JPP Journal of Pharmacy and Pharmacology

JPP Journal of Pharmacy and Pharmacology

JPP 2010, 62: 1711–1716

Received August 6, 2009
Accepted May 6, 2010
DOI
10.1111/j.2042-7158.2010.01145.x
ISSN 0022-3573

Research Paper

Lutein is a competitive inhibitor of cytosolic Ca^{2+}-dependent phospholipase A_2

Ho Sun Song, Hee Rae Kim, Myung Cheul Kim, Yeon Hee Hwang and Sang Soo Sim

College of Pharmacy, Chung-Ang University, 221 Huksuk-dong, Dongjak-gu, Seoul 156-756, Korea

Abstract

Objectives We have investigated the effect of lutein on phospholipase A_2 (PLA_2) isozymes.

Methods We measured arachidonic acid release in [^{3}H]arachidonic acid-labelled Raw 264.7 cells and PLA_2 activity using 1-palmitoyl-2-[^{14}C]arachidonyl phosphatidylcholine ([^{14}C]AA-PC) and 10-pyrene phosphatidylcholine *in vitro*.

Key findings Lutein suppressed the release of arachidonic acid and inhibited Raw 264.7 cell-derived cytosolic Ca^{2+}-dependent PLA_2 ($cPLA_2$)-induced hydrolysis of [^{14}C]AA-PC in a dose- and time-dependent manner. In contrast, lutein did not affect secretory Ca^{2+}-dependent PLA_2 ($sPLA_2$)-induced hydrolysis of [^{14}C]AA-PC. A Dixon plot showed that the inhibition by lutein on $cPLA_2$ appeared to be competitive with an inhibition constant, K_i, of 13.6 μM.

Conclusions We suggest that lutein acted as a competitive inhibitor of $cPLA_2$ but did not affect $sPLA_2$.

Keywords arachidonic acid; lutein; phospholipase A_2

Introduction

Phospholipase A_2 (PLA_2) cleaves the sn-2 ester bond of cellular phospholipids, producing arachidonic acid and lysophospholipid. Arachidonic acid is the biosynthetic precursor for the eicosanoid family of potent inflammatory mediators, such as prostaglandins, thromboxanes, leukotrienes and lipoxins. Eicosanoids play a role in a wide range of physiological and pathological processes such as immune responses, inflammation and pain perception.[1,2] The PLA_2 superfamily is composed of three main types of lipolytic enzymes including secretory PLA_2 ($sPLA_2$), cytosolic Ca^{2+}-dependent PLA_2 ($cPLA_2$) and Ca^{2+}-independent PLA_2 ($iPLA_2$).[3] $sPLA_2$ has a low molecular mass (~18 kDa) and can be activated at millimolar concentrations of Ca^{2+}. It is secreted by inflammatory cells upon their activation and by damaged tissues in inflammation-related diseases.[4] $cPLA_2$ is located in the cytosol, has a high molecular mass (~85 kDa), and can be activated by micromolar concentrations of intracellular Ca^{2+}. $iPLA_2$ is found in both the cytosol and membrane fractions, has a molecular mass ranging from 29 to 85 kDa, and does not require Ca^{2+} for activation.

Lutein is abundantly present in dark, leafy green vegetables, such as spinach and kale.[5] Humans cannot synthesize carotenoids *de novo*, and thus their presence in human blood and tissues is due to the intake of food. The chemical structures of lutein and its stereo isomer, zeaxanthin, are characterized by the presence of a hydroxyl group attached to each side of the molecule; these xanthophylls are more hydrophilic than other carotenoids found in blood and tissues.[6,7] In plants lutein has antioxidant activity and offers protection against photo-induced free radical damage.[8] In humans lutein is highly concentrated in the macula, a small area of the retina that is responsible for central vision.[9] Serum levels of lutein have an inverse relationship with the risk for ocular diseases, including age-related macular degeneration and cataracts.[10,11] Also, lutein may be important for skin and heart health as well as eye health as it helps maintain heart health by reducing the risk of atherosclerosis and skin health by reducing UV-induced damage.[12,13]

Recently, lutein was shown to inhibit nuclear factor kappa B (NF-κB)-dependent expression of genes, such as nitric oxide synthase and cyclooxygenase-2, through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-κB-inducing kinase pathways.[14] These data suggested that lutein has anti-inflammatory activity. Therefore, the aim

Correspondence: Sang Soo Sim, Department of Pathophysiology, College of Pharmacy, Chung-Ang University, 221 Huksuk-dong, Dongjak-gu, Seoul 156-756, Korea.
E-mail: simss@cau.ac.kr

of this study was to investigate the effect of lutein on PLA_2 isozymes, which are very important enzymes in the production of a variety of inflammatory mediators.

Materials and Methods

Chemicals

Pure lutein isolated from *Tagetes erecta* was supplied by Arlico Pharm Co (Seoul, Korea). PLA_2 from honey bee venom, melittin, bromoenol lactone and arachidonyl trifluoromethyl ketone ($AACOCF_3$) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dulbecco's modified Eagle minimum essential medium (DMEM) and fetal bovine serum were purchased from Invitrogen Co. (Grand Island, NY, USA). 10-Pyrene phosphatidylcholine (10-pyrene PC) was purchased from Molecular Probes (Leiden, Netherlands). [^{3}H]Arachidonic acid was obtained from NEN (Boston, MA, USA). 1-Palmitoyl-2-[^{14}C]arachidonyl phosphatidylcholine ([^{14}C]AA-PC) was obtained from Perkin Elmer (Boston, MA, USA). 1-Palmitoyl-2-arachidonyl phosphatidylcholine was obtained from Avanti Polar Lipid (Alabaster, AL, USA).

Cell culture

Raw 264.7 cells (mouse macrophage) were obtained from the Korean Cell Line Bank (Seoul, Korea). Cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum and antibiotic–antimycotic (100 IU/ml benzyl penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B) at 37°C with 5% CO_2.

Measurement of [^{3}H]arachidonic acid release

Raw 264.7 cells were harvested with Krebs buffer (in mM: 137 NaCl, 2.7 KCl, 0.4 Na_2HPO_4, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 5 glucose, and 10 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); pH 7.4)) and labelled with [^{3}H]arachidonic acid (0.4 μCi/ml) at 37°C for 2 h. The cells were washed with Krebs buffer containing 0.5 mg/ml bovine serum albumin to trap the liberated [^{3}H]arachidonic acid. Labelled cells were pre-incubated with indicated concentrations of lutein. Arachidonic acid release was induced by stimulating cells with 0.5 μM melittin for 30 min. After centrifugation, the supernatant and pellet were transferred to a liquid scintillation vial and scintillation fluid was added. Radioactivity was measured by liquid scintillation counter.[15] The percent release of [^{3}H]arachidonic acid was equal to [supernatant / (supernatant + pellet)] × 100.

Preparation of the Raw 264.7 cell-derived PLA_2 isozyme

Raw 264.7 cells were washed in 10 mM HEPES buffer (pH 7.5) containing 1 mM EDTA and 340 mM sucrose and then lysed by sonication. Cell lysates were centrifuged at 10 000*g* for 30 min at 4°C and the supernatant was used as the source of $iPLA_2$.

To prepare the source of $sPLA_2$ and $cPLA_2$, Raw 264.7 cells were washed and sonicated in 10 mM Tris-HCl buffer (pH 7.4) containing 100 mM NaCl, 2 mM EGTA (ethylene glycol tetra-acetic acid), 100 μM leupeptin, 150 μM aprotinin, and 1 mM Na_3VO_4. The lysate was centrifuged at 10 000*g* for 30 min at 4°C and the supernatants were stored at –70°C until used to supply $sPLA_2$ and $cPLA_2$.[16]

$cPLA_2$, $sPLA_2$ and $iPLA_2$ assay

PLA_2 activity was assayed by measuring [^{14}C]arachidonic acid hydrolysed from [^{14}C]AA-PC. For the measurement of inhibition of $cPLA_2$ activity, the cellular enzyme source and lutein were incubated for 30 min at room temperature in 100 mM Tris-HCl buffer (pH 8.5) containing 10 μM bromoenol lactone (as an $iPLA_2$ inhibitor), 5 mM $CaCl_2$ and 1 mM dithiothreitol (as an $sPLA_2$ inhibitor).[17,18] The reaction mixture was incubated for a given time with 0.025 μCi [^{14}C]AA-PC as a substrate. For the measurement of $sPLA_2$ activity, 10 μM arachidonyl trifluoromethyl ketone ($AACOCF_3$), a $cPLA_2$ inhibitor, was added instead of dithiothreitol.[19] For the measurement of $iPLA_2$ activity, dithiothreitol and 0.4 mM Triton X-100 were added to 100 mM HEPES buffer (pH 7.5), as was 5 mM EDTA to inhibit $cPLA_2$, while bromoenol lactone and $CaCl_2$ were omitted.

Each reaction mixture was incubated at 37°C for the indicated time and stopped by adding 560 μl modified Doles reagent (n-heptane / isopropyl alcohol / 1M H_2SO_4; 400 / 390 / 10).[20] After centrifugation, 150 μl of the upper phase was transferred to a new tube, to which 800 μl n-heptane and silica gel (10 mg) were added. The mixtures were mixed and centrifuged again for 2 min, after which 800 μl supernatant was moved into 1.0 ml scintillation solution and counted for radioactivity in a Packard Tri-carb liquid scintillation counter. The specific activity in picomoles per minute per milligram of protein (pmol/min/mg) was obtained by dividing the activity by the amount of enzyme protein. To calculate the specific activity, protein was analysed with a BCA (bicinchoninic acid) protein assay kit.

The hydrolysis ratio of [^{14}C]AA-PC was applied to compose a Dixon plot. This is a graphical method for determination of the type of enzyme inhibition and the inhibition constant (K_i) for an enzyme–inhibitor complex. For the Dixon plot, the concentration of the substrate was increased by adding 1-palmitoyl-2-arachidonyl phosphatidylcholine.

$sPLA_2$ assay with 10-pyrene phosphatidylcholine

PLA_2 activity was measured with a fluorospectrophotometer using pyrene-labelled phosphatidylcholine (10-pyrene PC) in the presence of serum albumin.[21] PLA_2 purified from honey bee venom was used as $sPLA_2$.[22] $sPLA_2$ and lutein were vortex-mixed and incubated for 10 min at room temperature. This enzyme mixture was incubated with a reaction mixture (1 ml; containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 10 μl 0.2 mM 10-pyrene PC (2 μM, final concentration), 10 μl 10% bovine serum albumin solution, and 6 μl 1 M $CaCl_2$) for 20 min. The fluorescence was measured using excitation and emission wavelengths of 345 and 398 nm, respectively, with a spectrophotometer (FL600, Microplate Fluorescence Reader, Bio-Tek Winooski, VT, USA).

Statistical analysis

Results are represented as means ± SD and were analysed statistically with analysis of variance, and differences between groups were determined with the Newman–Keul's test. The level of significance was set at less than 5%.

Table 1 Dose-response of melittin to [^{3}H]arachidonic acid release in Raw 264.7 cells

	Concn (μM)	% release
Control	0	4.7 ± 0.9
Melittin	0.05	6.6 ± 1.3
	0.1	23.7 ± 4.7*
	0.5	56.9 ± 3.7*
AACOCF$_3$ (10 μM) + melittin	0.5	36.8 ± 1.9†
Dithiothreitol (1 mM) + melittin	0.5	55.9 ± 2.5

Melittin dose-dependently induced [$_3$H]arachidonic acid release in Raw 264.7 cells. Melittin-induced [^{3}H]arachidonic acid release was significantly inhibited by 10 μM arachidonyl trifluoromethyl ketone (AACOCF$_3$), but not by 1 mM dithiothreitol. Results are means ± SD from four separate experiments. *$P < 0.05$, compared with control. $^\dagger P < 0.05$ compared with melittin 0.5 μM.

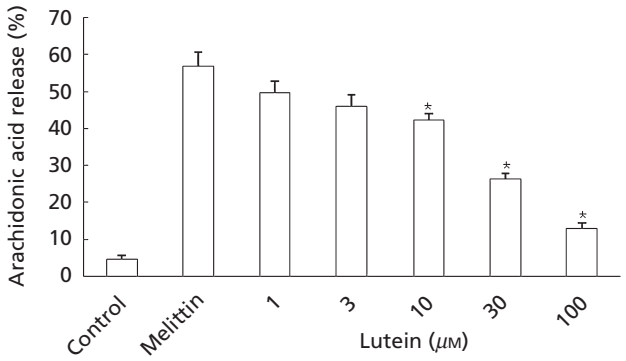

Figure 1 Effect of lutein on [^{3}H]arachidonic acid release in Raw 264.7 cells stimulated by melittin. The cells were incubated with lutein for 10 min and [^{3}H]arachidonic acid release was induced by melittin 0.5 μM. Results shown are means ± SD from four separate experiments. *$P < 0.05$ compared with melittin alone.

Results

Effect of lutein on melittin-induced arachidonic acid release in [^{3}H]arachidonic acid-labelled Raw 264.7 cells

To investigate the effect of lutein on PLA$_2$ activity in a cellular system, we measured melittin-induced arachidonic acid release in [^{3}H]arachidonic acid-labelled Raw 264.7 cells. Melittin increased arachidonic acid release in Raw 264.7 cells in a dose-dependent manner (Table 1), causing an increase in arachidonic acid release by 56.9% at a concentration of 0.5 μM. Melittin-induced [^{3}H]arachidonic acid release was significantly inhibited by 10 μM AACOCF$_3$, but not by 1 mM dithiothreitol. Lutein significantly decreased the release of [^{3}H]arachidonic acid induced by 0.5 μM melittin from 56.9 ± 3.7 to 42.2 ± 1.8%, 26.2 ± 1.6%, and 13.0 ± 1.6% at concentrations of 10, 30, and 100 μM, respectively (Figure 1).

Raw cell-derived PLA$_2$ activity

We measured sPLA$_2$, cPLA$_2$ and iPLA$_2$ activity using [^{14}C]AA-PC with the assay conditions as described in the Materials and Methods. The activity of Raw 264.7 cell-derived

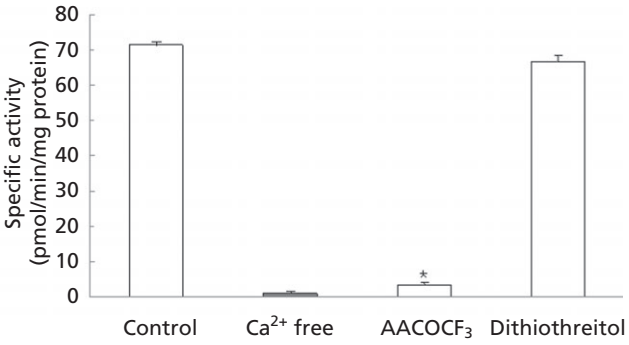

Figure 2 Raw 264.7 cell-derived PLA$_2$ activity in the presence of 5 mM CaCl$_2$. Raw 264.7 cell-derived PLA$_2$ (25 μg protein) was incubated with 1-palmitoyl-2-[^{14}C]arachidonyl phosphatidylcholine in the presence of 10 μM arachidonyl trifluoromethyl ketone (AACOCF$_3$; cPLA$_2$ inhibitor) or 1 mM dithiothreitol (sPLA$_2$ inhibitor) and in the absence of CaCl$_2$. Results are means ± SD from four separate experiments. *$P < 0.05$ compared with control.

Table 2 PLA$_2$ activity using 10-pyrene phosphatidylcholine

	Protein (μg)	Fluorescence value
None	0	1813 ± 98
Raw cell-derived PLA$_2$	2.5	1779 ± 75
	25	1765 ± 68
	250	1760 ± 80
Bee venom sPLA$_2$	0.25	1430 ± 120*
	0.5	1155 ± 104*
	1	678 ± 110*
AACOCF$_3$ (10 μM) + bee venom sPLA$_2$	1	736 ± 88
Dithiothreitol (100 mM) + bee venom sPLA$_2$	1	1305 ± 82†

Bee venom sPLA$_2$ and Raw 264.7 cell-derived PLA$_2$ activity using 10-pyrene phosphatidylcholine (10-pyrene PC), an sPLA$_2$-specific substrate. Bee venom sPLA$_2$ dose-dependently hydrolysed 10-pyrene PC and was significantly inhibited by 100 mM dithiothreitol, but not by 10 μM arachidonyl trifluoromethyl ketone (AACOCF$_3$). Raw 264.7 cell-derived PLA$_2$ did not show any PLA$_2$ activity. Results are means ± SD from four separate experiments. *$P < 0.05$ compared with nne. $^\dagger P < 0.05$ compared with 1 μg bee venom sPLA$_2$.

PLA$_2$ was significantly inhibited in the presence of 10 μM AACOCF$_3$ by 95.4%, but was not influenced by 1 mM dithiothreitol. In the absence of Ca^{2+}, the specific activity of Raw 264.7 cell-derived iPLA$_2$ was 20-fold lower than that of cPLA$_2$, so that the activity of iPLA$_2$ was too low to detect any inhibitory effect of lutein on iPLA$_2$ (Figure 2). In the assay using a sPLA$_2$-specific substrate, 10-pyrene PC, sPLA$_2$ obtained from honey bee venom dose-dependently hydrolysed 10-pyrene PC and was significantly inhibited by 100 mM dithiothreitol, but not by 10 μM AACOCF$_3$.[23] On the other hand, Raw 264.7 cell-derived PLA$_2$ did not show any PLA$_2$ activity with this substrate (Table 2). These data suggested that the Raw 264.7 cell-derived PLA$_2$ contained mainly cPLA$_2$.

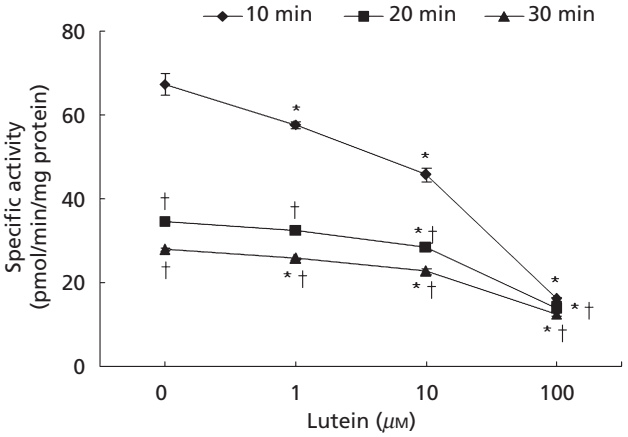

Figure 3 Dose- and time-dependent effects of lutein on Raw 264.7 cell-derived $cPLA_2$. Raw 264.7 cell-derived PLA_2 (25 μg protein) was incubated with 1-palmitoyl-2-[^{14}C]arachidonyl phosphatidylcholine in the presence of lutein for a given time. Results indicate means ± SD from four separate experiments. *$P < 0.05$ compared with control (without lutein) at the same time. †$P < 0.05$ compared with the value of 10 min at the same concentration.

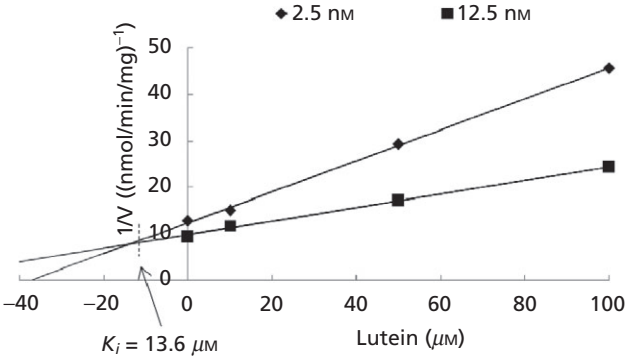

Figure 4 Determination of the inhibitory pattern of lutein on $cPLA_2$ using a Dixon plot. Raw 264.7 cell-derived PLA_2 (25 μg protein) was incubated with 2.5 or 12.5 mol 1-palmitoyl-2-arachidonyl phosphatidylcholine in the presence or absence of lutein for 30 min at 37°C. Results shown are means ± SD from four separate experiments. A Dixon plot is a graphical method for determination of the type of enzyme inhibition and the inhibition constant (K_i) for an enzyme–inhibitor complex.

Inhibitory effect of lutein on cytosolic PLA_2 activity using [^{14}C]AA-PC

Lutein showed significant inhibition of $cPLA_2$ in a dose- and time-dependent manner (Figure 3). Lutein inhibited $cPLA_2$ activity by 18.7 and 55.2% at concentrations of 10 and 100 μM, respectively. To determine the pattern of inhibition of $cPLA_2$ by lutein, Dixon plots were constructed from the hydrolysis rates for the substrate ([^{14}C]AA-PC at 2.5 and 12.5 nM), induced by $cPLA_2$, at various concentrations of lutein. Figure 4 illustrates that the apparent K_i value of lutein on $cPLA_2$ was 13.6 μM. Such an inhibitory pattern suggested that lutein acted as a competitive inhibitor against $cPLA_2$.

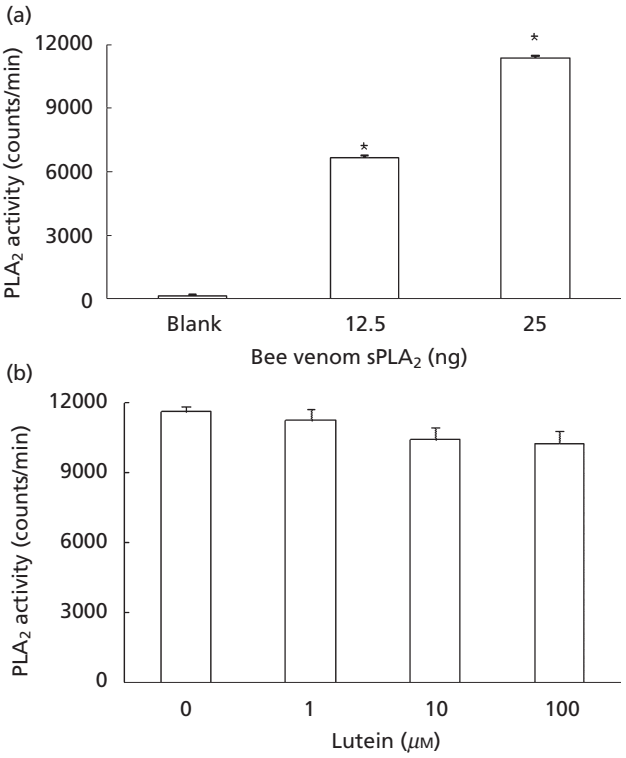

Figure 5 Effect of lutein on bee venom $sPLA_2$-induced 1-palmitoyl-2-[^{14}C]arachidonyl phosphatidylcholine hydrolysis. (a) $sPLA_2$ dose-dependently hydrolysed 1-palmitoyl-2-[^{14}C]arachidonyl phosphatidylcholine ([^{14}C]AA-PC). (b) Lutein did not affect $sPLA_2$ (25 ng)-induced [^{14}C]AA-PC hydrolysis. Results shown are means ± SD from four separate experiments.

Effect of lutein on $sPLA_2$ using [^{14}C]AA-PC and 10-pyrene phosphatidylcholine

To investigate the inhibitory effect of lutein on $sPLA_2$, we used purified $sPLA_2$ from honey bee venom. Using [^{14}C]AA-PC as substrate under the specific conditions described in the Materials and Methods section, purified $sPLA_2$ from honey bee venom hydrolysed [^{14}C]AA-PC in a dose-dependent manner (Figure 5a). Lutein did not affect purified $sPLA_2$ when using the [^{14}C]AA-PC substrate (Figure 5b). To confirm that lutein had no effect on $sPLA_2$, we measured PLA_2 activity using 10-pyrene PC as a $sPLA_2$ substrate and purified $sPLA_2$.[23] As shown in Figure 6, $sPLA_2$ was not affected by lutein, further indicating that lutein did not have any influence on Ca^{2+}-dependent $sPLA_2$ but selectively inhibited cytosolic Ca^{2+}-dependent PLA_2.

Discussion

In this study, lutein decreased melittin-induced [^{3}H]arachidonic acid release in Raw 264.7 cells in a dose-dependent manner. Melittin has been used as an endogenous PLA_2 activator by increasing intracellular Ca^{2+} concentration via receptor-operated calcium channels.[24] The observed inhibitory effect of lutein may have been related to the inhibition of PLA_2. However, arachidonic acid release is an indirect

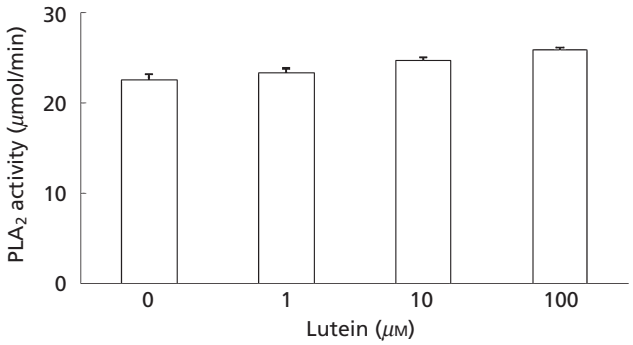

Figure 6 Effect of lutein on bee venom sPLA$_2$-induced 10-pyrene phosphatidylcholine hydrolysis. sPLA$_2$ (1 μg) significantly hydrolysed 10-pyrene phosphatidylcholine (an sPLA$_2$ substrate), but was not affected by lutein. Results shown are means ± SD from four separate experiments.

measure of PLA$_2$ activity because there are other enzymes, such as arachidonyl-CoA synthetase, CoA-dependent acyltransferase, and CoA-independent transacylase that are involved in free arachidonic acid production.[25] To circumvent this problem, we measured the direct effect of lutein on PLA$_2$ isozymes using [^{14}C]AA-PC, a substrate specific to PLA$_2$.

Since we did not prepare each PLA$_2$ isozyme individually, the lysates prepared from Raw 264.7 cells were used as the source of a mixture of PLA$_2$ isozymes. We found that Raw 264.7 cell-derived PLA$_2$ was completely inhibited by AACOCF$_3$ (a cPLA$_2$ inhibitor) but not by dithiothreitol (a sPLA$_2$ inhibitor) or bromoenol lactone (an iPLA$_2$ inhibitor). Since sPLA$_2$ contains several disulfide bonds, it was inactivated upon exposure to the reducing agent dithiothreitol.[26] On the other hand, Raw 264.7 cell-derived PLA$_2$ showed only a low level of activity in the absence of Ca^{2+}. These observations suggested that Raw 264.7 cell-derived PLA$_2$ contained mainly cPLA$_2$ rather than sPLA$_2$ or iPLA$_2$. In Raw 264.7 cells, iPLA$_2$ activity was found to be increased by exposure to 100 μM H_2O_2.[16] In this experiment, iPLA$_2$ activity isolated from 100 μM H_2O_2-treated Raw 264.7 cells, despite being elevated, was still too low to investigate the potentially inhibitory effect of lutein on iPLA$_2$. To further investigate the effect of lutein on sPLA$_2$, we used sPLA$_2$ purified from honey bee venom. This source of sPLA$_2$ dose-dependently hydrolysed [^{14}C]AA-PC, but lutein did not appear to affect sPLA$_2$-mediated hydrolysis of this substrate. Furthermore, a similar result was found using a different sPLA$_2$ substrate, 10-pyrene PC. These data suggested that lutein inhibited cPLA$_2$ but not sPLA$_2$, while the effect of lutein on iPLA$_2$ remains to be determined.

It has been reported that lutein modified dipalmitoylphosphatidylcholine membrane via interaction with sn-2- palmitic acid.[27] In this experiment, we used a specific PLA$_2$ substrate, 1-palmitoyl-2-arachidonyl phosphatidylcholine. Palmitic acid as a saturated fatty acid shows a linear form, but arachidonic acid as an unsaturated fatty acid includes four double bonds, forming bending structures. The structural difference between the two fatty acids could be affected differently by lutein. However, we could not rule out the possibility that the inhibitory effect of lutein on PLA$_2$ was related to the modification of PLA$_2$ substrate.

We next established the inhibitory pattern of lutein on cPLA$_2$ with Dixon plots. Lutein inhibited cPLA$_2$-induced hydrolysis of [^{14}C]AA-PC in a dose- and time-dependent manner. The Dixon plot constructed showed that the inhibition by lutein appeared to be competitive with a K_i value of 13.6 μM. It has been reported that cPLA$_2$ was inhibited by a trifluoromethyl ketone analogue of arachidonic acid (AACOCF$_3$), which presumably bound directly to the active site of cPLA$_2$.[19,28,29] The K_i values for cPLA$_2$ by lutein and AACOCF$_3$ were 13.6 and 0.65 μM, respectively, suggesting that the inhibitory potency of lutein on cPLA$_2$ was lower than that of AACOCF$_3$.[30] Considering this data, we suggest that lutein was a competitive inhibitor of cPLA$_2$ with a K_i value of 13.6 μM, but that it did not affect sPLA$_2$. PLA$_2$ activation resulted in the production of eicosanoid complex, which plays an important role in inflammatory responses. Therefore, PLA$_2$ inhibition by lutein may have caused the attenuation of the inflammatory response.

Conclusions

Lutein decreased melittin-induced [^{3}H]arachidonic acid release in Raw 264.7 cells and inhibited Raw 264.7 cell-derived cPLA$_2$-induced hydrolysis of [^{14}C]AA-PC in a dose- and time-dependent manner, but did not affect sPLA$_2$-induced hydrolysis. A Dixon plot showed that the inhibition of cPLA$_2$ by lutein appeared to be competitive with a K_i value of 13.6 μM. We suggest that lutein acted as a competitive inhibitor of cPLA$_2$ but did not affect sPLA$_2$.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by a Chung-Ang University Research grant in 2010.

References

1. Glaser KB. Regulation of phospholipase A$_2$ enzymes: selective inhibitors and their pharmacological potential. *Adv Pharmacol* 1995; 32: 31–66.
2. Calunga JL *et al*. Ozone oxidative post-conditioning in acute renal failure. *J Pharm Pharmacol* 2009; 61: 221–227.
3. Kudo I, Murakami M. Phospholipase A$_2$ enzymes. *Prostaglandins Other Lipid Mediat* 2002; 68–69: 3–58.
4. Murakami M *et al*. The functions of five distinct mammalian phospholipase A$_2$s in regulating arachidonic acid release. Type IIa and type V secretory phospholipase A$_2$s are functionally redundant and act in concert with cytosolic phospholipase A$_2$. *J Biol Chem* 1998; 273: 14411–14423.
5. Sommerburg O *et al*. Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *Br J Ophthalmol* 1998; 82: 907–910.
6. Granado F *et al*. Nutritional and clinical relevance of lutein in human health. *Br J Nutr* 2003; 90: 487–502.
7. Nakajima Y *et al*. Astaxanthin, a dietary carotenoid, protects retinal cells against oxidative stress in-vitro and in mice in-vivo. *J Pharm Pharmacol* 2008; 60: 1365–1374.

8. Demmig-Adams B *et al*. Carotenoids 3: in vivo function of carotenoids in higher plants. *FASEB J* 1996; 10: 403–412.
9. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys* 2001; 385: 28–40.
10. Mares-Perlman JA *et al*. Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third National Health and Nutrition Examination Survey. *Am J Epidemiol* 2001; 153: 424–432.
11. Brown L *et al*. A prospective study of carotenoid intake and risk of cataract extraction in US men. *Am J Clin Nutr* 1999; 70: 517–524.
12. Dwyer JH *et al*. Oxygenated carotenoid lutein and progression of early atherosclerosis – The Los Angeles Atherosclerosis Study. *Circulation* 2001; 103: 2922–2927.
13. Stahl W *et al*. Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. *Am J Clin Nutr* 2000; 71: 795–798.
14. Kim JH *et al*. The non-provitamin A carotenoid, lutein, inhibits NF-kB-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-kB-inducing kinase pathways: role of H_2O_2 in NF-kB activation. *Free Radic Biol Med* 2008; 45: 885–896.
15. Balboa MA *et al*. Regulation of arachidonic acid mobilization in lipopolysaccharide-activated P388D(1) macrophages by adenosine triphosphate. *J Biol Chem* 1999; 274: 36764–36768.
16. Martinez J, Moreno JJ. Role of Ca^{2+}-independent phospholipase A_2 on arachidonic acid release induced by reactive oxygen species. *Arch Biochem Biophys* 2001; 392: 257–262.
17. Ackermann EJ *et al*. Inhibition of macrophage Ca^{2+}-independent phospholipase A_2 by bromoenol lactone and trifluoromethyl ketones. *J Biol Chem* 1995; 270: 445–450.
18. Fonteh AN *et al*. Evidence that secretory phospholipase A_2 plays a role in arachidonic acid release and eicosanoid biosynthesis by mast cells. *J Immunol* 1994; 152: 5438–5446.
19. Riendeau D *et al*. Arachidonyl trifluoromethyl ketone, a potent inhibitor of 85-kDa phospholipase A_2, blocks production of arachidonate and 12-hydroxyeicosatetraenoic acid by calcium ionophore-challenged platelets. *J Biol Chem* 1994; 269: 15619–15624.
20. Dole VP, Meinertz H. Microdetermination of long-chain fatty acids in plasma and tissues. *J Biol Chem* 1960; 235: 2595–2599.
21. Radvanyi FI *et al*. A sensitive and continuous fluorometric assay for phospholipase A_2 using pyrene-labeled phospholipids in the presence of serum albumin. *Anal Biochem* 1989; 177: 103–109.
22. Wichmann O *et al*. Probing phospholipase A_2 with fluorescent phospholipid substrates. *Chembiochem* 2007; 8: 1555–1569.
23. Lusa S *et al*. Degradation of pyrene-labelled phospholipids by lysosomal phospholipases in vitro. Dependence of degradation on the length and position of the labelled and unlabelled acyl chains. *Biochem J* 1996; 315: 947–952.
24. Nielsen OH *et al*. Arachidonic acid and calcium metabolism in rnelittin stimulated neutrophils. *Mediators Inflamm* 1992; 1: 313–317.
25. Lio YC *et al*. Irreversible inhibition of Ca^{2+}-independent phospholipase A_2 by methyl arachidonyl fluorophosphonate. *Biochim Biophys Acta* 1996; 1302: 55–60.
26. Balsinde J *et al*. Regulation and inhibition of phospholipase A_2. *Annu Rev Pharmacol Toxicol* 1999; 39: 175–189.
27. Okulski W *et al*. Dipalmitoylphosphatidylcholine membranes modified with carotenoid pigment lutein: experiment versus Monte Carlo simulation study of the membrane organization. *Biochim Biophys Acta* 2008; 1778: 2105–2118.
28. Street IP *et al*. Slow- and tight-binding inhibitors of the 85-kDa human phospholipase A_2. *Biochemistry* 1993; 32: 5935–5940.
29. Bartoli F *et al*. Tight binding inhibitors of 85-kDa phospholipase A_2 but not 14-kDa phospholipase A_2 inhibit release of free arachidonate in thrombin-stimulated human platelets. *J Biol Chem* 1994; 269: 15625–15630.
30. Koutek B *et al*. Inhibitors of arachidonoyl ethanolamide hydrolysis. *J Biol Chem* 1994; 269: 22937–22940.