer (HeLa) cells and no cytoxic to normal cells (Vero). The apoptosis cell death inductions were demonstrated in MGs-treated HeLa cells. The inhibitory activity of MGs was higher in HeLa cell treated with the saturated fatty acid moiety than the unsaturated fatty acid of the same carbon chain length MGs but not depending on the carbon chain length of fatty acid moiety of long-chain MGs used.

Potential conflicts of interest

None.

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ผลการยับยั้งการเพิ่มจำนวนเซลล์มะเร็งปากมดลูกโดยอนุพันธ์ของสารกลุ่มโมโนกลีเซอไรด์สายโซ่ยาว

สุดารัตน์ รองปาน, สิริเนตร พลนอก, สิริรัตน์ บุญดิเรก, นิตยา ไตรภิญโญภาพ, เบญจมาส วงศ์สัตยนนท์

วัตถุประสงค์: ผู้วิจัยได้ศึกษาผลของอนุพันธ์สารกลุ่มโมโนกลีเซอไรด์สายโซ่ยาวต่อการยับยั้งการการเพิ่มจำนวนเซลล์มะเร็งปากมดลูก (HeLa) เปรียบเทียบผลกับเซลล์ปกติ (Vero)

วัสดุและวิธีการ: สารกลุ่มโมโนกลีเซอไรด์ (MGs) สายโซ่ยาวที่ใช้ในการศึกษาประกอบด้วย โมโนไมริสติน, โมโนพัลมิติน, โมโนสเตียริน, โมโนโอลิอิน และโมโนลิโนเลอิน โดยการศึกษาฤทธิ์ของสารต่อการยับยั้งการเพิ่มจำนวนเซลล์มะเร็งประเมินจากเมตาบอลิสมของเซลล์ที่ยังมีชีวิตรอดด้วยวิธี MTT assay ในเซลล์ไลน์สองชนิดคือเซลล์มะเร็งปากมดลูก (HeLa) และเซลล์ปกติ (Vero) ร้อยละของเซลล์ที่รอดชีวิตและค่าความเข้มข้นของสารที่สามารถยับยั้งการเพิ่มจำนวนเซลล์ได้ร้อยละ 50 ของเซลล์ทั้งหมด (IC50) จะถูกคำนวณภายหลังจากที่เซลล์ถูกบ่มด้วยสารโมโนกลีเซอไรด์ที่ความเข้มข้นแตกต่างกันเป็นเวลา 24 ชั่วโมง การศึกษาการเหนี่ยวนำให้เกิดการตายของเซลล์แบบ apoptosis จากการกระตุ้นของสารกลุ่มนี้ประเมินจากการแสดงออกของเอนไซม์ caspase-3 ด้วยวิธี ELISA immunoassay และตรวจสอบการแตกหักของดีเอ็นของเซลล์ด้วยวิธี DNA ladder assay

ผลการศึกษา: อนุพันธ์สารในกลุ่มโมโนกลีเซอไรด์สามารถยับยั้งการเพิ่มจำนวนเซลล์มะเร็งปากมดลูกได้มากขึ้นตามความเข้มข้นของสารที่ใช้แตกต่างจากผลการทดสอบกับเซลล์ปกติอย่างมีนัยสำคัญทางสถิติ ($p<0.001$) ที่น่าสนใจ คือสารกลุ่มดังกล่าวไม่มีความเป็นพิษต่อเซลล์ปกติ ถึงแม้จะใช้สารโมโนกลีเซอไรด์ที่ความเข้มข้นสูงถึง 1,000 ไมโครกรัมต่อมิลลิลิตร ในสารกลุ่มโมโนกลีเซอไรด์แบบโซ่ยาวที่มีกรดไขมันชนิดอิ่มตัวและไม่อิ่มตัว พบว่าโมโนไมริสติน (C18:2) และโมโนลิโนเลอิน (C18:2) มีฤทธิ์ในการยับยั้งการเพิ่มจำนวนเซลล์มะเร็งปากมดลูกต่ำที่สุดและพบว่ากรดไขมันชนิดอิ่มตัวมีประสิทธิภาพในการยับยั้งการเพิ่มจำนวนเซลล์มะเร็งปากมดลูกสูงกว่ากรดไขมันชนิดไม่อิ่มตัว ของสารโมโนกลีเซอไรด์ที่มีจำนวนคาร์บอนของกรดไขมันเท่ากัน (C18) และระดับในการยับยั้งการเพิ่มจำนวนเซลล์มะเร็งปากมดลูกไม่ขึ้นกับขนาดความยาวของคาร์บอนอะตอมของกรดไขมันที่ใช้ เมื่อวัดปริมาณ caspase-3 ซึ่งเป็นตัวบ่งชี้ของการเหนี่ยวนำการตายแบบ apoptosis ในเซลล์มะเร็งปากมดลูก HeLa ที่บ่มด้วยสารโมโนกลีเซอไรด์ พบว่ามีปริมาณเพิ่มสูงขึ้นอย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับเซลล์ที่ไม่ได้บ่มด้วยสารโมโนกลีเซอไรด์ ($p<0.001$) นอกจากนี้ยังพบรูปแบบการแตกหักของ ดีเอ็นเอ คล้ายขั้นบันไดในเซลล์มะเร็งปากมดลูกที่ได้รับการบ่มด้วยสารโมโนกลีเซอไรด์

สรุป: งานวิจัยนี้เป็นรายงานครั้งแรกที่แสดงว่าอนุพันธ์สารในกลุ่มโมโนกลีเซอไรด์สายโซ่ยาวสามารถยับยั้งการเพิ่มจำนวนเซลล์มะเร็งปากมดลูกและเหนี่ยวนำให้เกิดการตายแบบ apoptosis