Placenta 34 (2013) 751–756

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Lysophosphatidic acid increases the production of pivotal mediators of decidualization and vascularization in the rat uterus

J.S. Beltrame [a], M.S. Sordelli [a], M. Cella [b], S. Perez Martinez [c], A.M. Franchi [b], M.L. Ribeiro [a,*]

[a] Laboratorio de Fisiología y Farmacología de la Reproducción, CEFYBO (CONICET – UBA), Buenos Aires, Argentina
[b] Laboratorio de Fisiopatología de la Preñez y el Parto, CEFYBO (CONICET – UBA), Buenos Aires, Argentina
[c] Laboratorio de Biología de la Reproducción en Mamíferos, CEFYBO (CONICET – UBA), Buenos Aires, Argentina

ARTICLE INFO

Article history:
Accepted 4 June 2013

Keywords:
Decidualization
Vascularization
Implantation
Lysophosphatidic acid
Cyclooxygenase-2
Inducible nitric oxide synthase

ABSTRACT

Introduction: The decidual reaction and the formation of new vessels in the uterus are two crucial processes during embryo implantation. Previously, we observed that lysophosphatidic acid (LPA) increases cyclooxygenase-2 derived – prostaglandin E2 production during implantation in the rat uterus and that it augments the expression of decidualization (IGFBP-1) and vascularization (IL-10) markers. Both cyclooxygenase and nitric oxide synthase (NOS) are known enzymes involved in these processes. Thus, we became interested in studying which factors contribute to LPA receptor-specific role during the decidual and the vascular reaction at implantation.

Methods: We adopted a pharmacological approach *in vitro* incubating the uterus from rats on day 5 of gestation (day of implantation) with LPA, DGPP (a highly selective antagonist of LPA3, an LPA receptor) and cyclooxygenase and NOS selective and non-selective inhibitors. We determined NOS activity, prostaglandin E2 production and IGFBP-1 and IL-10 expression to evaluate decidualization and vascularization.

Results: We observed that LPA augmented the activity of the inducible NOS isoform through LPA1/LPA3. Inducible NOS activity participated in the induction of cyclooxygenase-2/prostaglandin E2 increase stimulated by LPA. Also, cyclooxygenase-2 derived prostaglandins mediated LPA-stimulatory action on NOS activity. Both cyclooxygenase-2 and inducible NOS mediated LPA effect on IGFBP-1 and IL-10 expression.

Conclusions: These results suggest the participation of LPA/LPA3 in the production of crucial molecules involved in vascularization and decidualization, two main processes that prepare the uterine milieu for embryo invasion during implantation.

1. Introduction

Implantation failure is common following natural conceptions and is an important clinical hurdle to overcome following assisted reproductive attempts. Differentiation of endometrial stromal cells to decidual cells and formation of new maternal blood vessels are critical for the establishment and maintenance of gestation. The decidua is a transient tissue that supports embryo growth and maintains early pregnancy [1]. The proper coordination of several vascular processes at the maternal–fetal interface ensures an adequate blood flow in response to the increasing metabolic demands of the embryo [2].

Based on published data we postulate that LPA might be a lipid promoter of vascularization during the first steps of embryo implantation. In trophoblast and human endometrial stromal cells, LPA enhances permeability, migration, proliferation and capillary tube formation of endometrial microvascular endothelial cells [3,4]. The group of Lin [5] showed that LPA up-regulates vascular endothelial growth factor-C expression in human umbilical vein endothelial cells and stimulates subsequent endothelial cell tube formation. These effects are mediated by LPA1 and LPA3 receptors [3–5]. Targeted deletion of LPA3 in mice resulted in significantly reduced litter size, which could be attributed to delayed implantation and altered embryo spacing. These two events led to delayed embryonic development, hypertrophic placentas shared by multiple embryos, and embryonic death, thus identifying LPA3 receptor-mediated signaling as a new influence on implantation [6].

* Corresponding author. Laboratorio de Fisiología y Farmacología de la Reproducción, CEFYBO (CONICET – Facultad de Medicina, UBA), Paraguay 2155, 16th Floor, CP(C1121ABG), Buenos Aires, Argentina. Tel.: +54 11 49624431; fax: +54 11 45083680.
E-mail address: marialribeiro@yahoo.com.ar (M.L. Ribeiro).

0143-4004/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.placenta.2013.06.001

Prostaglandins and nitric oxide are important mediators of decidualization and vascularization. NOS, that catalyzes the production of nitric oxide, exists in three isoforms: endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS). At implantation labeled iNOS cells are within the decidua and the ectoplacental cone, while eNOS is localized in vessels of the primary decidual zone [7]. Both iNOS and eNOS expression are higher at implantation sites. Moreover, *in utero* administration of a non-selective NOS inhibitor or of nitric oxide-donors during pre-implantation reduces implantation rates related to vascularization defects [8,9].

Prostaglandins derived from cyclooxygenase-2 (COX-2) are the most relevant in implantation. COX-2 is mostly restricted to implantation sites and COX-2$^{-/-}$ mice have defective implantation and decidualization [10,11]. Prostaglandin E2 (PGE2) and prostacyclin increase vascular permeability and decidualization [12,13] and their administration to LPA3$^{-/-}$ females rescues delayed implantation [6,14].

We recently published that LPA augments COX-2 derived PGE2 production in the rat uterus at implantation and that LPA stimulates decidualization and vascularization [15]. Also, we previously observed that NOS activity is higher at the implantation sites [16].

Based on these evidence, we hypothesized that under LPA effect, PGs and NO may interact, and that this crosstalk could be crucial for uterine decidualization and vascularization at the time of embryo implantation. Thus, the aims of the present study were: 1) to study LPA effect on NOS activity at implantation; 2) to investigate if COX-2 derived prostaglandins and nitric oxide interact under the effect of LPA during implantation; and 3) to determine if prostaglandins and nitric oxide mediate LPA effect on decidualization and vascularization.

2. Methods

2.1. Ethics statement

Experimental procedures were approved by the Animal Care Committee of our institute (CEFYBO – CONICET) and by the Institutional Committee for the Care and Use of Laboratory Animals, Permit Number: 2550/2010 (CICUAL, Comité Institucional para el Cuidado y Uso de Animales de Laboratorio) from the Facultad de Medicina (Universidad de Buenos Aires), and were carried out in accordance with the Guide for Care and Use of Laboratory Animals (NIH). All animals were provided by the animal facility of the Facultad de Odontología (Universidad de Buenos Aires).

2.2. Animals

Wistar females were housed in group cages under controlled conditions of light (12 h light, 12 h dark) and temperature (23–25 °C). Animals receive food and water *ad libitum*. Where mentioned, animals were sacrificed in a carbon dioxide chamber and all efforts were made to minimize suffering. Virgin females were mated with fertile males of the same strain. The morning the spermatozoa were observed in the vaginal fluid was defined as day 1 of pregnancy. Under the conditions of our animal facilities, spontaneous term labor occurs on day 22 of gestation.

Implantation is the process by which embryos make a close physical and physiological contact with the maternal endometrium for the establishment of pregnancy. In rats, implantation occurs in the evening of 5 days post-coitum and is preceded by embryo spacing, uterine edema and luminal closure resulting in an intimate apposition of the blastocyst with the uterine luminal epithelium. During this period, the endometrium undergoes pronounced structural and functional changes which prepare it to be receptive to invasion by the embryo. Thus, rats on day 5 of pregnancy were sacrificed at 9:00–10:00 in the morning and uterine horns were excised. Tissues were immediately cultured as described below (*in vitro* studies).

2.3. In vitro studies

Uterine slices were weighted and incubated in 24 wells plates. Where mentioned, tissues were incubated with different drugs in DMEM without phenol red (GIBCO, Invitrogen, Argentina) and supplemented with 10% fetal calf serum, 20 IU/ml penicillin G and 20 μg/ml streptomycin (final volume: 500 μl) for 6 and 12 h. Drugs used were: LPA (1-oleoyl-lysophosphatidic acid 18:0, Cayman Chemical, Migliore Laclaustra, Argentina), DGPP (diacylglycerol pyrophosphate 8:0, Sigma Chemical Company, Argentina), indomethacin (a non-selective COX-1 and COX-2 inhibitor, Montpellier, Argentina), NS-398 (a selective COX-2 inhibitor, Cayman Chemical, Argentina), 1400W (a selective iNOS inhibitor, Cayman Chemical, Migliore Laclaustra, Argentina) and L-NAME (a non-selective NOS inhibitor, Sigma Chemical Company, Argentina). Cultures were maintained in 5% CO_2 in air at 37 °C. After the culture, tissues were immediately frozen at −70 °C (NOS activity) or homogenized in Trizol (real time PCR, Genbiotech, Argentina). All supernatants were immediately frozen at −70 °C (prostaglandins' radioimmunoassays). Concentration response curves using DGPP and NOS and COX inhibitors were performed in order to determine the lowest concentration of the drugs used that do not affect basal levels of the determined mediators (data not shown).

2.4. Total NOS enzyme assay

NOS enzyme activity was quantified by the modified method of Bredt&Snyder as previously published by our group [16]. Enzyme activity was expressed as pmol L-citrulline/mg protein/15 min.

2.5. Prostaglandins radioimmunoassay

PGE2 concentration in culture supernatants was determined as previously described [17]. Values were expressed as pg of PGE2 released to the medium per mg of tissue protein.

2.6. Real time RT-PCR analyses

Real time RT-PCR was performed on RG6000 (Corvette) using Master Mix (Biodynamics, Argentina), gene specific primers [15] and 2.5 μl of cDNA as a template. The PCR conditions in all cases started with a denaturation step at 94 °C for 5 min and followed by up to 40 cycles of denaturation, annealing and primer extension [15]. IGFBP-1 and IL-10 mRNA levels were corrected to the levels of rat β-actin using the $2^{-\Delta\Delta Ct}$ method and normalized to the ratio produced in the control samples.

2.7. Statistical analysis

Statistical analysis was performed using the GraphPad Prism Software (San Diego, CA, USA). Comparisons between values of different groups were performed using one way ANOVA (analyze of variance) and *t*-Student. Significance was determined using Tukey's multiple comparison tests for unequal replicates. A number of 4–6 animals were used for each treatment. All values represent means ± S.E.M. Differences were considered significant when p was 0.05 or less.

3. Results

3.1. LPA increased inducible NOS activity on the implantation in the rat uterus

We observed that 50 μM LPA for 6 h increased NOS activity in the day of implantation (Fig. 1A). The incubation conditions were selected based on previously published results from our laboratory [15]. To study if LPA3 was mediating LPA effect in our experimental model, uterine strips were pre-incubated for 30 min with a highly selective LPA3 antagonist, 10 μM DGPP.

Afterwards tissues were incubated 6 h with 50 μM LPA in the presence of the antagonist. We observed that 10 μM DGPP blocked LPA effect on NOS activity (Fig. 1A). The incubation with 10 μM DGPP alone did not modify the activity of NOS enzyme (Table 1). Based on both binding and functional data described in the data sheet and on a previous work from our laboratory [15], DGPP at the selected concentration is a highly potent and selective antagonist for LPA3 receptor. However, since DGPP is a poor antagonist for LPA1 receptor (Ki = 6.6 μM), we could not discount effects of LPA mediated by LPA1 and LPA3 (Ki = 106 nM).

Then, we investigated which isoform of NOS was involved in LPA effect. Thus, uteri were pre-incubated for 30 min with 300 μM L-NAME or 1 μM 1400W. Afterwards tissues were incubated 6 h with 50 μM LPA in the presence of the inhibitors. The treatment with L-NAME or with 1400W inhibited LPA action to the control level (Fig. 1B).

These results suggest that LPA via LPA1/LPA3 increased the production of nitric oxide, an important mediator of vascularization and decidualization, by increasing iNOS activity during implantation in the rat uterus.

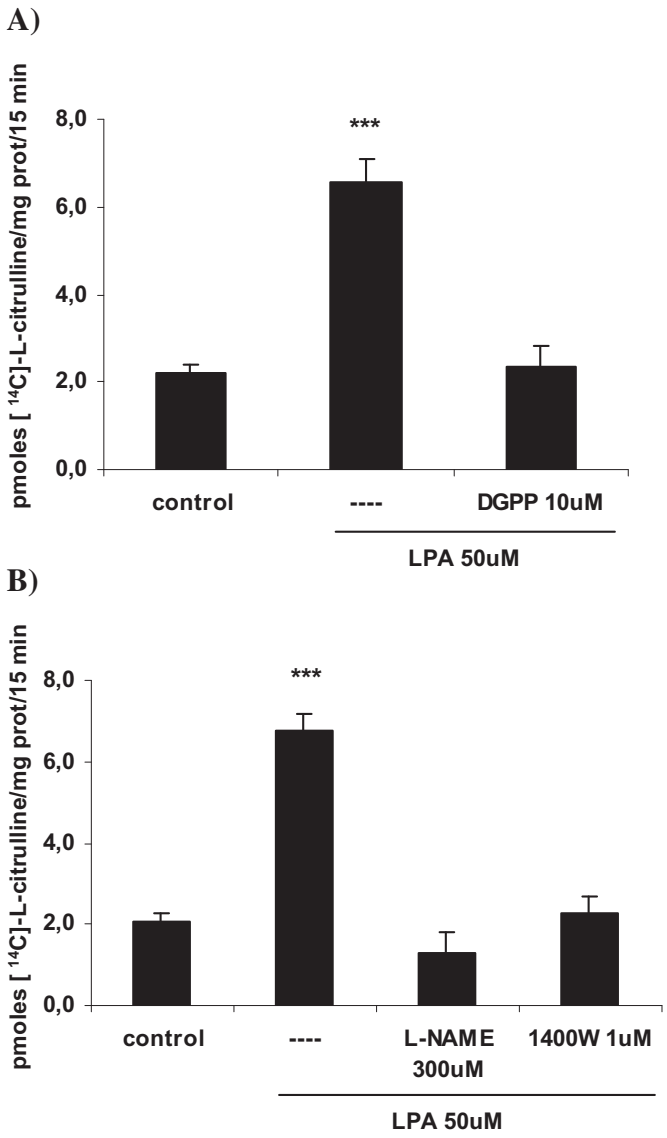

Fig. 1. LPA increased the activity of iNOS isoform through LPA3 receptor. Uterine strips from rats pregnant on day 5 of gestation were incubated with 50 μM LPA for 6 h in the presence of: A) 10 μM DGPP (a selective LPA3 antagonist); B) 300 μM L-NAME (a non-selective NOS inhibitor) or 1 μM 1400W (a selective iNOS inhibitor). NOS activity was determined. In A) $^{***}p < 0.001$ vs the rest; In B) $^{***}p < 0.001$ vs the rest. Results are shown as means ± S.E.M. $N = 4–6$ for each point.

3.2. Cyclooxygenase and nitric oxide synthase crosstalk under LPA effect during implantation in the rat uterus

Then, we investigated if prostaglandins and nitric oxide interact under the action of LPA during implantation.

Table 1
Effect of DGPP, indomethacin and NS-398 on NOS activity. Uterine strips from rats pregnant on day 5 of gestation were incubated for 6 h with 10 μM DGPP, 1 μM indomethacin or 1 μM NS-398. NOS activity was determined by the Bredt&Snyder modified technique.

	NOS activity, pmoles [^{14}C]-L-citrulline/mg prot/15 min
Control	2.0 ± 0.8
10 μM DGPP (LPA1/LPA3 antagonist)	2.1 ± 0.7
1 μM indomethacin (non-selective COX inhibitor)	2.5 ± 0.5
1 μM NS-398 (COX-2 selective inhibitor)	1.5 ± 0.9

To test this hypothesis, uterine strips were pre-incubated for 30 min with 1 μM indomethacin or 1 μM NS-398. Then, tissues were incubated with 50 μM LPA for 6 h in the presence of the inhibitors and NOS activity was determined. Both indomethacin and NS-398 blocked LPA stimulatory action (Fig. 2A), indicating that COX-2 derived prostaglandins mediated NOS increase induced by LPA. The incubation with 1 μM indomethacin or with 1 μM NS-398 alone did not modify the activity of NOS enzyme (Table 1). Then, uteri were pre-incubated for 30 min with 300 μM L-NAME or 1 μM 1400W and afterwards tissues were incubated with 50 μM LPA for 6 h in the presence of the inhibitors. PGE2 production was determined. Both L-NAME and 1400W prevented LPA stimulatory action on PGE2 production (Fig. 2B), indicating that iNOS derived nitric oxide mediated LPA effect on PGE2.

The incubation with 300 μM L-NAME or with 1 μM 1400W alone did not modify PGE2 liberation (Table 2). Concentrations of inhibitors were chosen based on previously published works [18–21].

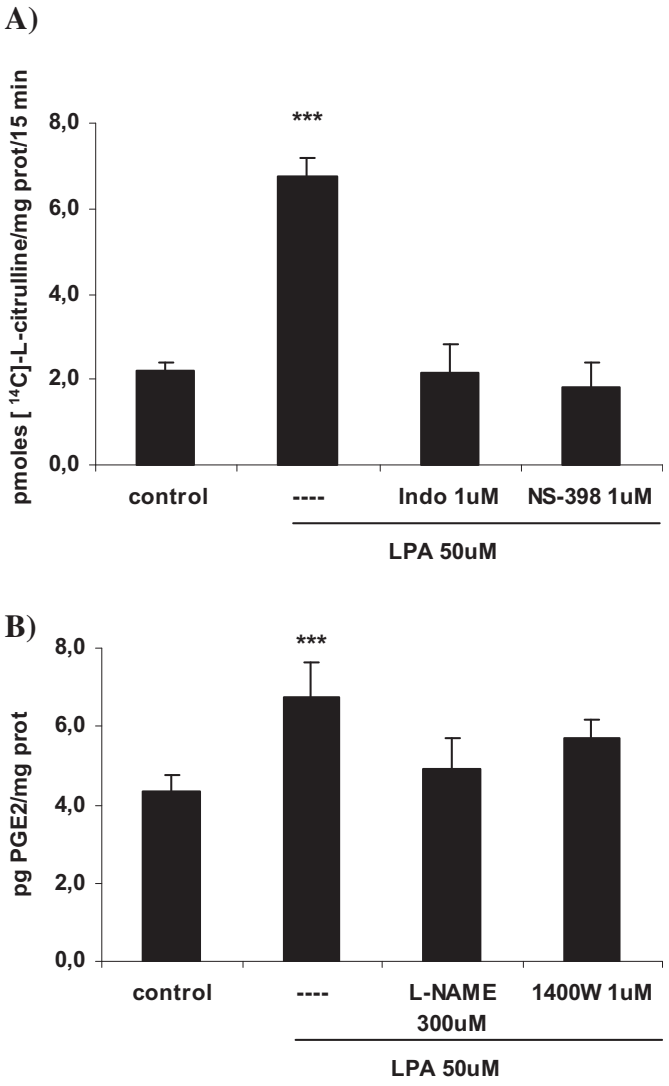

Fig. 2. COX-2 and iNOS crosstalk under LPA effect. Uterine strips from rats pregnant on day 5 of gestation were incubated with 50 μM LPA for 6 h in the presence of: A) 1 μM indomethacin (a non-selective COX inhibitor) or 1 μM NS-398 (a selective COX-2 inhibitor); B) 300 μM L-NAME (a non-selective NOS inhibitor) or 1 μM 1400W (a selective iNOS inhibitor). NOS activity (A) and PGE2 production (B) were determined. In A) $^{***}p < 0.001$ vs the rest; In B) $^{***}p < 0.001$ vs the rest. Results are shown as means ± S.E.M. $N = 4–6$ for each point.

3.3. COX-2 and iNOS participated in decidualization and vascularization induced by LPA

Afterwards, we investigated if iNOS derived nitric oxide and COX-2 derived prostaglandins participated in the stimulatory action that LPA elicited on decidualization and vascularization [15]. Rat uteri were pre-incubated for 30 min with 1 μM NS-398 or 1 μM 1400W. Then, tissues were incubated for 6 h (for IGFBP-1) or 12 h (for IL-10) with 50 μM LPA in the presence of the inhibitors. The expression of IGFBP-1, a decidualization marker, and IL-10, a vascularization marker, was determined by real time RT-PCR. We observed that NS-398 and 1400W decreased LPA stimulatory action on IGFBP-1 (Fig. 3A) and IL-10 (Fig. 3B) expression to the control levels. The incubation with NS-398 or 1400W alone did not exert any effect on IGFBP-1 or IL-10 basal expression (Table 3).

4. Discussion

In the present work we described that at implantation, LPA promotes decidualization and vascularization, by increasing crucial mediators of these processes as prostaglandins and nitric oxide synthase activity. Experiments in mice have directly shown that lipid molecules are essential during embryo invasion [for details see review [25]]. The establishment of successful pregnancy requires a profound reorganization of uterine tissues and the quality of implantation determines the quality of pregnancy and fetal well-being. Failure to achieve implantation risks pregnancy outcome. Some of the most widely studied lipid mediators are the phosphorylated lipids such as LPA. We recently published that the rat uterus expresses lysophospholipase-D during the window of implantation [15]. Lysophospholipase-D is the main enzyme involved in LPA synthesis, suggesting that the rat uterus is capable of producing LPA during this stage of pregnancy. As LPA, prostaglandins and nitric oxide participate in different female reproductive processes. Particularly, PGE2 and nitric oxide are known mediators of decidualization and vascularization at the sites of embryo implantation [8–13,22–24].

Our results suggest that LPA increases the production of nitric oxide as LPA augments iNOS activity through the activation of LPA1/LPA3 receptors. Previously, we observed that NOS activity is increased at the sites of embryo implantation and that this regulation depends on the presence of the blastocyst [16]. Also, nitric oxide has been demonstrated to participate in the decidual and vascular reactions [8,9]. Based on these evidence, the fact that LPA increases iNOS activity, strongly suggests that LPA is a local regulator of nitric oxide production in the uterus, regulating normal implantation. In this sense, Purcell and colleagues [7] described that iNOS isoform is expressed in the decidua and the endometrial stroma, which are in close contact with the embryonic cells during implantation. Hence, we postulate that LPA increases iNOS activity, promoting decidualization and vascularization at the time of embryo implantation.

Previously we published that during implantation, LPA augments the production of PGE2 by increasing COX-2 expression [15]. Here we described that iNOS and COX-2 pathways interact under the effect of LPA, which is relevant for the physiology and progress of decidualization and vascularization. NOS and COX crosstalk has been described by our group in other female reproductive events [19,20,26,27].

Rapid growth and differentiation of the endometrial fibroblasts into decidual cells are the earliest and most striking event in pregnancy [28]. The process of successful pregnancy also includes

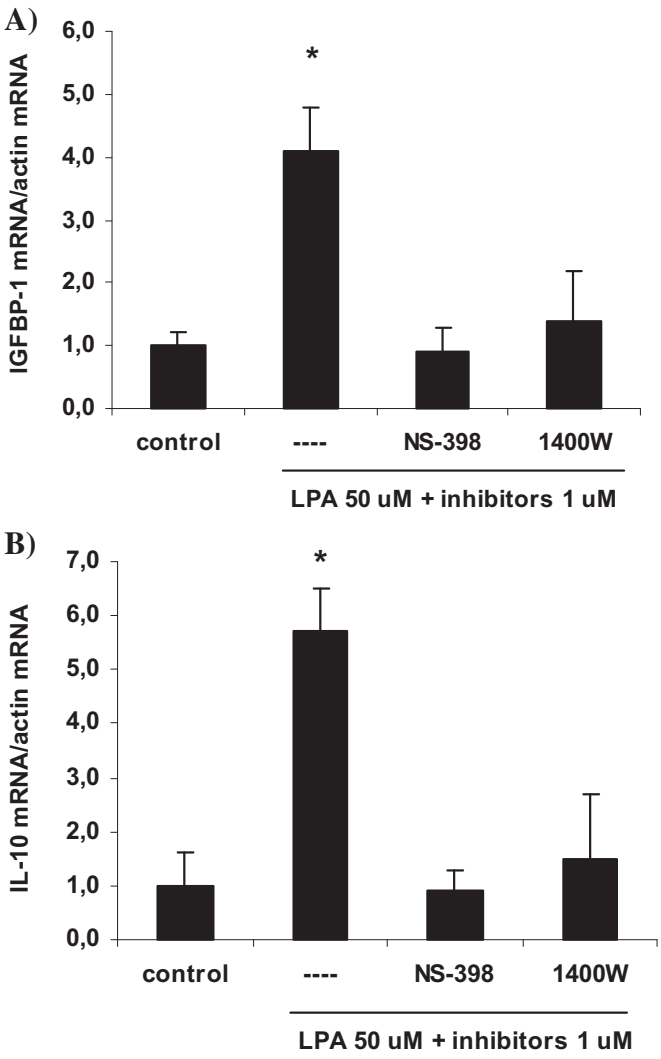

Fig. 3. COX-2 and iNOS mediated the increment in IGFBP-1 and IL-10 expression stimulated by LPA. Uterine strips from rats pregnant on day 5 of gestation were incubated with 50 μM LPA for 6 h (IGFBP-1) or 12 h (IL-10) in the presence of 1 μM NS-398 (a selective COX-2 inhibitor) or 1 μM 1400W (a selective iNOS inhibitor). The expression of IGFBP-1 (A) and IL-10 (B) was determined by real time RT-PCR. In A) $^{*}p < 0.05$ vs the rest; In B) $^{*}p < 0.05$ vs the rest. Results are shown as means ± S.E.M. $N = 4–6$ for each point.

Table 2
Effect of l-NAME and 1400W on PGE2 production. Uterine strips from rats pregnant on day 5 of gestation were incubated for 6 h with 300 μM L-NAME or 1 μM 1400W. PGE2 production was determined by radioimmunoassay.

	pg PGE2/mg prot
Control	4.3 ± 0.4
300 μM L-NAME (non-selective NOS inhibitor)	4.4 ± 1.0
1 μM 1400W (iNOS selective inhibitor)	4.1 ± 1.5

Table 3
Effect of NS-398 and 1400W on IGFBP-1 and IL-10 expression. Uterine strips from rats pregnant on day 5 of gestation were incubated for 6 h (for IGFBP-1) or 12 h (IL-10) with 1 μM NS-398 or 1 μM 1400W. IGFBP-1 and IL-10 mRNA expression was determined by real time RT-PCR.

	IGFBP-1 m RNA/actin	IL-10 m RNA/actin
Control	1.00 ± 0.10	1.00 ± 0.07
1 μM NS-398 (selective COX-2 inhibitor)	1.10 ± 0.12	1.06 ± 0.26
1 μM 1400W (selective iNOS inhibitor)	1.30 ± 0.10	0.71 ± 0.31

subsequent placentation with the development of neovasculatures. Our results suggest that LPA acts as a pro-implantation factor in the uterus during the first steps of the implantation process regulating the production and the interaction of fundamental mediators of decidualization and vascularization. The fact that LPA increases the expression of IGFBP-1 and IL-10 strongly supports our hypothesis [15]. Also, it is interesting that COX-2 derived prostaglandins and nitric oxide synthesized by iNOS participate in LPA effect, reinforcing the notion that LPA via LPA1 and/or LPA3 receptors modulated downstream signaling molecules involved in the physiology of the decidua and the uterine vasculature.

To our knowledge, it is for the first time that LPA is related with the transformation of the stroma into decidual cells. During the process of decidualization, cells undergo highly active apoptosis and differentiation. Many works have previously shown that LPA could promote apoptosis and/or cellular division and differentiation [for a detailed review see [29]]. Our results also suggest that LPA is a lipid promoter of vascularization during the first steps of embryo implantation. The group of Lin [5] showed that LPA upregulates vascular endothelial growth factor-C expression in human umbilical vein endothelial cells and stimulates subsequent endothelial cell tube formation. These are LPA1- and LPA3-dependent mechanisms and required COX-2 transactivation [5]. In trophoblast and human endometrial stromal cells, LPA enhances permeability, migration, proliferation and capillary tube formation of endometrial microvascular endothelial cells [3,4]. Hence, through LPA-induced signals, trophoblast cells and endometrial stromal cells may regulate angiogenesis during the window of implantation. Taken together our results with those previously published by other authors, we postulate that LPA may play a role in the angiogenesis of the endometrium and placenta during early pregnancy, through the regulation of crucial molecules as prostaglandins and nitric oxide.

The fact that LPA affects vascularization and decidualization through LPA3 is relevant based on previously published evidences. We published that LPA3 is expressed in the rat uterus during the window of implantation, specifically in the uterine endometrium, which is in close contact with the invasive trophoblast [15]. Also, patients displaying recurrent implantation failure express reduced levels of LPA3 in the endometrium compared with normal patients [30].

5. Conclusion

Understanding the relative roles and the interaction played by LPA and the molecules regulated downstream its receptors during the decidual and vascular reactions might help in the future to prevent implantation failure and late pregnancy-related diseases. Abnormal LPA signaling may result in aberrant regulation of decidualization and vascularization with consequent defects in trophoblast invasion and abnormal pregnancies. The fact that COX-2 derived PGE2 and iNOS interact under LPA effect and that they participate in LPA regulation on decidualization and vascularization, contributes to better understand the significance of ligand–receptor signaling with LPA as a possible effector in coordinating the series of events that leads to a successful pregnancy.

Acknowledgments

We thank Mrs. Ramona Morales and Mrs. Ana Inés Casella for their excellent technical support. We also thank Ricardo Horacio Orzuza (Animal Facility Technician, Facultad de Odontología, Universidad de Buenos Aires) for providing us the prepuber rats. Finally, we would like to thank Marcela Marquez and Enzo Cubas for their dedication in the management of our rats at CEFYBO's Animal Facility. This work was supported by PICT 2010 No 0813 (to Dr. Franchi) and PICT 2010 No 1974 (to Dr. Ribeiro).

References

[1] Dey SK, Lim H, Das SK, Reese J, Paria BC, Daikoku T, et al. Molecular cues to implantation. Endocr Rev 2004;25:341–73.
[2] Torry DS, Leavenworth J, Chang M, Maheshwari V, Groesch K, Ball ER, et al. Angiogenesis in implantation. J Assist Reprod Genet 2007;24:303–15.
[3] Chen SU, Lee H, Chang DY, Chou CH, Chang CY, Chao KH, et al. Lysophosphatidic acid mediates interleukin-8 expression in human endometrial stromal cells through its receptor and nuclear factor-kappaB-dependent pathway: a possible role in angiogenesis of endometrium and placenta. Endocrinology 2008;149:5888–96.
[4] Shee-Uan Chen, Chia-Hung Chou, Kuang-Han Chao, Hsinyu Lee, Chung-Wu Lin, Hsin-Fen Lu, et al. Lysophosphatidic acid up-regulates expression of growth-regulated oncogene-alpha, interleukin-8, and monocyte chemoattractant protein-1 in human first-trimester trophoblasts: possible roles in angiogenesis and immune regulation. Endocrinology 2010;151:369–79.
[5] Lin CI, Chen CN, Huang MT, Lee SJ, Lin CH, Chang CC, et al. Lysophosphatidic acid upregulates vascular endothelial growth factor-C and tube formation in human endothelial cells through LPA1/3, COX-2, and NF-κB activation- and EGFR transactivation-dependent mechanisms. Cell Signal 2008;20:1804–14.
[6] Ye X, Hama K, Contos JJA, Anliker B, Inoue A, Skinner MK, et al. LPA3-mediated lysophosphatidic acid signalling in implantation and embryo spacing. Nature 2005;435:104–8.
[7] Purcell TL, Given R, Chwalisz K, Garfield RE. Nitric oxide synthase distribution during implantation in the mouse. Mol Hum Reprod 1999;5:467–75.
[8] Novaro V, Gonzalez E, Jawerbam A, Rettori V, Canteros G, Gimeno MF. Nitric oxide synthase regulation during embryonic implantation. Reprod Fertil Dev 1997;9:557–64.
[9] Ota H, Igarashi S, Oyama N, Suzuki Y, Tanaka T. Optimal levels of nitric oxide are crucial for implantation in mice. Reprod Fertil Dev 1999;11:183–8.
[10] Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, et al. Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 1997;91:197–208.
[11] Bonventre JV, Huang Z, Taheri MR, O'Leary E, Li E, Moskowitz MA, et al. Reduced fertility and postischaemic brain injury in mice deficient in cytosolic phospholipase A2. Nature 1997;390:622–5.
[12] Lim H, Gupta RA, Ma WG, Paria BC, Moller DE, Morrow JD, et al. Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARdelta. Genes Dev 1999;13:1561–74.
[13] Cong J, Diao HL, Zhao YC, Ni H, Yan YQ, Yang ZM. Differential expression and regulation of cyloxygenases, prostaglandin E synthases and prostacyclin synthase in rat uterus during the peri-implantation period. Reproduction 2006;131:139–51.
[14] Hama K, Aoki J, Inoue A, Endo T, Amano T, Motoki R, et al. Embryo spacing and implantation timing are differentially regulated by LPA3-mediated lysophosphatidic acid signaling in mice. Biol Reprod 2007;77:954–9.
[15] Sordelli MS, Beltrame JS, Cella M, Gervasi MG, Perez Martinez S, Burdet J, et al. Interaction between lysophosphatidic acid, prostaglandins and the endocannabinoid system during the window of implantation in the rat uterus. Plos One 2012;7:e46059.
[16] Sordelli MS, Beltrame JS, Burdet J, Zotta E, Pardo R, Cella M, et al. The effect of anandamide on uterine nitric oxide synthase activity depends on the presence of the blastocyst. Plos One 2011;6:e18368.
[17] Sordelli Micaela S, Beltrame Jimena S, Cella Maximiliano, Franchi Ana M, Ribeiro Maria Laura. Cyclooxygenase-2 prostaglandins mediate anandamide-inhibitory action on nitric oxide synthase activity in the receptive rat uterus. Eur J Pharmacol 2012;685:174–9.
[18] Ribeiro ML, Perez Martinez S, Farina M, Ogando D, Gimeno M, Franchi AM. The effect of epidermal growth factor on prostaglandin synthesis of oestrogenized rat uterus is mediated by nitric oxide. Prostaglandins Leukot Essent Fatty Acids 1999;61:353–8.
[19] Ribeiro ML, Cella M, Farina M, Franchi AM. Crosstalk between nitric oxide synthase and cyclooxygenase metabolites in the estrogenized rat uterus. Prostaglandins Leukot Essent Fatty Acids 2003;68:285–90.
[20] Ribeiro ML, Ogando D, Farina M, Franchi AM. Epidermal growth factor modulation of prostaglandins and nitrite biosynthesis in rat fetal membranes. Prostaglandins Leukot Essent Fatty Acids 2004;70:33–40.
[21] Li S, Vana AC, Ribeiro R, Zhang Y. Distinct role of nitric oxide and peroxynitrite in mediating oligodendrocyte toxicity in culture and in experimental autoimmune encephalomyelitis. Neuroscience 2011;184:107–19.
[22] Novaro V, Pustovrh C, Colman-Lerner A, Radisky D, Lo Nostro F, Paz D, et al. Nitric oxide induces gelatinase A (matrix metalloproteinase 2) during rat embryo implantation. Fertil Steril 2002;78:1278–87.
[23] Song H, Lim H, Paria BC, Matsumoto H, Swift LL, Morrow J, et al. Cytosolic phospholipase A2alpha is crucial [correction of A2alpha deficiency is crucial] for 'on-time' embryo implantation that directs subsequent development. Development 2002;129:2879–89.
[24] Wang H, Ma WG, Tejada L, Zhang H, Morrow JD, Das SK, et al. Rescue of female infertility from the loss of cyclooxygenase-2 by compensatory up-regulation of cyclooxygenase-1 is a function of genetic makeup. J Biol Chem 2004;279:10649–58.

[25] Dey SK. Fatty link to fertility. Nature 2005;435:34–5.
[26] Cella M, Aisemberg J, Sordelli MS, Billi S, Farina M, Franchi AM, et al. Prostaglandins modulate nitric oxide synthase activity early in time in the uterus of estrogenized rat challenged with lipopolysaccharide. Eur J Pharmacol 2006;534:218–26.
[27] Aisemberg J, Vercelli CA, Billi S, Ribeiro ML, Ogando D, Meiss R, et al. Nitric oxide mediates prostaglandins' deleterious effect on lipopolysaccharide-triggered murine fetal resorption. Proc Natl Acad Sci U S A 2007;104:7534–9.
[28] Gu Y, Srivatstava RK, Clarke DL, Linzer DIH, Gibori G. The decidual prolactin receptor and its regulation by decidua-derived factors. Endocrinology 1996;137:4878–85.
[29] Noguchi K, Herr D, Mutoh T, Chun J. Lysophosphatidic acid (LPA) and its receptors. Curr Opin Pharmacol 2009;9:15–23.
[30] Achache H, Tsafrir A, Prus D, Reich R, Revel A. Defective endometrial prostaglandin synthesis identified in patients with repeated implantation failure undergoing in vitro fertilization. Fertil Steril 2010;94:1271–8.