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Resolution of subchorionic hematoma and symptoms of threatened miscarriage using vaginal alpha lipoic acid or progesterone: clinical evidences

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Abstract. – OBJECTIVE: Alpha Lipoic Acid (ALA) is a safe natural molecule that exerts a selective immunomodulating activity with antioxidant and anti-inflammatory properties. This randomized controlled clinical trial (RCT) tested the effect of the vaginal administration with ALA or Progesterone, in subchorionic hematoma resorption in women with threatened miscarriage.

PATIENTS AND METHODS: 400 mg of vaginal Progesterone or 10 mg of vaginal ALA were administered to sixty-two pregnant women, in the first trimester of gestation with threatened miscarriage and subchorionic hematoma. Controls were patients who chose not to receive any treatment.

RESULTS: In the ALA group the subchorionic hematoma was reabsorbed more quickly in comparison with the progression detected in Progesterone group ($p \leq 0.05$). The other parameters checked (pelvic pain and vaginal bleeding) did not show any significant difference and a smaller number of miscarriages was recorded in the ALA group, compared to Progesterone group.

CONCLUSIONS: Our data provides the first evidence of the efficacy of ALA, administered by vaginal route, in the healing process of patients with threatened miscarriage, thus supporting the normal course of pregnancy. Clinical trial registration number: NCT02601898 (ClinicalTrials.gov registry).

Key Words:
Threatened miscarriage, Vaginal alpha lipoic acid (ALA), Vaginal progesterone, Randomized controlled study, Subchorionic hematoma.

Introduction

Subchorionic hematoma is a gathering of blood in the subchorial area, between the membranes of the placenta and the chorion, deriving from a subchorionic hemorrhage. This kind of hematoma can appear during the first trimester of pregnancy (early pregnancy) and it is a typical anomaly of this gestational period. It is due to the partial separation of the chorion from the underlying decidua. Around 18% of all cases of vaginal bleeding in the first trimester are caused by a subchorionic hematoma[1], which is detected only via ultrasound scan. It shows a normal gestational sac close to an anechoic area, or echo free on a sonographic image, of variable size, having the typical form of a half-moon. The scan image plays a pivotal role to analyze any improvement or worsening. Also the clinical examination is required, because the sonographic results have to be related to clinical symptoms (i.e. bleeding, pelvic pain)[2]. The manifestation of a first-trimester subchorionic hematoma represents a very appropriate marker for identifying patients at greater risk for threatened miscarriage[3], a serious problem in the first 20 weeks of pregnancy, characterized by vaginal blood leaking, other than spotting, and pelvic pain. The presence of a large first-trimester subchorionic hematoma was related to a 46% risk of adverse pregnancy outcome, i.e. premature rupture of membranes and spontaneous abortion[4]. Consequently, its resorption is an essential goal to avoid early pregnancy loss. Among several causes of miscarriages, up to 80% are genetic[5], but also inflammatory processes and immunologic disorders can be mentioned[6,7]. A complex pathophysiological mechanism underlies the event of threatened miscarriage, where T helper (Th) cells (both Th1 and Th2), Th 17 and regulatory T (Treg) cells[8,9] are involved in combination with numerous signal molecules, exerting pro- or anti-inflammatory effects. Th1 cells are involved in cellular immunity, and Th2 in humoral immunity. This classifi-

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cation reflects the type and prevalence of cytokines secreted by each subset of cells[10,11]. Th17 cells play a central role in giving rise to inflammation[9,12,13], whereas Treg cells usually turn down the immune response[9]. Especially in the first months of pregnancy, inflammatory process is essential to protect the host from pathogens, allowing the continuation of pregnancy[14]. Interestingly, both excessive inflammation and immune suppression can prompt embryo resorption[8,14]. In general, an adequate balance between all the elements needs to be kept or restored for the maintenance of a healthy gestation.

The therapeutic effects of Progesterone in pregnancy, in condition of threatened miscarriage, is approved by therapeutic protocols, but its efficacy is strongly put in doubt and criticized[15-20]. In this context, Alpha Lipoic Acid (ALA), a multifunctional natural molecule, is revealing a very interesting profile. This compound, given orally or i.v., is safe at therapeutic doses, and exerts worthwhile biological activity, that are beneficial also for modulating several mechanisms underlying threatened miscarriage[21-23]. Vaginal administration of ALA is a new approach which can provide a direct effect at vaginal and uterine level.

The aim of this pilot study was to preliminary compare the therapeutic efficacy of ALA *vs*. Progesterone, by vaginal administration, on subchorionic hematoma resorption in women at the first trimester of pregnancy with threatened miscarriage. Furthermore, also the effects on pelvic pain and vaginal bleeding were evaluated.

Patients and Methods

Patients

Gravid women with threatened miscarriage were enrolled from January 2015 to August 2015 at the Women's Health Center, Azienda USL Ferrara – Italy. The inclusion criteria were: patients age 24-40 years and in the 7th to 12th week of physiological gestation, with pelvic pain and with or without moderate vaginal bleeding (comparable to the heaviest normal menstrual flow), and subchorionic hematoma, observed by sonography. The exclusion criteria were: lack of fetus, absence of fetal heart tone, uterine anomaly or fetal anomaly, presence of multiple pregnancy, pre-pregnancy or gestation pathologies (such as maternal autoimmune diseases, antiphospholipid syndrome, arterial hypertension), diagnosis of allergic reaction to progesterone, therapies with anti-coagulant or anti-hypertensive drugs. Furthermore, patients with previous miscarriage (no more than three miscarriages) underwent to examination to exclude karyotype abnormalities and any possible clinical factor linked to recurrent pregnancy loss. All the patients gave an informed consent before entering the study. The protocol was approved by the Ethics Committee.

Study Design and Treatment Regimen

The study was a Randomized Controlled Trial (RCT) with allocation concealment of patients in two treatments groups (1:1 ratio): in one group patients received 400 mg Progesterone (Progeffik®, Effik Italia srl, two vaginal soft gel per day, before sleeping), and in the second one (case study) 10 mg of ALA (DAV® vaginal capsules, Lo.Li. Pharma srl, Rome, Italy, one vaginal capsule per day, before sleeping). Randomization did not involve a third group which was formed by twenty-two patients who decided not to receive any treatment and it was used as control (Figure 1). Treatments were given until the total resolution of the clinical picture.

Outcomes and Follow-up

The primary outcome was the resolution of subchorionic hematoma linked to threatened miscarriage. The evaluation of the hematoma significance was done comparing its size with that one of the gestational sac during the ultrasound examination, according to the method reported by Nagy et al[4] which allows one to classify the subchorionic hematoma as small (< 20% of the gestational sac), medium (20%-50% of the gestational sac), or large (> 50% of the gestational sac). Changes in hematoma resorption (% improvement/ worsening) during the treatment were obtained for each patient by calculating the Δ percentage between two subsequent time points. Then the average values for each group were compared. The secondary outcomes were reduction/disappearance of the subjective (pelvic pain) and objective (vaginal bleeding) symptoms. A sheet was given to all patients to record the evolution of these symptoms, which later had to be statistically analysed.

Follow-up checks were performed at twenty days (t = 1), and sixty days (t = 2) from the beginning of the treatment (baseline, t = 0) and both clinical signs and symptoms were recorded by investigators. The incidence of miscarriage was evaluated in both groups and the treatment was judged successful if pregnancy went over 20 weeks.

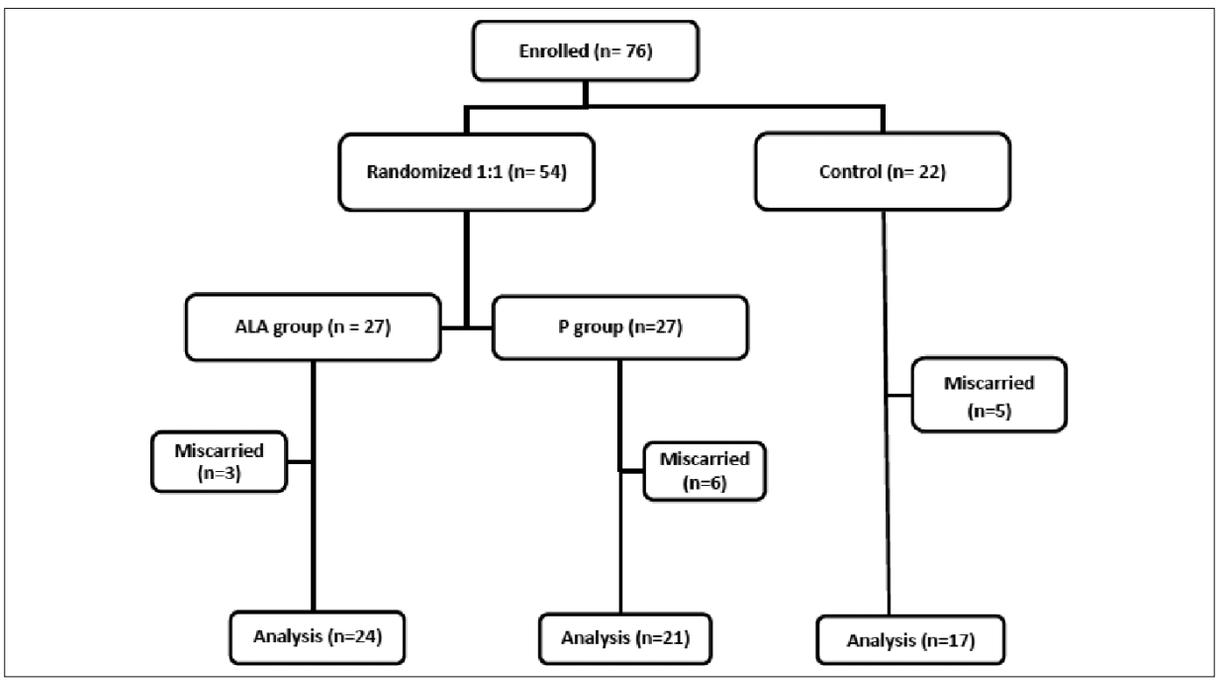

Figure 1. Flow chart of participants over the course of the trial (Progesterone: P).

Statistical Analysis

Percentages and mean values of sign and symptoms were calculated excluding the number of patients who miscarried. Statistical analysis of the clinical data was performed by SPSS software (SPSS Inc., Chicago, IL, USA) employing Wilcoxon test for nonparametric data. A Mann-Whitney U test, with SPSS software, was used to make the comparison between the single groups. The difference was considered statistically significant if the *p*-value was ≤ 0.05.

Results

Among eighty-four evaluated subjects, with threatened miscarriage, a total number of seventy-six pregnant women were included in the trial according to the inclusion criteria. Patients characteristics at baseline were homogeneous in both groups, as shown in Table I.

The outcomes were evaluated excluding the number of patients who miscarried during the study.

Table I. Baseline patients features in the three groups.

Parameter	Group ALA n = 27	Group P n = 27	Group C n = 22	*p*-value
Age (years)	29.8 ± 0.7	31.2 ± 1.6	30.4 ± 1.3	n.s.
Gestational age (weeks)	8.8 ± 0.2	9.1 ± 0.3	8.9 ± 0.2	n.s.
Nulliparous (%)	13 (48%)	11 (41%)	9 (41%)	n.s.
Multiparous (%)	0	0	0	n.s.
Previous C-section (%)	2 (7%)	3 (11%)	1 (4%)	n.s.
Previous miscarriages	12 (44%)	10 (37%)	10 (66%)	n.s.
-1 miscarriage	6	6	5	n.s.
-2 miscarriages	5	3	3	n.s.
-3 miscarriages	1	1	2	n.s.
Vaginal bleeding (%)	13 (48%)	14 (52%)	14 (64%)	n.s.
Hematoma size				n.s.
-Medium	24 (89%)	25 (92%)	20 (94%)	n.s.
-Large	3 (11%)	2 (7%)	2 (9%)	n.s.

Data are expressed as mean ± SE or as number and percentage (Progesterone: P).

A blind investigator controlled the patients after twenty days (t = 1), and sixty days (t = 2) from the baseline (t = 0) by vaginal ultrasound scan to check and register the evolution of subchorionic hematoma. The ALA group was found to be statistically different from Progesterone and control groups. The result was significant at $p \leq 0.05$ (Figures 2 and 3). Patients treated with Progesterone did not show any significant difference *vs*. controls.

According to the inclusion criteria, pelvic pain was present in all the patients at the baseline and it was recorded only in 3 subjects treated with Progesterone and in 2 subjects who did not receive treatment at the first medical examination (t1) and nobody at the second one (t2). Also regarding vaginal bleeding, the effects due to the treatments were similar.

Although not significantly different, a smaller number of miscarriages was registered in the ALA group: this is an interesting, positive trend, which should be further verified in a following trial with a large cohort of patients (Table II).

No adverse effects on foetus were detected during the treatments and until the final check-up of the study. Four patients in the case study group reported sporadic episodes of mild vaginal burning which did not require suspension or discontinuation of the therapy.

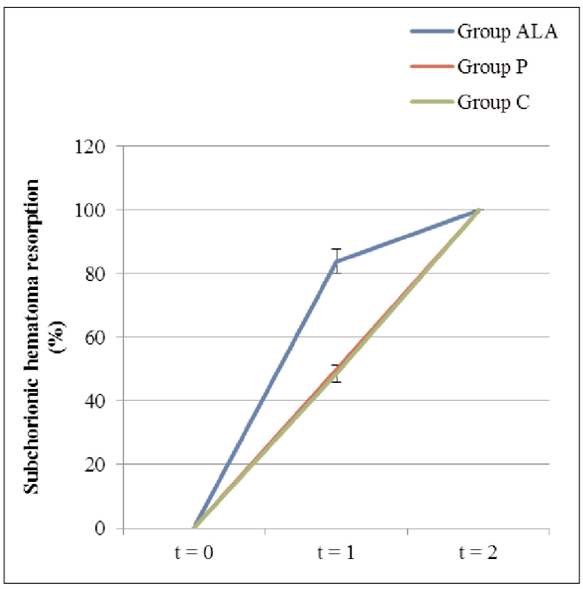

Figure 2. The progress of subchorionic hematoma resorption was detected by ultrasound in ALA group (n = 24), Progesterone (P) group (n = 21) and Control group (n=17) at different time points of treatment and the data are shown as Δ percentage of Mean ± SEM. Percentages and mean values were calculated excluding the number of patients who miscarried. The size of the hematoma was compared (%) with the size of the gestational sac during the examination. Progressive hematoma resorption during treatment was calculated as Δ percentage between two subsequent time points for each patient and the medium value for each group has been obtained and compared.

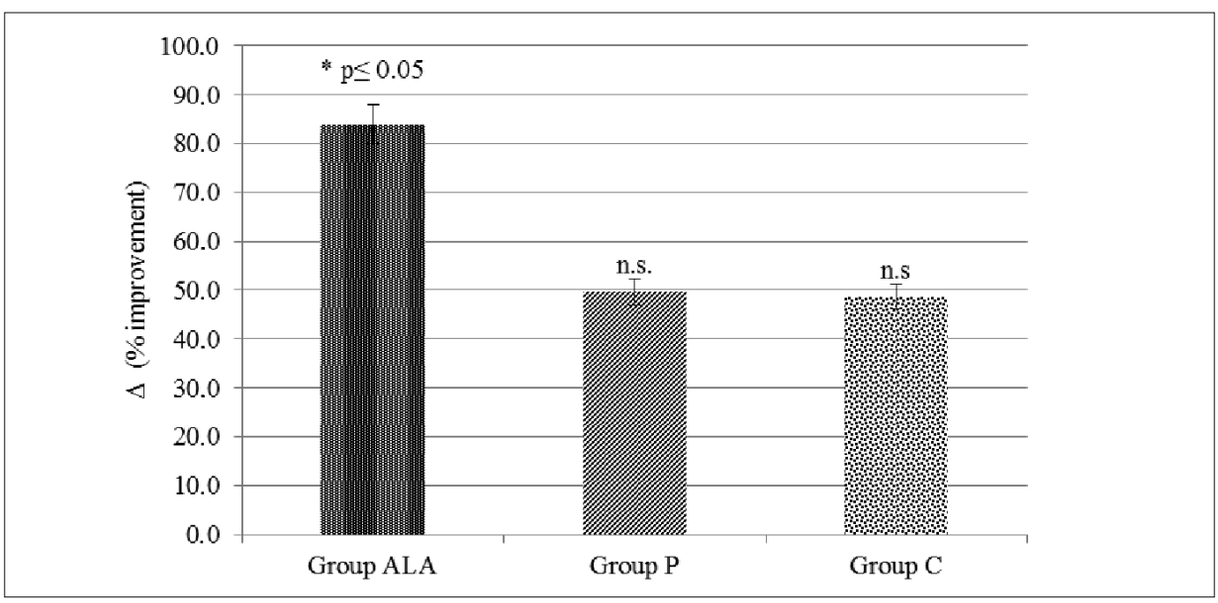

Figure 3. ***A,*** Statistical significance of Δ (% improvement) at t = 1 for the subchorionic hematoma resorption: ALA, Progesterone (P) and control groups (mean ± SEM). Wilcoxon and Mann-Whitney U test were used. The ALA group was found significantly different ($p \leq 0.05$) from Progesterone and control groups. Patients treated with Progesterone did not show any significant difference vs controls.

Table II. Effects of the treatment with ALA, Progesterone (P) or no treatment (C) on symptoms and incidence of miscarriage.

	Baseline med. exam. t_0			1st Medical control t_1			2nd Medical control t_2			Miscarriage		
Symptoms	ALA (n = 24)	P (n = 21)	C (n =17)	ALA (n = 24)	P (n = 21)	C (n =17)	ALA (n = 24)	P (n = 21)	C (n =17)	ALA (n = 27)	P (n = 27)	C (n = 22)
Pelvic pain	24 (100%)	21 (100%)	17 (100%)	0 (0%)	3 (14%)	2 (12%)	–	0 (0%)	0 (0%)	3	6	5
Vag. bleed	13 (54%)	12 (57%)	11 (65%)	0 (0%)	0 (0%)	2 (12%)	–	–	0 (0%)			

Data are given as sample size (number and percentage) of each group at different time points: t_0: baseline medical examination; t_1: 20 days after baseline medical examination; t_2: 60 days after baseline medical examination. Percentages were calculated excluding the number of patients who miscarried.

Discussion

As shown by our data, the subchorionic hematoma has shown a faster resorption as result of the vaginal administration with ALA, than with Progesterone. These findings are in full agreement with a very recent paper where the authors have found an interesting effect due to the oral administration of ALA plus Progesterone by vaginal route vs only Progesterone[24].

Data obtained in previous researches and studies can help in explaining these clinical results, focusing the role played by some cytokines and T helper 1 (Th1) and T helper 2 (Th2) cells in subchorionic hematoma formation and miscarriage. The cytokine network is deeply involved in the positive or negative development of the ongoing pregnancies[25], even though the knowledge of the specific underlying mechanisms is in evolution. Th1 cells are essential in cellular immunity, whereas Th2 cells are involved in humoral immunity. There are some peculiarities that distinguish them: IFN-gamma and TNF-beta are released only by Th1, whereas IL-4 and IL-5 only by Th2. Moreover, Th1 predominantly secrete IL-2, TNF-alpha, TGF-beta, and other (e.g. IL-10) in small amounts. On the other hand, Th2 release higher quantities of IL-3, IL-6, IL-9, IL-10, IL-13, TGF-beta, but little TNF-alpha and IL-2[26,27]. Th1 cells, through their cytokines, stimulate macrophages, lymphocytes, and PMNs to destroy bacterial pathogens[11]. Furthermore, they induce the development of cytotoxic T cells, which play an essential role in the cell-mediated immune response against the aggression of foreign agents such as viruses and tumor cells. This pivotal function exerted by Th1 cells in immune system sometimes can give rise to their over activation or misdirected attacks against some of our own tissues, making Th1 cells central players in autoimmune diseases and also when extraneous cells develop[11].

Th2 cells stimulate strong antibody responses and eosinophil accumulation and also inhibit several functions of phagocytic cell[11].

Saito and his team[8] have suggested an intriguing explanation involving, Th1, Th2, Th17 and regulatory T (Treg) cells in pregnancy. Th17 cells produce IL-17, an proinflammatory cytokine, and exert a pivotal role in giving rise to inflammation[9,13,14]. Th 17 cells can induce pathogenetic mechanisms in autoimmunity and acute transplant rejection, affecting also the development of gestation. It is worth of note that Th17 cells and their released cytokine, IL-17, might exert a dual action on pregnancy, like IL-1. Indeed, increased Th17 cells in pregnancy decidua might be disadvantageous for the maintenance of physiological process[28]. On the other side, Treg cells usually turn down the immune response[9], playing a pivotal role in the processes of immunoregulation and in the induction of tolerance. They secrete anti-inflammatory cytokines: IL-10, IL-35, TGF-beta, which, directly or indirectly, inhibit the release or the activity of pro-inflammatory cytokines. Treg cells inhibit cytotoxic activity of natural killer (NK) cells, immunoglobulin production by B cells, and dendritic cells (DCs) maturation[29,30]. Th1/Th2/Th17 and Treg lineages are associated with each other, and, in some cases, they are able to convert to other lineages[8]. Now, it holds to be true that in normal development of pregnancy inflammation is necessary for successful implantation[14] but excessive inflammation can cause embryo resorption, a process that can be counteracted in the uterus by Treg cells. On the whole, we have to keep in mind both excessive inflammation and immune

suppression can cause embryo resorption[8,14]. A healthy pregnancy needs an adequate balance between all the elements, without any unidirectional overstimulation.

The treatment with progesterone, an immune suppressant, to ward off the risk of miscarriage is approved by therapeutic protocols, but its real efficacy in preventing spontaneous abortion is disputed[15-20]. In this context, Alpha Lipoic Acid (ALA), a safe multifunctional molecule, is revealing an interesting and innovative therapeutic activity. In addition to being synthesized in small amounts by humans, ALA can be assimilated in food, taken as dietary supplement and, much better, vaginally administered in order to directly reach the site of action. It is a powerful antioxidant (called also the "ultimate antioxidant") because of the wide range of its actions in regulating oxidative stress pathways and the capability to pass through biological membranes[31,32]. ALA is not an immune suppressant, but an immune modulator, which regulates many parameters, such as the secretion of inflammatory cytokines, increases Treg-cell number, inhibits the production of vascular and intracellular adhesion molecules (VCAM-1 and ICAM-1), reduces the expression of CD4 on the surface of blood mononuclear cells, and blocks the activation and cytotoxicity of natural killer (NK) cells[21,22,33,34]. Treatments with ALA inhibits the expression of matrix metalloproteinase-9 (MMP-9)[35] which is involved in the degradation of the extracellular matrix, and it also reduces the secretion prostaglandin E2 (PGE2)[36].

In particular, because subchorionic hematoma appears to be due to immunological vasculitis in the decidual vessels, vaginal treatment with ALA may strongly support its resorption through its immunomodulating activity.

In addition, several studies have highlighted its efficacy in contrasting the weakening of human fetal membranes[37,38]. It is ascertained that ALA deficiency is responsible for the deficit in development of the foetus[39-41].

Conclusions

In pregnancy there is cross talk among different immune cells, which communicate by means of messenger molecules (mainly cytokines) strictly related in a network. In situation such as threatened abortion, ALA, modulating this network, can play a pivotal role in ameliorating significantly the medical conditions of mothers and foetus.

Our results have shown that ALA vaginal administration can efficiently improve the medical picture of women with threatened miscarriage, positively affecting the hematoma resorption. In this context, a controlled inflammatory process is indispensable in many steps of a healthy pregnancy, also in the first trimester. Progesterone is known to exert an immunosuppressive action, whereas ALA is able to finely modulate the complex of cells and molecules involved without a unidirectional activity. The use of this careful modulation should provide the best therapeutic choice to treat patients with threatened miscarriage, reducing, also markedly, the main clinical sign and symptoms. Our preliminary evidences support this new approach proving the first example on the vaginal use of ALA in the clinical practice.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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