Lysophosphatidic Acid Up-Regulates Expression of Interleukin-8 and -6 in Granulosa-Lutein Cells through Its Receptors and Nuclear Factor-κB Dependent Pathways: Implications for Angiogenesis of Corpus Luteum and Ovarian Hyperstimulation Syndrome

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Context: Lysophosphatidic acid (LPA) was found at significant amounts in follicular fluid of pre-ovulatory follicle. The lysophospholipase D activity of serum from women receiving ovarian stimulation was higher than women with natural cycles. Angiogenic cytokines, including IL-6, IL-8, and vascular endothelial growth factor, increased in plasma and ascites of patients with ovarian hyperstimulation syndrome. The role of LPA in ovarian follicles is unclear.

Objective: Our objective was to investigate the expression of LPA receptors and function of LPA in granulosa-lutein cells.

Design: Granulosa-lutein cells were obtained from women undergoing in vitro fertilization. We examined the expression of LPA receptors using RT-PCR. The effects of LPA on the expression of IL-6, IL-8, and vascular endothelial growth factor were examined. Signal pathways of LPA were delineated. The functions of secretory angiogenic factors were tested using human umbilical vein endothelial cells.

Results: The LPA1, LPA2, and LPA3 receptors’ mRNA was identified in granulosa-lutein cells. LPA enhanced IL-8 and IL-6 expressions in a dose- and time-dependent manner. LPA functioned via LPA receptors, Gi protein, MAPK/ERK, p38, phosphatidylinositol 3-kinase/Akt, and nuclear factor-κB, and transactivation of epidermal growth factor receptor. LPA induced IL-8 and IL-6 through different pathways. LPA-induced IL-8 and IL-6 increased permeability of human umbilical vein endothelial cell monolayer.

Conclusions: LPA induces IL-8 and IL-6 expressions through LPA receptors and nuclear factor-κB dependent pathways in granulosa-lutein cells. The LPA in preovulatory follicles may play a role in the angiogenesis of corpus luteum. Large amounts of LPA-induced IL-8 and IL-6 from multiple corpora luteae of stimulated ovaries may be one of the pathophysiological causes of ovarian hyperstimulation syndrome. (J Clin Endocrinol Metab 93: 935–943, 2008)
obscure. A number of growth factors, cytokines, and phospholipids may exert modulatory effects (2, 3). These include IL-6, vascular endothelial growth factor (VEGF), secreted protein acidic and rich in cysteine, IL-8, thrombospondin, angiogenin, basic fibroblast growth factor, angiotensin 1 and 2, and sphingosine 1-phosphate (3–12).

Lysophosphatidic acid (LPA), a biologically active phospholipid, plays critical roles in physiological and pathological processes, including inflammation, cell proliferation, angiogenesis, wound healing, and cancer invasion (13, 14). It could be produced through the hydrolysis of phospholipids by extracellular lysophospholipase D (15) or by activated platelets, leukocytes, epithelial cells, and tumor cells (16–18). The serum concentration of LPA in healthy subjects ranges from 0.1–6.3 μM (19). LPA had been detected to exist at considerable amounts in follicular fluid of preovulatory follicles at levels up to 25 μM (20). However, the physiological role of LPA in human follicles remains elusive.

Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication of ovarian stimulation. In severe cases a critical condition develops with massive ascites, pleural effusion, hemoconcentration, and oliguria. The underlying mechanism is due to an increase in the capillary permeability with acute fluid shift out of the intravascular space (21, 22). Several angiogenic cytokines, including IL-6, IL-8, and VEGF, have been abundant in the plasma and ascites of OHSS patients, and been attributed to pathogenetic factors (23, 24). It is thought that these factors are mainly secreted by multiple corpora luteae (25, 26). The lysophospholipase D activity of the serum of women receiving ovarian stimulation was significantly higher than that of women with natural cycles (20). The relation of LPA and increased angiogenic cytokines in patients undergoing ovarian stimulation merits further investigation.

LPA exhibits pleiotropic functions via the interaction with specific G protein (Go, Gs, Gi, or G12/13)-coupled endothelial differentiation gene (Edg) receptors, including LPA1/Edg2, LPA2/Edg4, or LPA3/Edg7 (27). In humans, whereas the expression of the LPA receptors has been reported in some healthy tissues and cancer cells (14), the expression of the LPA receptor isoforms in the granulosa-lutein cells is unknown. In the present study, we attempt to determine the possible role of LPA in ovulation, angiogenesis of corpus luteum, and OHSS. We first investigated the LPA receptors of granulosa-lutein cells. The effects of LPA on granulosa-lutein cells regarding expressions of angiogenic factors of IL-6, IL-8, and VEGF, and the signaling pathways were explored. We then examined whether LPA-induced angiogenic factors modified migration, permeability, capillary tube formation, or proliferation of endothelial cells.

Patients and Methods

Granulosa-lutein cell culture

This study was approved by the ethics committee of the National Taiwan University Hospital. Granulosa-lutein cells were obtained from women undergoing oocyte retrieval for in vitro fertilization treatment. Informed consent was obtained from each patient. Follicular fluid from all follicles was collected and then centrifuged at 350 × g for 5 min. The cells were resuspended in 10 ml HEPES-buffered human tubal fluid medium. The cell suspensions were added on 10 ml Ficoll (Sigma-Aldrich, St. Louis, MO). After centrifugation at 450 × g for 15 min, the interphase cells were collected. The cells were treated with 80 IU/ml hyaluronidase in 1 ml human tubal fluid for 10 min. The cells were washed and suspended in RPMI-1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.25 mg/ml amphotericin B. The granulosa-lutein cells were seeded in a flask. On the second day since collection, the cells were washed to remove remaining red blood cells or leukocytes because these did not adhere to the plastic surface. The cells were then incubated at 37 °C in a humidified atmosphere with 5% CO2 in air.

Antibodies and reagents

Pertussis toxin (PTX), Ki16425, AG1478, LY294002, PD98059, SB203580, I-Oleoyl-LPA, and fatty acid-free BSA were purchased from Sigma-Aldrich. LPA was dissolved in vehicle of PBS containing 1% fatty acid-free BSA. Recombinant human IL-8 and IL-6 neutralizing antibodies were obtained from R&D Systems, Inc. (Minneapolis, MN). Antibodies to human phospho-p38, ERK, and Akt were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

RT-PCR

The total RNA was isolated from the granulosa-lutein cells using the RNAzol B reagent (Biotecx Laboratories, Houston, TX). cDNA was then prepared from 2 μg of the total RNA with random hexamer primers (Inviselect II RT system; Promega, Southampton, UK). The specific oligonucleotide primer pairs were as follows: LPA1, LPA2, and LPA3 described by Fang et al. (28); IL-8, 5′-ACT TCC AAC CTG GCC GTG CGT CTC TCT GCA-3′ and 5′-TGA ATC CTT ACC CCT CTT CAA AAA CTT CTC-3′ (295 bp); IL-6, 5′-CTT CGG CTT AGT TGC CT 3′ and 5′-AGG AAC TCC TTA AAG CTG CTG-3′ (609 bp); and β-actin, 5′-CTT CTA AAA TGA GCT CGG TG-3′ and 5′-TCA TGA GGT AGT CAC TGA GG-3′ (305 bp).

Real-time quantitative RT-PCR

We further quantified IL-6 and IL-8 mRNA expression in various conditions. The IL-6 and IL-8 cDNA was analyzed using a fluorescein quantitative real-time PCR detection system (LightCycler DNA Master SYBR Green I; Roche Molecular Biochemicals, Indianapolis, IN). The primer pairs were: for IL6, 5′-GCC TTC CTT GTG CCA GTT GCC TT-3′ and 5′-GCA GAA TGA TAT GAG TGT TC-3′; for IL-8, 5′-TTC TTG CAG CTC TCT GTG AGG-3′ and 5′-CTG CTG TTG TTG TTG CTT CTC-3′; and for glyceraldehyde-3-phosphate dehydrogenase, 5′-GGG AAG GTG AAT GTC GGA-3′ and 5′-TGG ACT CTA CGA CGT ACT CAG-3′. Amplification was followed by melting curve analysis to verify the correctness of the amplification. A negative control without cDNA was run with every PCR to assess the specificity of the reaction. Analysis of data was performed using LightCycler software (Roche Diagnostics Ltd., Burgess Hill, UK). PCR efficiency was determined by analyzing a dilution

![FIG. 1. The mRNA expression of LPA receptors in human granulosa-lutein cells. The total RNA of the cultured granulosa-lutein cells (GLC), HUVEC (H), and SK-OV3 cells (S) was extracted under normal culture conditions. LPA1, LPA2, and LPA3 receptors were detected using RT-PCR with specific primers.](image-url)
series of cDNA (external standard curve). The amount of IL-6 or IL-8 mRNA was normalized by that of glyceraldehyde-3-phosphate dehydrogenase mRNA and is presented in arbitrary units, with 1 U corresponding to the value in cells treated with a vehicle control.

**Enzyme immunoassay (EIA)**

Granulosa-lutein cells were plated into six-well culture plates at a density of $2 \times 10^5$ cells per well. After cell attachment, the culture medium was removed. Cell layers were washed and incubated with serum-free medium for 24 h. The cells then were treated with either vehicle or indicated conditions. After 24 h, the supernatant was collected. Levels of IL-6, IL-8, and VEGF were determined using EIA kits (R&D Systems).

**Promoter construction and reporter assays**

The human IL (hIL)-6 and hIL-8 promoters in a luciferase activity reporter system were constructed. Transfections of phIL6–1.2Kb, phIL8–1.4Kb, or nuclear factor (NF)-κB binding site-driven luciferase plasmids (BD Biosience, Palo Alto, CA) into granulosa-lutein cells were performed in six-well plates using the Transfast transfection reagent (Promega). At 24 h after transfection, cells were serum starved for 24 h and then treated with the indicated conditions. To control the transfection efficiency, cells were cotransfected with pSV-β-galactosidase, and data normalizations after all transient transfections were conducted using triplicate cultures.

**RNA interference**

Small interfering RNA duplexes (siRNA) were purchased from Santa Cruz Biotechnology. The targeted siRNA of LPA1, LPA2, and LPA3 were sc-43746, sc-39926, and sc-37088, respectively. Granulosa-lutein cells were transfected with siRNA at the concentrations of 25 nM in serum-free Opti-MEM by using the Oligofectamine method (Invitrogen Corp., Carlsbad, CA).

**Western blotting**

The granulosa-lutein cell lysates were centrifuged at 12,000 rpm for 25 min at 4°C. The protein concentration then was measured using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). A 50-μg protein sample was separated using SDS-PAGE, transferred onto polyvinylidene difluoride membrane, and immunoblotted with various antibodies.

**NF-κB and activator protein (AP)-1 decoy oligodeoxynucleotides to granulosa-lutein cells**

Synthetic double-stranded oligodeoxynucleotides were used as “decoy” cis-elements to block the binding of NFs to promoter regions of the targeted genes, thus inhibiting gene transactivation. We used the EMSA to examine the specific effect of NF-κB decoy and AP-1 decoy (29). For transfection of granulosa-lutein cells, the NF-κB decoy, AP-1 decoy, or scrambled decoy was mixed with the Transfast transfection reagent for 15 min and then incubated with the cells in a serum-free medium.

**Human umbilical vein endothelial cells (HUVECs) trans-well migration assay**

A total of $2 \times 10^5$ HUVECs in 200 μl culture medium was added to the upper chamber of the 24-well Millicell inserts ($8 \mu$m pore; Millipore Corporate, Bedford, MA), with 500 μl culture medium added to the lower chamber. When the cells attached to the insert (~6-h incubation), the medium was changed to serum-free medium in the upper chamber and to the indicated conditioned medium (CM) in the lower chamber. After 6 h, the migrated HUVECs were counted.

**HUVEC monolayer permeability assay**

HUVECs were cultured in trans-well chambers (0.4 μm pore-size polycarbonate filters; Costar Corp., Cambridge, MA). After reaching confluence, the cells were washed, and then the medium was replaced with the indicated conditions (0.3 ml in the upper chamber and 1 ml in the lower chamber). Horseradish peroxidase molecules (Type VI-A, 44 kD; Sigma-Aldrich) at a concentration of 0.126 μM were added to the upper compartment. After incubation for 1 h, the medium in the lower compartment was assayed for enzymatic activity using a photometric guaiacol substrate assay (Sigma-Aldrich).

**HUVEC capillary tube formation assay**

HUVECs ($5 \times 10^4$) in the medium of indicated conditions were plated on growth factor-reduced Matrigel (BD Biosciences, Franklin Lakes, NJ) coated 24-well plates. After incubation for 6 h, the wells were examined...
for tube formation under a phase-contrast microscope and photographed.

**HUVEC proliferation assay**

HUVECs were seeded in 96-well plates at densities of $1 \times 10^6$ cells per well using 0.2 ml endothelial cell medium. After 24 h for cell attachment, the medium was changed to the indicated conditions. After incubation for 72 h, the number of HUVECs was analyzed using the Trypan blue exclusion assay.

**Statistics**

In this study each experiment was repeated at least three times on different occasions. Data were presented as mean ± so. The data were examined with one-way ANOVA, followed by a Tukey test for multiple comparisons. The significance level was set as $P < 0.05$ (n = 5). SAS software version 8.01 (SAS Institute Inc., Cary, NC) was used for calculation.

**Results**

**Human granulosa-lutein cells express LPA-specific receptors**

We first detected the mRNA of LPA1, LPA2, and LPA3 receptors using RT-PCR in granulosa-lutein cells, in comparison with the SK-OV3 cell line, which was a well-known ovarian cancer cell line proved to express LPA1, LPA2, and LPA3, and HUVEC, which was proved to express LPA1 only (Fig. 1). The results indicated that LPA1, LPA2, and LPA3 mRNA was all expressed in the granulosa-lutein cells of four individual cases.

**LPA enhances IL-8 and IL-6 expression in human granulosa-lutein cells at both protein and mRNA levels**

Using EIA (Fig. 2A), we found that LPA significantly increased IL-8 (6.7 ± 1.4-fold) and IL-6 (4.9 ± 1.1-fold) protein secretions of granulosa-lutein cells in 24 h. However, LPA did not induce VEGF secretion. Human chorionic gonadotropin (HCG) significantly increased VEGF secretion (1.9 ± 0.2-fold) but did not enhance IL-8 and IL-6 secretions. In 48-h LPA treatment, IL-8 protein level increased to 8.4 ± 1.6-fold and IL-6 to 6.1 ± 1.2-fold. In addition, VEGF remained unchanged. Of HCG treatment, VEGF secretions increased to 3.4 ± 0.4-fold, and IL-8 and IL-6 levels did not increase.

Using real-time quantitative RT-PCR (Fig. 2B), we found that mRNA expressions of IL-8 and IL-6 increased in a time-dependent manner when treated with LPA. After the 4-h treatment, the levels of IL-8 and IL-6 mRNA (3.2 ± 0.3-fold in IL-8 and 2.4 ± 0.2-fold in IL-6, compared with controls) reached the maximum. We examined the dosage effect of LPA on IL-8 and IL-6 expressions using promoter reporter assays (Fig. 2C). The results revealed that the fold of induction of IL-8 promoter luciferase activity increased in a dose-dependent manner from 1–10 µM (6.3 ± 1.5 to 17 ± 4-fold). At the concentration of 20 µM (16 ± 2.6-fold), the elevated level of IL-8 promoter activity was not significantly different from that of 10 µM. A similar pattern was also found in the induction of IL-6 promoter activity from 1–10 µM (3.0 ± 1.5 to 13.3 ± 4-fold). At the concentration of 20 µM (14.6 ± 3.2-fold), the elevated level was not significantly distinct from that of 10 µM.

**Signal transduction pathways involved in LPA-mediated IL-8 and IL-6 expressions in granulosa-lutein cells**

Using real-time quantitative RT-PCR (Fig. 3A), we found that LPA-enhanced IL-8 mRNA expression was significantly diminished by Ki16425, G protein inhibitor (PTX), and epidermal growth factor receptor (EGFR) inhibitor (AG1478). On the other hand, LPA-enhanced IL-6 mRNA expression was significantly reduced by PTX. However, Ki16425 and AG1478 did not have inhibitory effects. We further applied specific LPA receptor siRNA to affect mRNA expressions of LPA receptors in granulosa-lutein cells (Fig. 3B). We found that the LPA1 siRNA reduced LPA-inducing IL-8 secretion, and the LPA2 siRNA decreased the LPA-inducing IL-6 secretion. The results indicated an involvement of the PTX-sensitive Gi protein-coupled LPA receptors in the LPA-enhanced IL-8 and IL-6 expressions.
IL-6 expressions. LPA induced IL-8 expression through LPA1, and IL-6 expression through LPA2. LPA-enhanced IL-8 expressions were partially through transactivation of the EGFR.

We further explored the signal transduction mediators involved in LPA-induced IL-8 and IL-6 expressions. Using real-time quantitative RT-PCR (Fig. 4A), we found that LPA-induced IL-8 mRNA expression was significantly reduced by MAPK/ERK inhibitor (PD98059), phosphatidylinositol 3-kinase (PI3K)/Akt inhibitor (LY294002), and p38 inhibitor (SB203580). However, JNK inhibitor (SP600125) did not have an inhibitory effect. On the other hand, LPA-induced IL-6 mRNA expression was significantly decreased by p38 inhibitor (SB203580). Using Western blotting, we verified that LPA-induced phosphorylation of Akt, ERK, and p38. In addition, the phosphorylation was diminished using PTX pretreatment (Fig. 4B). LPA also induced EGFR phosphorylation that was inhibited by PTX pretreatment (Fig. 4C). The phosphorylation of ERK, but not of Akt and p38, was significantly reduced by the EGFR inhibitor (AG1478) (Fig. 4D). Therefore, LPA may transactivate EGFR through the Gi protein and then activate the ERK. The results indicated that LPA induced IL-8 expression via MAPK/ERK, PI3K/Akt, and p38, as well as induced IL-6 expression via p38.

We verified the specific binding effect of the NF-κB decoy and AP-1 decoy using EMSA with antibody supershift analysis (Fig. 5A). We further found that the NF-κB decoy, but not the AP-1 decoy, significantly inhibited LPA-induced IL-8 and IL-6 secretions (Fig. 5, B and C). LPA strongly enhanced the NF-κB binding site-driven luciferase activity (8.7 ± 0.9-fold) that was significantly reduced by inhibitors of Gi (PTX), Akt (LY294002), ERK (PD98059), and p38 (SB203580) (Fig. 5D). These results indicate that NF-κB plays a critical role in LPA-induced IL-8 and IL-6 expressions.

**LPA-induced IL-8 and IL-6 protein secretions from granulosa-lutein cells enhance angiogenesis**

Using trans-well migration assay (Fig. 6A), we observed increased migration of HUVECs when incubated with LPA-treated CM (Fig. 6A, left panel, b), compared with vehicle-treated CM (Fig. 6A, left panel, a). LPA-treated CM preincubated with IL-8 neutralizing antibodies significantly diminished this enhancing effect (Fig. 6A, right panel, c). LPA-treated CM preincubated with...
IL-6 neutralizing antibodies did not inhibit the enhancing effects (Fig. 6A, left panel). The quantitative results demonstrated the specificity and direct effect of LPA-induced IL-8 in mediating HUVEC migration. LPA-treated CM preincubated with isotype IgG preserved the enhancing capability (Fig. 6A, right panel).

With monolayer permeability assay (Fig. 6B), we detected increased permeability of HUVEC monolayer when incubated with LPA-treated CM (Fig. 6B, lane 2), compared with vehicle-treated CM (Fig. 6B, lane 1). LPA-treated CM preincubated with IL-8 neutralizing antibodies significantly diminished the enhancing effects (Fig. 6B, lane 3). LPA-treated CM preincubated with IL-6 neutralizing antibodies also significantly reduced the enhancing effects (Fig. 6B, lane 4). LPA-treated CM preincubated with isotype IgG maintained the enhancing capability (Fig. 6B, lane 5). These results suggested the specificity and direct effects of LPA-induced IL-8 and IL-6 in increasing permeability of HUVEC monolayer.

In the test of HUVEC capillary tube formation (Fig. 6C), we found increased tube formation of HUVECs when incubated with LPA-treated CM (Fig. 6C, left panel), compared with vehicle-treated CM (Fig. 6C, left panel). LPA-treated CM preincubated with IL-8 neutralizing antibodies did not enhance HUVEC tube formation (Fig. 6C, left panel). However, LPA-treated CM preincubated with IL-6 neutralizing antibodies maintained the enhancing effects (Fig. 6C, left panel). The quantitative data indicated the specificity and direct effects of LPA-induced IL-8 in mediating HUVEC tube formation (Fig. 6C, right panel).

In the examination of HUVEC proliferation (Fig. 6D), we observed increased growth of HUVECs when incubated with LPA-treated CM (Fig. 6D, lane 2), compared with vehicle-treated CM (Fig. 6D, lane 1). LPA-treated CM preincubated with IL-8 neutralizing antibodies significantly reduced the enhancing effects (Fig. 6D, lane 3). LPA-treated CM preincubated with IL-6 neutralizing an-
tibodies did not diminish the enhancing effects (Fig. 6D, lane 4). The results implied the specificity and direct effects of LPA-induced IL-8 in augmenting HUVEC proliferation.

The representation of LPA function through LPA receptors and signaling pathways was schematically summarized in Fig. 7.

**Discussion**

In the present study, we first demonstrate that human granulosa-lutein cells express LPA1, LPA2, and LPA3 receptors. In primary culture of granulosa-lutein cells, LPA up-regulates IL-8 and IL-6 expressions in a concentration- and time-dependent mode. We further verify that LPA-induced IL-8 protein functionally enhances multistep processes of angiogenesis, including migration, permeability, tube formation, and proliferation of endothelial cells, and IL-6 enhances permeability. The presence of significant concentrations of LPA in preovulatory follicles may mediate granulosa-lutein cells to secrete IL-8 and IL-6, and regulate neovascularization. Therefore, LPA may play a role in the angiogenesis of the corpus luteum.

High concentrations of VEGF, IL-6, and IL-8 have been found in the plasma and ascites of patients with OHSS (23, 24). These angiogenic cytokines are mainly secreted from multiple corpora lutea after ovarian stimulation (25, 26). Previous studies indicated that the secretion of VEGF of granulosa-lutein cells was principally induced by LH or HCG (5). However, the regulation of the IL-6 and IL-8 secretions remained unclear. These were enhanced by other cytokines, such as TNF-α, IL-1α, and IL-1β (30, 31). In our study we found that HCG induced VEGF secretion in granulosa-lutein cells, but not IL-8 and IL-6. LPA induced IL-8 and IL-6 secretions, but not VEGF.

The primary mechanism of OHSS is due to increased capillary permeability induced by angiogenic cytokines that results in acute fluid and albumin loss from the intravascular space into the third space (21, 22). LPA-induced IL-8 and IL-6 in granulosa-lutein cells functionally increased permeability of endothelial cells. Therefore, we suggest that excessive IL-8...
and IL-6 secretions from multiple corpora lutea induced by LPA may be a contributing cause of OHSS. Intravenous albumin administration has been applied to prevent and treat OHSS (26). It has been found that serum albumin and other LPA-binding proteins modify the cellular function of LPA (32–34). The therapeutic effects of albumin administration for OHSS may be partly through reducing LPA function, which deserves further investigation.

Ki16425 is an LPA receptor antagonist, especially on LPA1 and LPA3, that blocks some biological actions of LPA (35). Boucharaba et al. (36) studied the effects of Ki16425 in a human breast cancer cell line using nude mice models. They found that Ki16425 reduced IL-6 and IL-8 secretions of breast cancer cells that diminished osteoclast activity and prevented bone metastasis. In our study we found that Ki16425 blocked IL-8 secretion of granulosa-lutein cells. Gomez et al. (37) used dopamine agonist (cabergoline) to reverse VEGF receptor 2-dependent vascular permeability and treat OHSS in a rat model. It could be suggested that the inhibition of LPA action in granulosa-lutein cells using LPA receptor antagonist may be another therapeutic target for studying the treatment of OHSS.

Inflammatory reaction is one of the processes of ovulation. IL-8 and IL-6 have been found to elevate in the preovulatory follicles and have been implicated in the involvement of ovulation (7, 38). The IL-8 and IL-6 may possess leukocyte chemoattractive activity for neutrophils and monocytes that may contribute to tissue degradation in follicle rupture by the release of proteolytic factors (8). Therefore, LPA may play a role in ovulation through induction of IL-8 and IL-6 in granulosa-lutein cells, which deserves further study.

Here, we verify that through LPA1, the Gi, MAPK/p38, PI3K/Akt, and NF-κB signal pathways are involved in the LPA-induced IL-8 expression. LPA also transactivates EGFR via Gi and then activates ERK to induce IL-8 secretion. Through the LPA2 receptor, the Gi, MAPK/p38, and NF-κB pathways are involved in the LPA-induced IL-6 expression, and perhaps LPA3 alters something else that remains to be investigated. Recently, LPA was found to induce IL-8 expression in human bronchial epithelial cells and in HUVECs, as well as IL-8 and IL-6 expressions in dendritic cells (17, 39, 40). In addition, these enhancement effects are NF-kB dependent (17, 39). Together, LPA appears to be an important controlling factor for IL-8 or IL-6 expression in human tissues.

Conclusions

We demonstrate that LPA1, LPA2, and LPA3 receptors are expressed in human granulosa-lutein cells. In addition, LPA induces IL-8 and IL-6 expressions through LPA receptors via Gi-dependent NF-κB signaling pathways, but with different LPA receptors and different signal transduction mediators. Furthermore, LPA induction of IL-8 and IL-6 proteins stimulates angiogenesis. Therefore, the presence of significant amounts of LPA in a preovulatory follicle may play a role in ovulation and the neovascularization of corpus luteum. In addition, excessive IL-8 and IL-6 secretions induced by LPA from multiple corpora lutea of superovulated ovaries may be a cause of OHSS.

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References


FIG. 7. Schematic signaling and possible function of LPA-enhanced IL-8 and IL-6 expressions in granulosa-lutein cells.


