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Vaginal Alpha-Lipoic Acid shows an anti-inflammatory effect on the cervix, preventing its shortening after primary tocolysis.

A pilot, randomized, placebo-controlled study

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Abstract

Introduction: Inflammation might be an important underlying cause of preterm birth. Our aim is to explore whether vaginal administration α-lipoic acid reduces cervical inflammation and shortening after primary tocolysis.

Matherials and Methods: Singleton pregnancies between 24-30 weeks remaining undelivered after hospitalization for preterm labor were randomly allocated to placebo (20 women, 15 analyzed) or vaginal ALA 400 mg daily (20 women, 17 analyzed) for 30 days. A cervical swab to quantify pro-inflammatory (IL1, IL2, IL6, IL8, TNFα) and anti-inflammatory (IL4, IL10) cytokines as well as transvaginal ultrasound cervical length measurement (CL) were performed before and after treatment.

Results: The % changes of pro-inflammatory cytokines do not differ between treatment groups, while IL4 significantly increases by vaginal ALA in comparison to placebo (118.0±364.3% vs. 29.9±103.5%, p=0.012). Combined anti-inflammatory cytokines show same trend (292.5±208.5% vs. 64.5±107.4, p=0.03). CL remains similar in vaginal ALA group (from 23.1±6.6 to 20.80±7.9 mm), while it significantly decreased in placebo group (from 20.4±6.5 to 13.8±7.5 mm, p<0.001 vs. Baseline; p=0.003 vs. vaginal ALA).

Conclusion: Vaginal ALA significantly stimulates anti-inflammatory ILs in the cervix of undelivered women after a preterm labor episode. This effect is associated with a stabilization of the CL.

Keywords: Vaginal Alpha-Lipoic acid, preterm birth, tocolysis, cervical length, cytokines, inflammation, chemokines, preterm labor.

1. Introduction

1.1 Background

The overall rate of preterm birth (PTB) is steadily increasing in the developed world [1,2]. Recent clinical and experimental evidence supports the syndromic nature of PTB [3] which derives from four pathogenic processes, sharing a common final biological pathway leading to uterine contractions and cervical changes with or without premature rupture of membranes: 1) the decidual-chorioamniotic or systemic inflammation, 2) the decidual haemorrhage, 3) the activation of the maternal or fetal hypothalamic-pituitary-adrenal axis, 4) pathological distention of the uterus [4].

Strategies to prevent preterm labor/PTB can now be taken into account in view of the availability of drugs and devices. Among them, treatment with progestogens to prevent PTB has represented the major research focus in the last years. Several studies have demonstrated the efficacy of either vaginal progesterone (P) or intramuscular 17α-hydroxyprogesterone caproate for the prevention of PTB in asymptomatic women with singleton pregnancy with a history of a previous preterm birth [5-7]. Moreover, evidence support the efficacy of vaginal P in asymptomatic women presenting with an objectively evaluated short cervix at mid pregnancy [8]. On the other hand, the efficacy of progestogens in women remaining undelivered after an episode of PTL remain to be demonstrated, as controversial data have been published [9-12]. For this reason new approaches to PTB prevention are needed [13], namely in the field of the so called "secondary or maintenance tocolysis".

Alpha-lipoic acid (ALA), also known as thioctic acid, and its reduced form, dihydrolipoic acid (DHLA), are naturally occurring compounds [14] with a well-known anti-oxidant and anti-inflammatory profile [15], probably via the inhibition of nuclear factor κ beta (NF-κB) signaling pathway [16]. NF-κB plays a fundamental role in the expression of various genes involved in inflammatory response and in cell apoptosis processes and it has a central role in labor-associated activation pathways. Its expression is increased in the myometrium in the later stages of pregnancy

and NF-κB activity mediates cyclo-oxygenase (COX)-2 expression in the human amnion during labor [17]. Its stimulation is connected to the cell's exposure to lipopolysaccharide, inflammatory cytokines (tumor necrosis factor (TNF)-α and interleukin (IL)-1), lack of progesterone, growth factors, free radicals and many other physiological and nonphysiological stimuli [18]. It has been observed that treatment with ALA was effective both in inhibiting the degradation of the IκB, a protein that inhibits NF-κB, and in directly reducing the expression of NF-κB and of matrix metalloproteinase-9 (MMP-9), an enzyme responsible for the degradation of the extracellular matrix [19]. This enzyme has been associated to PTB as its increased levels in amniotic fluid and its enhanced gene expression in fetal membranes have been associated with preterm rupture of membranes compared with other causes of PTB and TNFα-induced MMP-9 expression results in a significant decrease in the force needed to rupture human term fetal membranes [20]. LA *in vitro* treatment inhibited TNFα-induced weakening of fetal membranes and cytokine-induced MMP9 and prostaglandins production in both intact fetal membranes and amnion cells suggesting that its supplementation during pregnancy might prove clinically useful in prevention of preterm premature rupture of fetal membranes [21]. Recently it was also shown by preliminary trials that ALA, administered by oral or vaginal route, is able to potentiate the effect of progesterone in the healing process of threatened miscarriage and it may speed up the process of subchorionic hematoma resorption, by enhancing the levels of Vascular Endothelial Growth Factor (VEGF) and alpha Smooth Muscle Actin (alpha-SMA) and decreasing the expression of NF-κB and MMP-9 [22, 23].

1.2 Rationale

ALA, acting on different pathways involved in the pathogenesis of PTB [24], could be a molecule of interest. Thus, we have decided to investigate the vaginal administration of ALA looking at its effects on the cervix, in women remaining undelivered after an episode of preterm labor.

2. Materials and Methods

This was a pilot, prospective, randomized, placebo-controlled, parallel group, monocenter trial. The study was conducted from January 2015 to February 2016 in the Azienda Ospedaliero-Universitaria Policlinico of Modena in the Unit of Obstetrics and Gynaecology. The randomization allocation was 1:1 (vaginal ALA:placebo) and was accomplished using an electronic randomization list.

2.1 Subjects

A written informed consent was collected from all patients prior to inclusion in the study. The inclusion criteria were: women with a singleton pregnancy, at a gestational age ranging 24-30 weeks, hospitalized for a first preterm labour episode (regular uterine contractions with cervical change documented by transvaginal ultrasound. Cervical change was defined as a length < 25 mm or a documented cervical length decrease of more than 10 mm within the previous four weeks of gestation. Exclusion criteria included: previous cervical surgery (conization, cervical cerclage), cervical length < 5 or > 35 mm, cervical dilatation > 2 cm, use of progestagens or other vaginal suppositories in the last 30 days.

At hospital discharge, in women were randomized to receive either alpha-lipoic acid vaginal tablets 400 mg, 1 tablet per day before sleep (Dav ®, LO. LI. Pharma, Rome, Italy) per 30 days or apparently similar tablets composed by the very same excipients as the active drug product except the active ingredient (placebo).

During hospitalization, patients were treated according to the standard practice of our Institution, i.e, intravenous fluids, tocolytic therapy using Atosiban, steroid administration for respiratory Distress Syndrome prophylaxis, if clinically indicated.

2.2 Outcomes of the study

The primary outcome of the study was the change in the profile of pro-inflammatory and anti-inflammatory cytokines in cervical fluid. Monthly change of the cervical length (CL) was considered as a second outcome of the trial. Other outcome included the frequency of adverse events in patients allocated to placebo or ALA group. All outcomes were determined and the database was locked prior to the unsealing of the randomization code.

2.3 Determination of pro- and anti-inflammatory cytokines

All the samples for cytokine assays were obtained from the cervical fluid. Both sides of a Pre-weighted Dacron swab were applied for 10 seconds. Swab was then weighed again, stirred in 1.5 ml saline solution, centrifuged and stored at -20°C, until assay. Cytokine concentrations were measured following the manufacturer's instructions by the Ciraplex ® immunoassay kit (Aushon BioSytem Inc, Massachussets, US), a multiplex sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA). Each well of the 96-well microplate is pre-spotted with protein specific antibodies. After the removal of excess detection antibody, streptavidin-horseradish peroxidases (SA-HRP) is added and the luminescent signal detected by Cirascan ® Imaging System (Aushon BioSytem Inc, Massachussets, US). Lower Limit of Quantification (LLOQ) and Upper Limit of Quantification (ULOQ) (pg/ml) with back-calculated concentration <20% and relative error <25% of the kit are: IL1α: 0.439;1,800, IL1β: 0.049; 200, IL4: 0.049; 200, IL6: 0.028; 114, IL8: 0.391;400, IL10: 0.049; 200, TNFα: 0.391; 400.

Changes in pro- and anti-inflammatory cytokines were considered as concentrations (pg/ml), mean of the percent changes (%) and the mean of the percent changes of the pro- (IL1, IL2, IL6, IL8, TNFα) and anti-inflammatory (IL4, IL10) cytokines.

2.4 Determination of Cervical Length

All the ultrasound evaluations of the CL were performed by the same operator in the Preterm Birth Clinic (LP) by using a 25 Gold Mylab® (Esaote SpA®, Genoa, Italy) with an endo-vaginal probe model equipped for color Doppler imaging and power Doppler angiography (Model EC 1123).

With the woman's bladder emptied, the vaginal transducer was inserted into the anterior fornix of the vagina and positioned so that the endocervical canal is visualized. The ultrasound probe should be gradually withdrawn until the image is just visible to ensure there is not excessive pressure on the probe. A minimum of 3 CL measurements should be obtained by placing calipers at the internal and external os. The shortest and best measurement was recorded [25].

2.5 Statistical analysis

Data were analyzed by one of the authors (GG) who was blinded to the specific treatment of each group. Statistical analysis was performed by the statistical package StatView (version 5.01.98, SAS Institute Inc., Cary, NC). Within-group comparison was performed with the t-test for paired data and by the Wilcoxon signed-rank test for normal and non-normal data distribution, respectively. For all analyses, the null hypothesis was rejected at two-tailed p value < 0.05. Results are expressed as the mean±standard deviation (SD).

3. Results

The flowchart of the study is reported in Figure 1. A total of 57 women were eligible according to the inclusion/exclusion criteria of the study and they were asked to participate. Of these, 40/57 (70.2%) subjects accepted, signed the informed consent and were then randomized to the treatment groups (vaginal ALA vs. placebo) in a a ratio of 1:1 (20 subjects per group). No significant differences were found in subjects that reach final follow up visit [17/20 (85%) in vaginal ALA group, 15/20 (75%) in placebo group, p=0.72].

The baseline features of the women included in the study are reported in Table 1 and comparable between groups, except that subjects in the vaginal ALA were more frequently included presenting both contractions and cervical changes at admission (p=0.03).

3.1 Changes in the concentrations of cytokines in cervical fluid

Baseline and after treatment values of cytokines are reported in Table 2. Baseline levels were not different between groups.

The percent changes of pro- and anti-inflammatory cytokines were then considered. No significantly different changes were observed between groups as far as pro-inflammatory cytokines are concerned (vaginal ALA vs. placebo) (Figures 2a and 2c). On the contrary, vaginal ALA induced a significant increase of IL4 in comparison to the placebo group (118.0±364.34% vs. 29.9±103.5%, p=0.012) (Figure 2b). Once combined with IL10 changes, the increase of both anti-inflammatory cytokines was significantly more pronunced in vaginal ALA group in comparison to placebo group (292.5±208.5% vs. 64.5±107.4%, p=0.03) (Figure 2c).

3.2 Changes of cervical length

Overall, cervical length remains similar in vaginal ALA group (from 23.1±6.6 to 20.8±7.9 mm), while it significantly decreased in the placebo group (from 20.4±6.5 to 13.8±7.5 mm, p=<0.001 vs. Baseline; p=0.003 vs. vaginal ALA).

The women were then divided according to cervical length at inclusion < 20 mm (n=11) (Figure 2a) or ≥ 20 mm (n=21) (Figure 2b). The general findings reported above were true for both subgroups (< 20 mm: p=0.014 vs. Vaginal ALA, p=0.009 vs. Baseline, ≥ 20 mm: p=0.05 vs. Vaginal ALA, p=0.002 vs. Baseline) (Figure 3a-b).

3.3 Tolerability

The only adverse effect reported in the vaginal ALA group was transient vaginal discomfort in 2/17 (11.8%) of the subjects while none in the placebo group. The incidence of adverse effects was not different between groups (p=0.170).

4. Discussion

4.1 Principal findings of the study

In this pilot study, we have shown that vaginal ALA administration is associated with a specific anti-inflammatory effect at the cervical level in comparison to placebo, namely stimulating anti-inflammatory cytokines. Vaginal ALA is more effective than placebo in limiting the monthly cervical shortening observed in women remaining undelivered after a preterm labor episode. Such effect is present both in women with a short cervix (< 20 mm) as well as in those with a cervical length above this cut-off. However, whether this biological property of vaginal ALA could lower the risk of PTB (thus affecting neonatal morbidity/mortality) or not has to be evaluated in large randomized clinical trial.

4.2 Interpretation

The aetiology of PTB is complex and unfortunately still not understood. After an episode of threatened preterm labor with intact membranes, which is initially treated with tocolysis and corticosteroids for 48 h, women remain at risk for preterm delivery, as they deliver before 37 weeks at a rate ranging 38-75% [26-28].

Accumulated research indicate that infection and inflammation (most often subclinical) is an important underlying cause of PTB. In particular a recent systematic review has shown that IL6 and C-reactive protein in amniotic fluid, but not in plasma, are strongly associated with increased risk of spontaneous PTB among asymptomatic women, indicating that inflammation at the maternal–fetal interface rather than a systemic one, may play a major role in the etiology of such spontaneous PTB. These data are confirmed by the elevation of IL6 levels in the cervico-vaginal fluid which are also strongly associated with spontaneous PTB, suggesting that the local level presents the most important and subtle changes in women with PTB. However, there was insufficient evidence for the association between other inflammatory cytokines, like IL1, IL2, IL8 and TNF-α and spontaneous PTB among asymptomatic women [29]. The role of these mediators has not yet been entirely

elucidated, but it is likely that inflammatory cytokines bring about parturition through increasing prostaglandin levels by affecting prostaglandins production, metabolism, or both.

In this study, we focused on the activity of vaginal ALA at the cervical level. In ongoing experiments in female rats, addressed to verify ALA vaginal absorption, it was demonstrated by immunohistochemistry that the endometrial epithelial and stromal cells and the cervical squamous epithelium and muscular tissue were stained with intense amount of anti-ALA antibody, proving this route of administration very effective for ALA [30].

In our study the active treatment did not affect pro-inflammatory cytokines, including IL6. On the other hand, vaginal ALA stimulated the cervical secretion of IL4, an anti-inflammatory cytokine that induces differentiation of naive helper T cells to Th2 cells. It is well-known that a shift in the bias of overall maternal reactivity to the more conducive Th2 type, or at least a reduction in strong Th1 bias, may provide the best milieu to favor pregnancy [31].

Moreover, polymorphisms of IL4 have been associated with spontaneous PTB [32] while its dysregulation has found to be correlated with Toll-Like receptor activity in cases of preterm neonates [33].

Our findings are in agreement with the growing body of evidences suggesting the clinical use of LA when the abnormal regulation of the inflammatory response is recognized as an important component of the disease as in the case of diabetes, Alzheimer's disease and multiple sclerosis [15]. In such conditions, LA exhibits anti-inflammatory properties by inhibiting IL6 and IL17 production and T cell proliferation and activation while possibly maintaining IL10 synthesis [15, 16, 30].

On the other hand, CL is a marker of clinical value in the prediction of PTB, the shorter is the cervix, the higher is the risk of PTB [34, 35]. However, a progressive shortening of the cervix has been reported throughout physiological pregnancy. Namely, between 30 and 34 weeks the cervix shortens by 3 mm [36], the same change we observed in women receiving vaginal ALA. Considering that the cervix shorten the double in our women receiving placebo, the active treatment, slowing down cervical remodelling, reduces the theoretical lower risk of PTB.

4.3 Implications for further research

The use of ALA represents a safe and well-tolerate treatment in many circumstances up to doses of 2400 mg/day [37], also in pregnant women. We confirm tolerability in our small trial and demonstrated that vaginal ALA exerts a direct anti-inflammatory effect on the cervix, preventing its shortening after primary tocolysis. Such premise allows the planning of a clinical trial to explore the efficacy of this treatment in the prevention of PTB.

4.4 Strenghts and Limitations

The major limitation of this study is the small sample size, thus it should be considered as a pilot study. Furthermore, the study was conducted in a single center and the results cannot be generalized. For all these reasons, results should be considered preliminary, although worthy to be explored in larger comparative investigations.

4.5 Conclusion

To the best of our knowledge, this is the first study investigating the effects of ALA administration through vaginal route after primary tocolysis. Such treatment induces significant changes in the cervical ILs pattern in undelivered women after a preterm labor episode, namely stimulating anti-inflammatory cytokines. Moreover, the treatment is associated with a stabilization of the cervix, which otherwise undergoes to a shortening.

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6. Declaration of interest statement: Fabio Facchinetti was invited by Lo.Li Pharma to sponsored symposia within International Congresses. Other authors: nothing to declare. There was no funding for this trial except Lo.Li Pharma provided both active and placebo treatments and supply diagnostic kits.

7. References

1] World Health Organization (WHO), Preterm birth Fact sheet N°363 Updated November 2015, http://www.who.int/mediacentre/factsheets/fs363/en/

2] March of Dimes, 2015 Premature Birth Report Cards, http://www.marchofdimes.org/mission/prematurity-reportcard.aspx

3] Romero R, Mazor M, Munoz H, Gomez R, Galasso M, Sherer DM. The preterm labor syndrome. Ann N Y Acad Sci 1994; 734: 414-29.

4] Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science 2014: 15;345:760-5.

5] Meis PJ, Klebanoff M, Thom E, et al. Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. N Engl J Med 2003; 348: 2379e85.

6] Da Fonseca EB, Bittar RE, Carvalho MH, et al. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomised placebo-controlled double-blind study. Am J Obstet Gynecol 2003;188:419e24.

7] Spong CY, Meis PJ, Thom EA, et al. Progesterone for prevention of recurrent preterm birth: impact of gestational age at previous delivery. Am J Obstet Gynecol 2005; 193:1127e31.

8] Romero R, Nicolaides KH, Conde-Agudelo A et al. Vaginal progesterone decreases preterm birth ≤ 34 weeks of gestation in women with a singleton pregnancy and a short cervix: an updated meta-analysis including data from the OPPTIMUM study. Ultrasound Obstet Gynecol 2016;48:308-17.

9] Suhag A, Saccone G, Berghella V. Vaginal progesterone for maintenance tocolysis: a systematic review and metaanalysis of randomized trials. Am J Obstet Gynecol 2015;213:479-87.

10] Saccone G, Suhag A, Berghella V. 17-alpha-hydroxyprogesterone caproate for maintenance tocolysis: a systematic review and metaanalysis of randomized trials. Am J Obstet Gynecol 2015;213:16-22.

11] Martinez de Tejada B, Karolinski A, et al; 4P trial group. Prevention of preterm delivery with vaginal progesterone in women with preterm labour (4P): randomised double-blind placebo-controlled trial. BJOG 2015;122:80-91.

12] Palacio M, Cobo T, Antolín E et al; PROMISE Collaborative Group. Vaginal progesterone as maintenance treatment after an episode of preterm labour (PROMISE) study: a multicentre, double-blind, randomised, placebo-controlled trial. BJOG 2016 Mar 30.

13] Kurjak A. Preterm birth--prediction, prevention, and consequences: an unmet challenge to perinatal medicine, science, and society: the declaration of Dubrovnik. J Matern Fetal Neonatal Med 2010;23:1286-7.

14] Shay KP, Moreau RF, Smith EJ, et al. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. Biochim Biophys Acta 2009;1790:1149-1160.

15] Salinthone S, Yadav V, Schillace RV, et al. Lipoic acid attenuates inflammation via cAMP and protein kinase A signaling. PLoS One 2010;5(9).

16] Li G, Fu J, Zhao Y, et al. Alpha-lipoic acid exerts anti-inflammatory effects on lipopolysaccharide-stimulated rat mesangial cells via inhibition of nuclear factor kappa B (NF-κB) signaling pathway. Inflammation 2015; 38: 510-9.

17] Allport VC, Pieber D, Slater DM, Newton R, White JO, Bennett PR. Human labor is associated with nuclear factor-kappaB activity which mediates cyclooxygenase- 2 expression and is involved with the 'functional progesterone withdrawal'. Mol Hum Reprod 2001; 7: 581–586.

18] Grandi G, Mueller M, Bersinger N, et al. Progestin suppressed inflammation and cell viability of tumor necrosis factor-α stimulated endometriotic stromal cells. Am J Reprod Immunol 2016; In press.

19] Hye-Soon K, Hye-Jin K, Keun-Gyu P, et al. α-Lipoic acid inhibits matrix metalloproteinase-9 expression by inhibiting NF-κB transcriptional activity. Exp Mol Med 2007; 39: 106-113.

20] Ferrand PE, Parry S, Sammel M, et al. A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans. Mol Hum Reprod 2002; 8:494– 501.

21] Moore RM, Novak JB, Kumar D, et al. Alpha-lipoic acid inhibits tumor necrosis factor-induced remodeling and weakening of human fetal membranes. Biol Reprod 2009; 80: 781-7.

22] Porcaro G, Brillo E, Giardina I, et al. Alpha Lipoic Acid (ALA) effects on subchorionic hematoma: preliminary clinical results. Eur Rev Med Pharmacol Sci 2015; 19: 3426-32.

23] Costantino M, Guaraldi C, Costantino D. Resolution of subchorionic hematoma and symptoms of threatened miscarriage using vaginal alpha lipoic acid or progesterone: clinical evidences. Eur Rev Med Pharmacol Sci 2016; 20: 1656-63.

24] MacIntyre DA, Sykes L, Teoh TG, Bennett PR. Prevention of preterm labour via the modulation of inflammatory pathways. J Matern Fetal Neonatal Med 2012;25:17-20.

25] Society for Maternal-Fetal Medicine (SMFM). McIntosh J, Feltovich H, Berghella V, et al. The role of routine cervical length screening in selected high- and low-risk women for preterm birth prevention. Am J Obstet Gynecol 2016 Apr 28. [Epub ahead of print]

26] Facchinetti F, Paganelli S, Comitini G, et al. Cervical length changes during preterm cervical ripening: effects of 17-alpha-hydroxyprogesterone caproate. Am J Obstet Gynecol 2007; 196:453.e1-4.

27] Lyell DJ, Pullen KM, Mannan J, et al. Maintenance nifedipine tocolysis compared with placebo: a randomized controlled trial. Obstet Gynecol 2008;112:1221-6.

28] Valenzuela GJ, Sanchez-Ramos L, Romero R, et al. Maintenance treatment of preterm labor with the oxytocin antagonist atosiban. The Atosiban PTL-098 Study Group. Am J Obstet Gynecol 2000; 182: 1184-90.

29] Wei SQ, Fraser W, Luo ZC. Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review. Obstet Gynecol 2010; 116: 393-401.

30] Monastra G, De Grazia S, Cilaker Micili S, et al. Immunomodulatory activities of alpha lipoic acid with a special focus on its efficacy in preventing miscarriage. Expert Opin Drug Deliv 2016 Jun 24:1-14. [Epub ahead of print]

31] Makhseed M, Raghupathy R, Azizieh F, et al. Th1 and Th2 cytokine profiles in recurrent aborters with successful pregnancy and with subsequent abortions. Hum Reprod. 2001;16:2219-26.

32] Harmon QE, Engel SM, Olshan AF, et al. Association of polymorphisms in natural killer cell-related genes with preterm birth. Am J Epidemiol 2013; 178:1208-18.

33] Heinzmann A, Mailaparambil B, Mingirulli N, et al. Association of interleukin-13/-4 and toll-like receptor 10 with preterm births. Neonatology 2009; 96: 175-81.

34] van Baaren GJ, Vis JY, Wilms FF, et al. Predictive value of cervical length measurement and fibronectin testing in threatened preterm labor. Obstet Gynecol 2014; 123: 1185–92.

35] DeFranco EA, Lewis DF, Odibo AO. Improving the screening accuracy for preterm labor: is the combination of fetal fibronectin and cervical length in symptomatic patients a useful predictor of preterm birth? A systematic review. Am J Obstet Gynecol 2013; 208: 233 e1–6.

36] Silva SV, Damião R, Fonseca EB, et al. Reference ranges for cervical length by transvaginal scan in singleton pregnancies. J Matern Fetal Neonatal Med 2010; 23: 379-82.

37] Gorąca A, Huk-Kolega H, Piechota A, et al. Lipoic acid - biological activity and therapeutic potential. Pharmacol Rep 2011; 63: 849-58.

8. Tables

Table 1. Baseline features of the subjects randomized for the two different treatment groups (Placebo (n=20) and Vaginal ALA (n=20)). Values are expressed in mean±standard error (SE).

	Placebo (n=20)	**Vaginal ALA (n=20)**	**P**
Gestational Age (days)	207.7±7.4 (160-237)	216.6±3.6 (184-237)	0.234
Age (years)	30.8±1.5	28.5±1.5	0.305
Nulliparous (%)	12/20 (60%)	13/20 (65%)	0.744
Cervical lenght at inclusion (mm)	20.4±1.9	23.1±1.5	0.270
Need of tocolysis (%)	8/20 (40%)	10/20 (50%)	0.525
Betamethasone need (%)	18/20 (90%)	19/20 (95%)	0.548
Indication for admission - Contractions only (%) - Cervical change only (%) - Both contractions and cervical changes (%)	 7/20 (35%) 4/20 (20%) 9/20 (45%)	 2/20 (10%) 1/20 (5%) 17/20 (85%)	0.030

Table 2. Baseline and after treatment concentration (pg/ml) in the cervico-vaginal fluid of pro-inflammatory (IL1, IL2, IL6, IL8, TNFα) and anti-inflammatory (IL4, IL10) cytokines of the subjects randomized for the two different treatment groups (Placebo (n=15) and Vaginal ALA (n=17). Values are expressed in median (inter-quartile ranges). * p< 0.05

Cytokines		Baseline	After treatment
Pro-inflammatory			
IL1(pg/ml)	Vaginal ALA (n=17)	192.12 (56.18; 266.78)	297.18 (113.85;386.75)
	Placebo (n=15)	343.43 (87.55;567.65)	590.31 (246.48;713.54)
IL2 (pg/ml)	Vaginal ALA (n=17)	0.70 (0.31;1.35)	0.48 (0.18;1.93)
	Placebo (n=15)	1.11 (0.83;1.47)	0.73 (0.17;1.78)
IL6 (pg/ml)	Vaginal ALA (n=17)	33.45 (1.04;79.86)	84.20 (0.27;166.77)
	Placebo (n=15)	6.25 (1.71;14.50)	3.00 (2.50;25.50)
IL8 (pg/ml)	Vaginal ALA (n=17)	146.50 (0.35;1,586.00)	652.70 (135.70;1,470.00)
	Placebo (n=15)	674.20 (394.1;1,455.00)	283.00 (89.35;1,410.00)
TNFα (pg/ml)	Vaginal ALA (n=17)	0.27 (0.10;1.39)	0.16 (0.08;1.89)
	Placebo (n=15)	0.40 (0.21;0.67)	1.35 (0.41;3.22)
Anti-inflammatory			
IL4 (pg/ml)	Vaginal ALA (n=17)	0.03 (0.01;0.14)	0.09 (0.03;0.21)*
	Placebo (n=15)	0.07 (0.03;0.10)	0.05 (0.02;0.07)
IL10 (pg/ml)	Vaginal ALA (n=17)	0.16 (0.01;0.35)	0.59 (0.04;3.44)
	Placebo (n=15)	0.09 (0.04;0.17)	0.29 (0.04;1.33)

9. Legends for Figures

Figure 1. Flow-chart of the study.

Figure 2. Percent changes of cytokines concentrations in the cervical secretion after 30 days of treatment with vaginal ALA or placebo. Changes of pro-inflammatory cytokines (IL1, 2, 6, 8 and TNFα) are reported separately **(Figure 2a)** from those of anti-inflammatory cytokines (IL4 and IL10) **(Figure 2b).** The bottom panel reports them (pro- and anti-inflammatory) combined **(Figure 2c).** Bars represent scattergram with medians. Continuous line: ALA group. Dashed line: Placebo Group.

* $p<0.05$ vs. Vaginal ALA

Figure 3. Changes of CL after 30 days of treatment with vaginal ALA or placebo in subjects with a cervical length at inclusion <20 mm (n=11) **(Figure 3a)** or ≥20 mm (n=21) **(Figure 3b).** Bars represent means±standard error (SE).

* $p<0.05$ vs. Vaginal ALA

** $p<0.01$ vs. Baseline