



Anti-Cancer Effect of Phosphatidylcholine Containing Conjugated Linoleic Acid at *sn*-2 Position on MCF-7 Breast Cancer Cell Line

Riska Rian Fauziah^{1,2}, Chin Rie¹, Tomoyuki Yoshino¹, Shinjiro Ogita¹, Yukihiro Yamamoto^{1,*}

¹Faculty of Life and Environmental Science, Prefectural University of Hiroshima, 5562 Nanatsuka-cho, Shobara, Hiroshima, 727-0023, Japan

²Faculty of Agricultural Technology, University of Jember, Jalan Kalimantan No 37, Jember, East Java, 68121, Indonesia

Correspondence: E-mail: yyamamoto@pu-hiroshima.ac.jp

ABSTRACTS

Conjugated linoleic acid (CLA) exhibits anti-cancer effects; however, most studies have only investigated its free fatty acid forms (CLA-FFA). Here, we compared the anticancer effect of CLA with its lipid forms (FFA and phosphatidylcholine [PC]) on the breast cancer cell line MCF-7 with Soy-PC as a control. CLA-FFA, CLA-PC, and Soy-PC caused a dose- and time-dependent inhibition of MCF-7 growth. However, the lowest antiproliferative activity was observed with CLA-PC. These results were consistent with Hoechst staining and protein expression of apoptosis markers Bax and Bcl-2. Interestingly, CLA-PC uptake was four times lower than that of Soy-PC, which may have affected its antiproliferative effect. Many papers showing that phospholipid forms of functional compounds have superior activity than intact forms have been reported, however, our results suggest that the PC form of CLA was inferior to its FFA form in inhibiting MCF-7 cell growth. This is the first report showing that the phospholipid form is not always the superior form.

© 2022 Tim Pengembang Jurnal UPI

ARTICLE INFO

Article History:

Submitted/Received 19 May 2022

First revised 17 Jun 2022

Accepted 16 Aug 2022

First available online 18 Aug 2022

Publication date 01 Sep 2022

Keyword:

Anticancer,
Conjugated linoleic acids,
Free fatty acid,
Phosphatidylcholine,
MCF-7 cells.

1. INTRODUCTION

Conjugated linoleic acid (CLA) is the positional and geometric isomer of linoleic acid (LA, C18:2 [n-6]). CLA has been studied for its anticarcinogenic activity since its discovery in 1987 (Ha et al., 1987). Later, it was reported to exert many other physiological functions, such as anti-obesity, anti-diabetic, and antihypertensive effects. Thus, CLA can be effective in preventing lifestyle-related diseases and metabolic syndromes (Koba & Yanagita, 2004). The United States Food and Drug Administration has approved CLA as “generally regarded as safe” for human consumption (Basak & Duttaroy, 2020). CLA is currently one of the most popular and commercially available nutraceuticals.

The World Health Organization has reported that cancer is the leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, with breast cancer being the most common cancer, accounting for 2.26 million cases (Sung et al., 2021). Even in Japan, breast cancer shows the highest incidence and more than fourteen thousand deaths in 2020 (Cancer Information Service, National Cancer Center, Japan, 2020).

The anticarcinogenic activity of CLA has been demonstrated in a chemically induced rat mammary tumor model with an effective dietary range of 0.1–1% in the diet (Ip et al., 1991). This CLA content is ordinarily found in ruminant meat and its dairy products (Schmid et al., 2006). Additionally, CLA has been shown to exert anticancer effects against breast, colon, and prostate cancer cell lines (Maggiore et al., 2004). Ip et al. (2003) suggested that CLA may be an excellent candidate for preventing breast cancer (Ip et al., 2003). Thus, a lot of concern with CLA to prevent breast cancer has got attention.

The mechanism of anticarcinogenic effects of CLA in breast cancer cells is mostly mediated by the apoptosis pathway (Majumder et al., 2002; Rakib et al., 2013;

Islam et al., 2008). Islam et al. reported that different isomers of CLA exert different anticancer effects (Rakib et al., 2013). It has been reported that *t,t*-CLA (a mixture of 1.2% *t7,t9*-CLA; 4.1% *t8,t10*-CLA; 43.5% *t9,t11*-CLA; 42.4% *t10,t12*-CLA; 5.0% *t11,t13*-CLA; and 3.8% *t12,t14*-CLA) exerted stronger anticancer activity than *c9,t11*-CLA, *t10,c12*-CLA, and LA.

The *t,t*-CLA treatment effectively induced a cytotoxic effect in a time-and concentration-dependent manner and increased the levels of tumor suppressor proteins p53 and Bax, but suppressed the expression of Bcl-2 protein. Similarly, Roz et al. reported that *t9,t11*-CLA was more efficient than *c9,t11*-CLA, and *t10,c12*-CLA in decreasing MCF-7 proliferation and inducing apoptosis after 24 h treatment (Le et al., 2019).

The majority of the studies investigating the anticancer effects of CLA used the free fatty acid (FFA) form. Phosphatidylcholine (PC) is commonly used as an emulsifier in food processing and as a component of drug delivery systems. Additionally, soy lecithin-derived liposomes are commonly used as drug delivery systems in cancer treatment, brain targeting, and vaccinology (Gandola et al., 2014). Gandola et al. (2014) reported that PC nanoparticles exerted mitogenic effects on MCF-7 cells (Gandola et al., 2014). Moreover, PC can be combined with certain nutrients to provide further health benefits (Li et al., 2015).

Thus, because phospholipid form has many advantages, we have studied phosphatidylation of functional compounds, such as terpenes, ricinoleic acid, and panthenol. Anti-cancer effects of terpenes such as geraniol, perillyl alcohol, myrtenol, and nerol were increased by phosphatidylation (Yamamoto et al., 2008).

The anti-inflammatory effects of ricinoleic acid and panthenol were also increased by phosphatidylation (Yamamoto et al., 2019;

Yamamoto *et al.*, 2020). However, no study has been reported that is showing a negative effect of the phosphatidylation.

Considering the use of PC in cancer treatment, we investigated the effects of the molecular form of CLA. We compared the individual effects of CLA in the FFA (CLA-FFA) form and PC-conjugated form (CLA-PC; with CLA at the *sn*-2 position) on proliferation and apoptosis in MCF-7 breast cancer cells, using commercially available Soy-PC as a control.

2. METHOD

2.1. Materials

CLA-FFA was commercially available (CLA Gold Gym™, Tokyo, Japan), whereas CLA-PC was prepared by phospholipase A₂-catalyzed esterification as reported previously (Fauziah *et al.*, 2020). Both samples contained CLA as a mixture of *c*9,*t*11-CLA, and *t*10,*c*12-CLA at similar concentrations. Soy-PC and glycerophosphocholine (GPC) were purchased from Holstein Co., Ltd. (Tokyo, Japan). Choline and LA were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan).

Tocopherols in the samples were removed using activated charcoal. The fatty acid compositions of CLA-FFA, CLA-PC, and Soy-PC were analyzed using gas chromatography as reported previously (Dachev *et al.*, 2021) and are summarized in **Table 1**. All solvents and

other chemicals used were of analytical grade.

2.2. Cell Culture and Sample Treatment

The MCF-7 breast cancer cell line was obtained from the Japanese Collection of Research Bioresources (JCRB0134, Osaka, Japan) and cultured in Eagle's Minimum Essentials Media (EMEM, Sigma Aldrich, Osaka, Japan) supplemented with essential amino acids (Nacalai Tesque, Kyoto, Japan), solution of 100 U/mL penicillin and 100 mg/mL streptomycin (Fujifilm Wako Pure Chemical Corp.), and 10% fetal bovine serum (FBS, Sigma Aldrich, St Louis, MO, USA) at 37 °C in a humidified 5% CO₂ incubator.

Briefly, cells were grown to 80% confluence and subcultured following detachment with 0.1% trypsin-EDTA (Denka Seiken, Tokyo, Japan). The cells were subcultured or treated with the samples for further analysis. Samples were dissolved in ethanol and the final ethanol concentration in the culture medium was ≤0.1%. Soy-PC was used as the control for CLA-PC. Cells were treated with the sample after 24 h preculture.

2.3. Analysis

For easy understanding, a flowchart of the experiment in this study is illustrated in **Figure 1**.

Table 1. Fatty acid composition of cla-ffa, cla-pc, and soy-pc used in this study.

Fatty acid	CLA-FFA (%)	CLA-PC (%)	Soy-PC (%)
Palmitic acid	5.1 ± 0.6	36.1 ± 0.9	13.6 ± 0.1
Stearic acid	1.5 ± 0.1	12.1 ± 0.4	3.1 ± 0.0
Oleic acid	12.9 ± 0.6	8.1 ± 0.5	6.9 ± 0.0
LA	1.3 ± 0.4	ND	68.5 ± 0.1
<i>c</i> 9, <i>t</i> 11-CLA	36.6 ± 0.3	19.6 ± 0.8	ND
<i>t</i> 10, <i>c</i> 12-CLA	36.9 ± 0.7	16.6 ± 0.6	ND
Linolenic acid	ND	ND	7.9 ± 0.0
n.i.	5.7 ± 0.9	7.5 ± 1.1	ND

Abbreviations: n.i., not identified; CLA, conjugated linoleic acid; FFA, free fatty acid; PC, phosphatidylcholine; LA, linoleic acid. Data are expressed as the mean ± SD (n = 3).

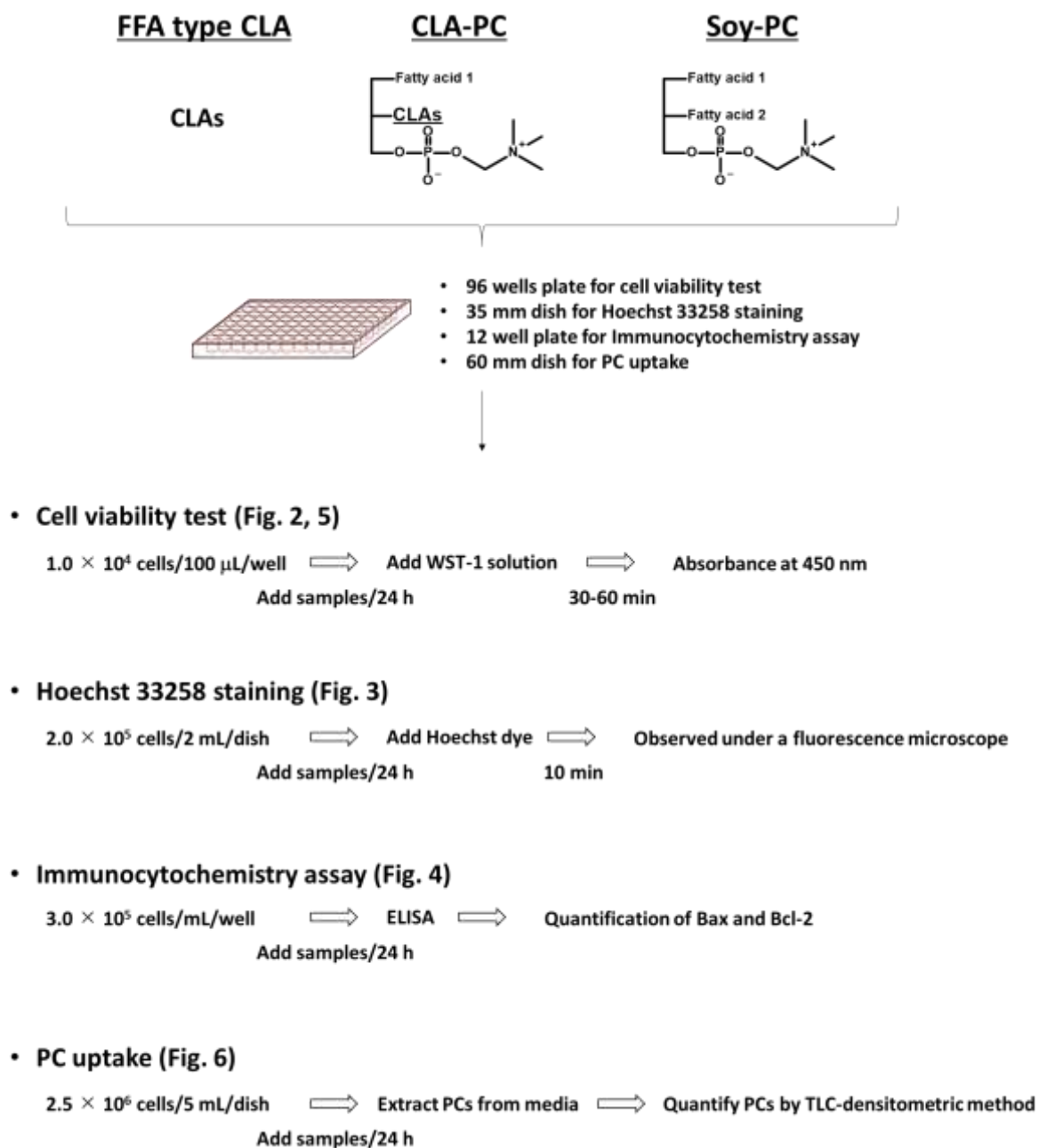


Figure 1. Flowchart of experimental of this study.

2.4. Cell Viability Test

Cell viability was assessed using 2- (4-iodophenyl) -3- (4-nitrophenyl) -5- (2,4-disulfophenyl)-2H-tetrazolium sodium salt (WST-1, Fujifilm Wako Pure Chemical). MCF-7 cells were seeded at a density of 1.0×10^4 cells/well in 96-well microplates and cultured in 100 μ L medium/well for 24 h. The samples (CLA-FFA, CLA-PC, and Soy-PC) were dissolved in ethanol at the desired

concentrations and 10 μ L of the ethanoic solution was added to each well. After 24 h incubation at 37 $^{\circ}$ C in a humidified 5% CO₂ incubator, 10 μ L of WST-1 solution was added to each well and the plate was incubated for an additional 30–60 min. Cell viability was measured at 450 nm using a microplate reader (Varioskan, Thermo Fisher Scientific, Waltham, MA, USA) and calculated as a percentage of the control.

2.5. Hoechst 33258 Staining

Treated cells were also stained with Hoechst 33258 to observe apoptosis. The cell density was 2.0×10^5 cells/2 mL/dish. Following 24 h treatment, the media was aspirated and cells were stained with 5 $\mu\text{g/mL}$ Hoechst dye (Dojindo Laboratories, Kumamoto, Japan) for 10 min and incubated at 37 °C and 5% CO_2 . The stain was aspirated and the cells were washed three times with D-PBS (Fujifilm Wako Pure Chemical Corp.) and observed under a fluorescence microscope (BZ-X700 Series; Keyence, Itasca, IL, USA).

2.6. Immunocytochemistry Assay of BAX and Bcl-2

Immunofluorescence analysis was performed to analyze the expression of Bax and Bcl-2 proteins using the Human Bax SimpleStep ELISA Kit and Bcl-2 Human ELISA Kit (both from Abcam, Cambridge, UK), respectively, as per the manufacturer's instructions. Cell culture conditions were similar to those used for WST-1 cell viability assay, except that the cells were at a density of 3×10^5 cells/mL/well in 12-well plates and treated with 200 μM of the samples.

2.7. Phosphatidylcholine (PC) Uptake

PC uptake analysis was performed to evaluate the availability of samples in the different PC forms. Cell culture conditions were similar to those used for WST-1 cell viability assay, except that the cells were seeded at a density of 5×10^5 cells/mL in 5-mL Petri dishes (60 mm) and treated with 200 μM of the samples.

PC content in the media was measured before and after 24 h treatment. PC in the media was extracted using chloroform and methanol at chloroform:methanol:water ratio of 3:10:5 (v/v/v) and until PC separated and evaporated into the chloroform layer. PC content was then measured using silica gel thin-layer chromatography (TLC), followed by

quantification using the ImageJ software (<https://imagej.nih.gov>).

First, a standard curve was prepared using Soy-PC and oleic acid (C18:1, Fujifilm Wako Pure Chemical Corp) as the internal standards in TLC plates (Merck, Darmstadt, Germany). Then, an equation with the weight ratio (Soy-PC/C18:0) and area ratio (Soy-PC/C18:1) was defined: $y = 0.4973x + 0.1528$, $R^2 = 0.9944$. Chloroform:methanol:water (65:25:4, v/v/v) was used as the mobile phase, and I_2 vapor was used for detection. The resulting spots were converted to gray-colored 16-bit images and their densities were quantified. Finally, the PC uptake was quantified using Eq. (1):

$$\text{PC uptake } (\mu\text{g/plate}) = \text{Blank} - \text{remained PC} \quad (1)$$

where "Blank" is the PC content obtained from the media-only with added PCs.

2.8. Statistical Analysis

All experiments were performed in triplicates. Data are expressed as the mean \pm standard deviation (SD). Significances were determined using Scheffe's test ($P < 0.01$).

3. RESULTS AND DISCUSSION

3.1. Growth Inhibition of Treatments on MCF-7 Cells

The inhibitory effects of the different molecular forms of CLA on cell growth were evaluated at various concentrations (50–200 μM). All samples inhibited the growth of MCF-7 cells in a dose- and time-dependent manner (**Figure 2**). Although Soy-PC showed a similar inhibitory effect to that observed with 200 μM CLA-FFA, CLA-FFA was more effective at inhibiting cell growth than Soy-PC. The lowest inhibitory effect was observed with CLA-PC. It was surprising that CLA-PC, which is phosphatidylated CLA, showed a lower effect than CLA-FFA, which is before phosphatidylation. As mentioned in the introduction section, several reports have shown that phosphatidylated functional compounds exert higher physiological activity than that before phosphatidylation

(Yamamoto et al., 2008; Yamamoto et al., 2019; Yamamoto et al., 2020). The reason why phospholipid form shows superior activity have supposed that phospholipid form has higher solubility to the media and affinity to the cell membrane (Takami and Suzuki, 1994). However, the results obtained by this study showed the opposite effect.

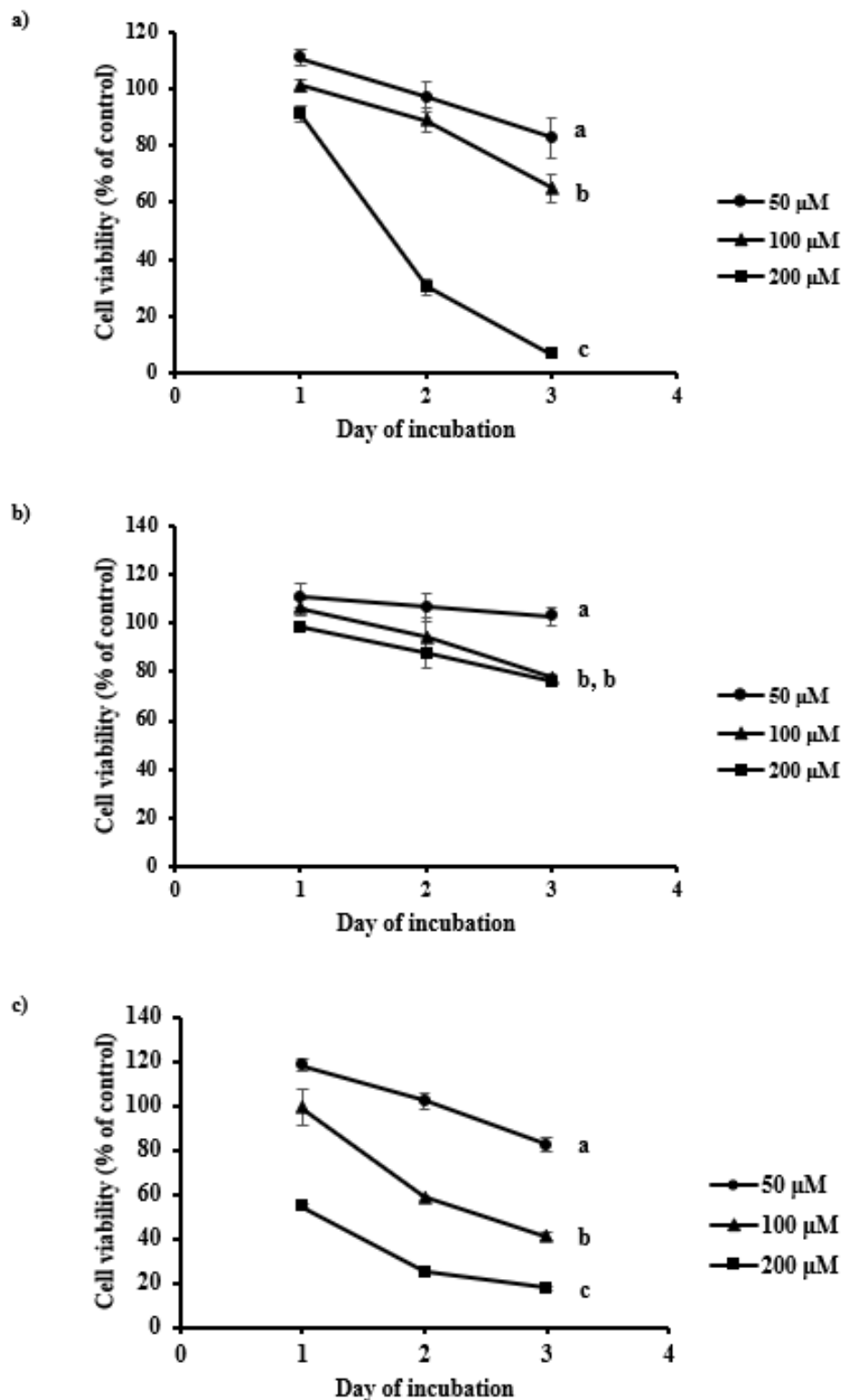


Figure 2. Dose- and time-dependent effect of (a) conjugated linoleic acid (CLA)- free fatty acid (FFA), (b) CLA- phosphatidylcholine (PC), and (c) Soy-PC on the growth of MCF-7 cells. Values are presented as a percentage (%) of the control. Different letters represent significant difference between the groups ($P < 0.01$, Scheffe's test).

3.2. Induction of Cell Apoptosis

Apoptosis is one of the most common mechanisms underlying the anticarcinogenic effect of CLA. The mechanism of apoptosis-mediated growth inhibition of MCF-7 cells by CLAs was illustrated in **Figure 3**.

Hoechst staining was performed to observe apoptosis following treatment with CLA-FFA, CLA-PC, and Soy-PC. Hoechst stain would be intact with the DNA of the cell, so when apoptosis occurred, the cells shrunk and condensed nuclei appeared under the fluorescence microscope as blue light (Islam *et al.*, 2008). The highest number of apoptotic cells was observed in CLA-FFA-treated cells, followed by Soy-PC- and CLA-PC-treated cells (**Figure 4**).

To further investigate apoptosis, an immunocytochemistry assay was performed

to analyze the protein expression of the pro-apoptotic Bax and anti-apoptotic Bcl-2. The expression of Bax and Bcl-2 proteins affects apoptosis in MCF-7 cells (Kudo and Murakami, 1999). The reciprocal expression of these proteins is used to determine whether the observed cell death is due to apoptosis (Majumder *et al.*, 2002; Rakib *et al.*, 2013; Islam *et al.*, 2008).

In the present study, CLA-FFA showed the highest Bax and lowest Bcl-2 expression (**Figure 5**). This is consistent with our finding that CLA-FFA is the most effective in inhibiting the growth of MCF-7 cells compared to CLA-PC and Soy-PC. Similarly, Islam *et al.* reported that *t,t*-CLA treatment increased the expression of Bax, but suppressed the expression of Bcl-2 (Islam *et al.*, 2008).

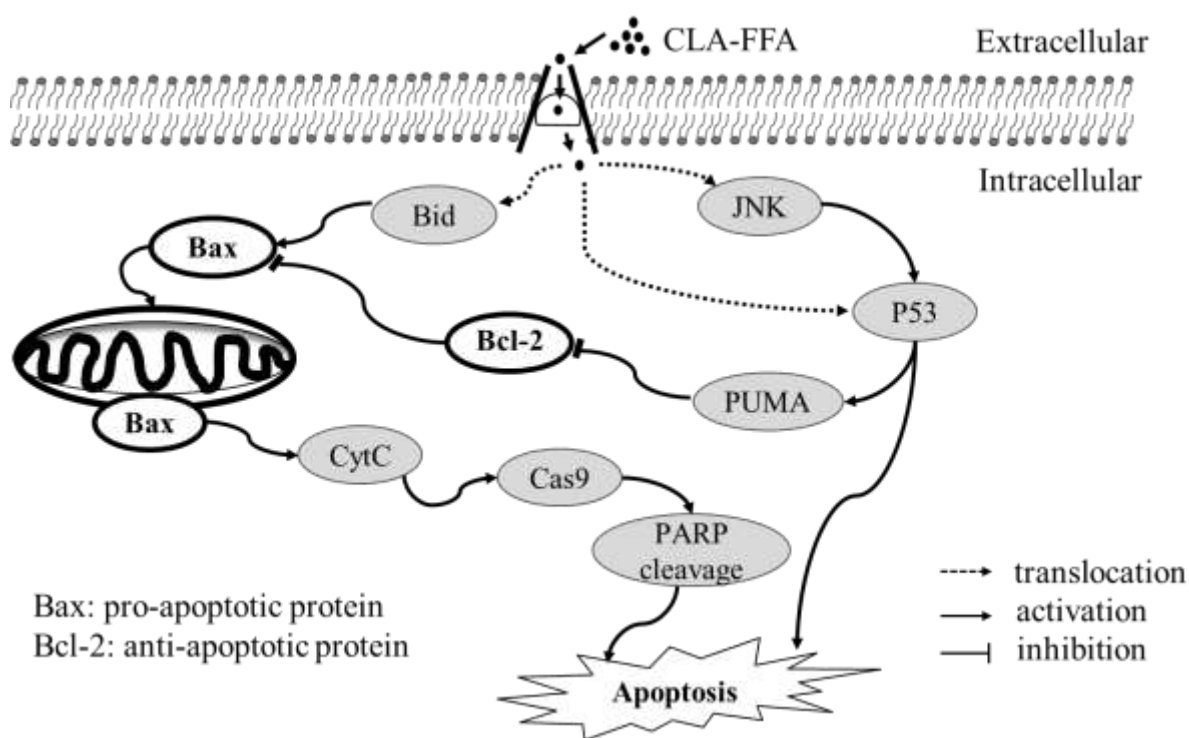


Figure 3. Mechanism of apoptotic-mediated growth inhibition of MCF-7 cells by CLAs (modified from Koronowicz *et al.*)

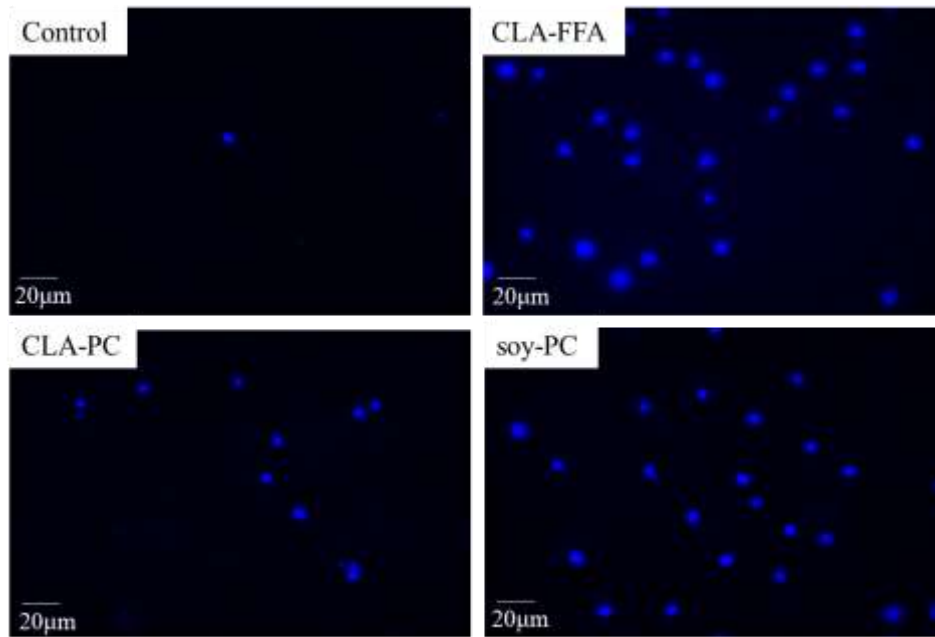


Figure 4. Hoechst stained MCF-7 cells treated for 24 h with 200 µM CLA-FFA, CLA-PC, and Soy-PC. Abbreviations: CLA, conjugated linoleic acid; FFA, free fatty acid; PC, phosphatidylcholine.

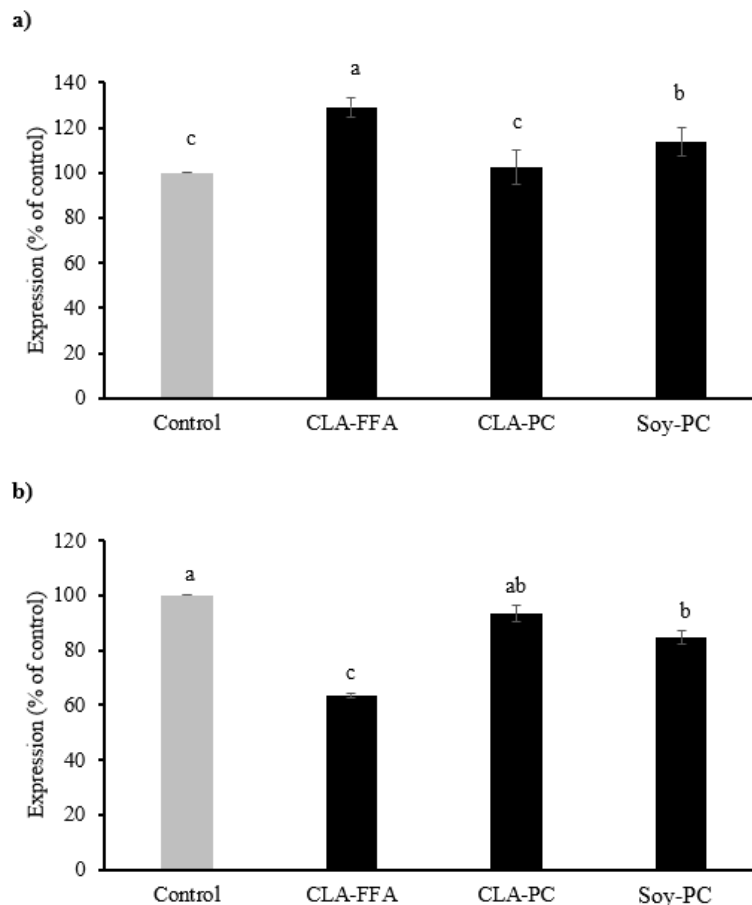


Figure 5. Protein expression of (a) Bax and (b) Bcl-2 in MCF-7 cells treated with 200 µM CLA-FFA, CLA-PC and Soy-PC. Values are presented as a percentage (%) of the control. Different letters represent significant difference between the groups ($P < 0.01$, Scheffe's test). Abbreviations: CLA, conjugated linoleic acid; FFA, free fatty acid; PC, phosphatidylcholine.

3.3. Effect of PC Component on MCF-7 Cells Growth

To determine which component of Soy-PC caused the observed inhibition of MCF-7 growth, the effect of individual PC components, choline, glycerol, GPC, and LA, on MCF-7 cell growth was investigated. CLA-FFA, CLA-PC, and Soy-PC were also used for comparison. As shown in **Figure 6**, choline, glycerol, and GPC did not significantly inhibit MCF-7 cell growth, and only LA, the major fatty acid in Soy-PC, inhibited MCF-7 growth; however, the inhibition was not as strong as that of CLA-FFA. It was suggested that Soy-PC, even though it did not contain CLA, could inhibit MCF-7 growth because it contained LA, which is the component that inhibits MCF-7 cell growth. Because Soy-PC was more easily taken up by the cell than CLA-PC (as shown later), the effect of LA, a component of Soy-PC, is stronger than that of CLA, a component of CLA-PC. LA might be released from PC at cell/perinuclear membranes by cytosolic/ Ca^{2+} -independent phospholipase A_2 -mediated hydrolysis (Kudo and Murakami, 1999).

3.4. PC Uptake by MCF-7 Cells

Inhibition of MCF-7 cell growth was higher with Soy-PC treatment than with CLA-PC

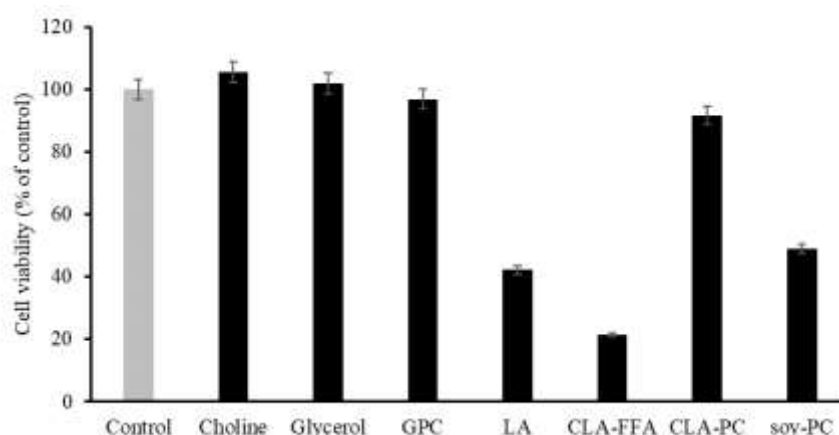


Figure 6. Cell viability of MCF-7 cells treated with PC components (Choline, Glycerol, GPC, and LA) compared to treatment with 200 μM CLA-FFA, CLA-PC, and Soy-PC. Values are presented as the mean \pm SD, a percentage (%) of the control. Different letters represent significant difference between the groups ($P < 0.01$, Scheffe's test). Abbreviations: CLA, conjugated linoleic acid; FFA, free fatty acid; PC, phosphatidylcholine.

treatment (**Figure 2, 5**). To gain insight into this phenomenon, PC uptake in MCF-7 cells treated with CLA-PC and Soy-PC was monitored. Richard and Richard reported that PC itself is supposed to be incorporated into the cell membrane followed by internalization through endocytosis and localized in the endoplasmic reticulum and Golgi apparatus in the experiment using Chinese hamster fibroblasts (Richard & Richard, 1984).

Further, Villa et al. also reported that PC is incorporated into the cell membrane, internalized, and localized in the endoplasmic reticulum of MCF-7 cells using a PC-fluorescent derivative as a sample (Villa et al., 2005). Interestingly, the uptake in CLA-PC-treated cells was significantly lower (four times lower) than that in Soy-PC-treated cells (**Figure 7**). Therefore, it can be assumed that CLA-PC exerts lower inhibition than Soy-PC on MCF-7 growth because of lower CLA-PC.

Additionally, as shown in **Table 1**, the concentration of CLA in CLA-FFA and LA in Soy-PC was approximately 70%, whereas the concentration of CLA in CLA-PC was only 36.2%; i.e., CLA content in CLA-PC was half of that in CLA-FFA and half of the LA content in Soy-PC. This difference may have affected the effectiveness of MCF-7 growth inhibition.

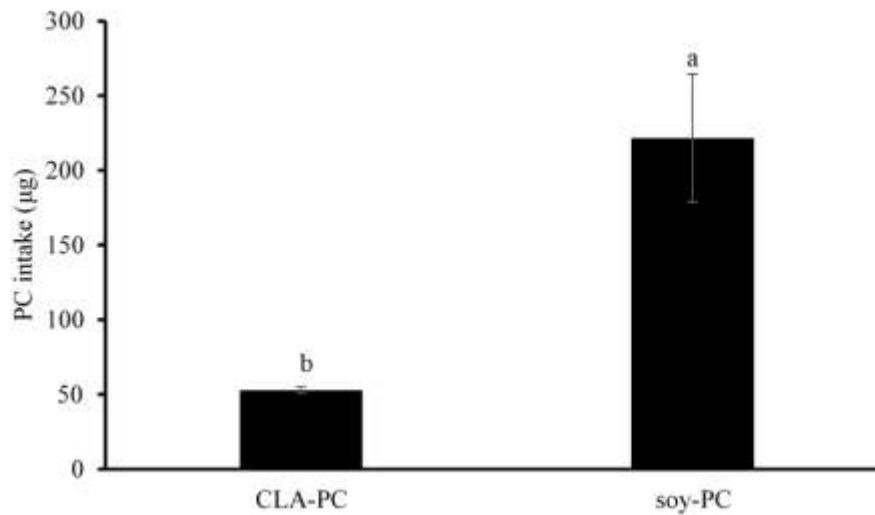


Figure 7. PC uptake in MCF-7 cells treated with 200 μ M CLA-PC and Soy-PC. Values are presented as a percentage (%) of the control. Different letters represent significant difference between the groups ($P < 0.01$, Scheffe's test). Abbreviations: CLA, conjugated linoleic acid; PC, phosphatidylcholine.

4. CONCLUSION

In the present study, treatment with CLA-FFA, CLA-PC, and Soy-PC inhibited the growth of MCF-7 cells in a dose- and time-dependent manner. CLA and its phosphatidylated form, CLA-PC exerted an antiproliferative effect on MCF-7 cells. Although the underlying mechanism was the same, the degree of the effect varied; CLA-FFA was more effective than CLA-PC in inhibiting MCF-7 cell growth, as CLA-PC was associated with lower cellular uptake. Further studies are required to

elucidate why the cellular uptake of CLA-PC is lower than CLA-FFA in MCF-7 cells.

5. ACKNOWLEDGMENTS

This study has been supported by a grant from Mishima Kaiun Memorial Foundation.

6. AUTHORS' NOTE

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article. The authors confirmed that the data and the paper are free of plagiarism.

7. REFERENCES

- Basak, S., and Duttaroy, A. K. (2020). Conjugated linoleic acid and its beneficial effects in obesity, cardiovascular disease, and cancer. *Nutrients*, 12(7), 1-7.
- Dachev, M., Bryndova, J., Jakubek, M., Moucka, Z., and Urban, M. (2021) The effects of conjugated linoleic acids in cancer. *Processes*, 9, 454.
- Fauziah, R.F., Ogita, S., Yoshino, T., and Yamamoto, Y. (2020). Effect of molecular form of conjugated linoleic acid on oxidative stability: Comparison of triacylglycerol and phosphatidylcholine form. *Journal of Oleo Science*, 69, 801-807.
- Gandola, Y. B., Perez, S.E., Irene, P.E., Sotelo, A.I., Miquet, J.G., Carradi, G.R., Carlussi, A.M., and Gonzalez, L. (2014). Mitogenic effects of phosphatidylcholine nanoparticles on mcf-7 breast cancer cells. *Biomed Research Internatioinal*, 2014, 1-13.
- Ha, Y. L., Grimm, N. K., and Pariza, M. W. (1987). Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. *Carcinogenesis*, 8(12), 1881-1887.

- Ip, C., Chin, S. F., Scimeca, J. A., and Pariza, M. W. (1991). Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Research*, 51(22), 6118-6124.
- Ip, M. M., Masso-Welch, P. A., and Ip, C. (2003). Prevention of mammary cancer with conjugated linoleic acid: Role of the stroma and the epithelium. *Journal of Mammary Gland Biology and Neoplasia*, 8(1), 103-118.
- Islam, M. A., Kim, Y. S., Jang, W. J., Lee, S. M., Kim, H. G., Kim, S. Y., and Ha, Y. L. (2008). A mixture of trans, trans conjugated linoleic acid induces apoptosis in MCF-7 human breast cancer cells with reciprocal expression of Bax and Bcl-2. *Journal of Agricultural and Food Chemistry*, 56(14), 5970-5976.
- Koba, K., and Yanagita, T. (2014). Health benefits of conjugated linoleic acid (CLA). *Obesity Research and Clinical Practice*, 8(6), e525-e532.
- Koronowicz, A.A., Drozdowska, M, Banks, P., Piasna-Slupecka, E., Domagala, D., and Leszczynska, T. (2018). Fatty acids of CLA-enriched egg yolks can induce mitochondrial pathway of apoptosis in MCF-7 breast cancer cells. *Anticancer Research*, 38, 2861-2870.
- Kudo, I., and Murakami, M. (1999). Phospholipase A2. *Tanpakushitsu Kakusan koso. Protein, Nucleic Acid, Enzyme*, 44(8 Suppl), 1013-1024.
- Le, N. T. T., Cao, V. D., Nguyen, T. N. Q., Le, T. T. H., Tran, T. T., and Hoang Thi, T. T. (2019). Soy lecithin-derived liposomal delivery systems: Surface modification and current applications. *International Journal of Molecular Sciences*, 20(19), 4706.
- Li, J., Wang, X., Zhang, T., Wang, C., Huang, Z., Luo, X., and Deng, Y. (2015). A review on phospholipids and their main applications in drug delivery systems. *Asian Journal of Pharmaceutical Sciences*, 10, 81-98.
- Maggiore, M., Bologna, M., Ceru, M.P., Possati, L., and Angelucci, A. Climini, A., Miglietta, A., Bozzo, F., Margiotta, C., Muzio, G., and Canuto R.A. (2004). An overview of the effect of linoleic and conjugated-linoleic acids on the growth of several human tumor cell lines. *International Journal of Cancer*, 112, 909-919.
- Majumder, B., Wahle, K.W.J., Moir, S., Scofield, A., and Choe, S.N., Farquharson, A., Grant, I, and Heys, S.D. (2002). Conjugated linoleic acids (CLAs) regulate the expression of key apoptotic genes in human breast cancer cells. *The FASEB Journal*, 16, 1447-1449.
- Rakib, M.A., Lee, W.S., Kim, G.S., Han, J.H., Kim, J.O., and Ha, Y.L. (2013). Antiproliferative action of conjugated linoleic acid on human MCF-7 breast cancer cells mediated by enhancement of gap junctional intercellular communication through inactivation of NF- κ b. *Evidence-Based Complementary and Alternative Medicine*, 2013, 429393.
- Richard, G.S, and Richard, E.P. (1984). Transport of a fluorescent phosphatidylcholine analog from the plasma membrane to the golgi apparatus. *Journal of Cell Biology*, 99, 742-751.
- Schmid, A., Collumb, M., Sieber, R., and Bee, G. (2006). Conjugated linoleic acid in meat and meat products: A review. *Meat Science*, 73, 29-41.
- Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., and Soerjomataram, I., Jemal, A., and Bray F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer Journal for Clinicians*, 71, 209-249.

- Takami, M., and Suzuki, Y. (1994). Enzymatic synthesis of novel phosphatidylcholine, and its enhanced cytotoxicity. *Bioscience Biotechnology and Biochemistry*, 58, 1897-1898.
- Villa, A.M., Caporizzo, E., Papagni, A., Miozzo, L., Del Buttero, P, Grilli, M. D., Amboldi, N., Fazio F., Doglia, S.M., and Giglioni, B. (2005) Choline and phosphatidylcholine fluorescent derivatives localization in carcinoma cells studied by laser scanning confocal fluorescence microscopy. *European Journal of Cancer*, 41, 1453-1459.
- Yamamoto, Y., Harada, K., Kasuga, S., and Hosokawa, M. (2019). Phospholipase A2-mediated preparation of phosphatidylcholine containing ricinoleic acid and its anti-inflammatory effect on murine macrophage-like RAW264.7 cells. *Biocatalysis and Agricultural Biotechnology*, 19, 101141.
- Yamamoto, Y., Hosokawa, M., Kurihara, H., Maoka, T., and Miyashita, K. (2008). Synthesis of phosphatidylated-monoterpene alcohols catalysed by phospholipase D and their antiproliferative effects on human cancer cells. *Bioorganic and Medicinal Chemistry Letters*, 18, 4044-4046.
- Yamamoto, Y., Suzuri, K., Kunii, T., Kurihara, H., Miyashita, K., Hosokawa, M. (2020). Preparation of phosphatidyl-pantenol by phospholipase D-mediated transphosphatidylation and its anti-inflammatory activity on macrophage-like RAW264.7 cells. *Biocatalysis and Agricultural Biotechnology*, 26, 101629.