Alpha Lipoic Acid (ALA) effects on subchorionic hematoma: preliminary clinical results

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Abstract. – OBJECTIVE: The clinic use of alpha Lipoic Acid (ALA) is linked to its capability to exert antioxidant effects and, more interestingly, to counteract the pathologic changes of complex networks of cytokines, chemokines and growth factors, restoring their physiological state. The aim of this randomized controlled clinical trial was to test the contribution of oral supplementation of ALA to the standard treatment with Progesterone vaginal suppositories, in healing subchorionic hematomas in patients with threatened miscarriage. Controls were administered only Progesterone suppositories.

PATIENTS AND METHODS: Nineteen pregnant women in the first trimester of gestation, with threatened miscarriage and ultrasound evidence of subchorionic hematoma, were included in the trial and randomly divided in two groups: controls, treated with 400 mg Progesterone (200 mg 2 times per day), given by vaginal suppositories, and case study treated with the same Progesterone dosage, plus ALA, given orally at the dose of 600 mg (300 mg 2 times per day, DAV®, Lo.Li. Pharma srl, Italy). Sixteen patients completed the trial. Treatment was performed until complete resolution of the clinical picture.

RESULTS: In both groups, the subjects improved significantly but, in general, a better and faster evolution in the major signs of threatened miscarriage was observed in the subjects treated with ALA and Progesterone. In these patients, the speed of resorption of subchorionic hematoma was significantly ($p \leq 0.05$) superior compared to controls. The ALA and Progesterone group showed a faster decrease or disappearance of all symptoms than that observed in the control group, however the difference was not significant.

CONCLUSIONS: These preliminary results suggest that ALA supplementation significantly contributes to speed up the process of restoration of physiological conditions in threatened miscarriage and ameliorates the medical conditions of both the mothers and the foetus, probably modulating the networks of cytokines, growth factors and other molecules.

Introduction

Threatened miscarriage is characterized by vaginal bleeding, other than spotting, and pelvic pain within the first 20 weeks of gestation. It affects around 20% of women, and spontaneous abortion occurs approximately in half of these pregnancies[1]. Up to 80% of these losses appear to be caused by genetic abnormalities[2]. Also inflammatory processes and immunologic disorders can induce miscarriage[3-4]. The presence of a first-trimester subchorionic hematoma is a suitable marker of patients at greater risk for adverse pregnancy outcome[5]. In particular, the presence of a very large first-trimester subchorionic hematoma has been suggested to be associated with a 46% risk of adverse pregnancy outcome (spontaneous abortion and premature rupture of membranes)[6]. Therefore, its resorption represents a fundamental target to prevent preterm delivery.

Th1, Th2, Th17 and regulatory T (Treg) cells[7] are essential in immunity[8] and pregnancy[9] and are involved in boosting and/or dampening inflammation[10-12]. Th1 cells secrete inflammatory cytokines, such as Tumor Necrosis Factor (TNF) and Interferon-gamma (IFN-gamma), but also Interleukine-10 (IL-10). Instead, Th2 cells produce several Interleukins, such as IL-4, IL-10, etc.[8] Finally, Th17 cells, similarly involved in pregnancy[13], produce the proinflammatory cytokine IL-17[7,14-15]. On the other side, Treg cells play an essential role in the development of immunoregulation and induction of tolerance. IL-1 beta, a pro-inflammatory cytokine, mainly released by monocytes and macrophages, is also

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involved in gestation, exerting either an aborto-genic effect (probably prevailing) or a protection from miscarriage[16]. The maintenance of a healthy gestation needs an adequate interplay between Th1- and Th2-mediated immunity. Furthermore, a broad spectrum of different molecules, such as growth factors, cytokines and chemokines, are implicated in acute wound and in its healing. TNF-alpha, IL-1 and IL-6 induce inflammation in the acute wound (as well known, inflammation is required for fighting infection), and are involved also in reepithelialization[17]. Vascular Endothelial Growth Factor (VEGF) stimulates epithelialization and collagen deposition in wounds[18], and Alpha-Smooth Muscle Actin (alpha-SMA) takes part in fibrogenesis[19], i.e. vasculogenesis and angiogenesis. Inflammation, extracellular matrix (ECM) degradation and remodeling of new ECM are positively affected also by matrix metalloproteases (MMPs), though a selective control of their activity is necessary to avoid the wound worsening[20].

The use of progesterone in the managing of threatened miscarriage, is prescribed by therapeutic protocols, although, few evidences support its efficacy[21].

Our aim was to verify whether alpha lipoic acid (ALA), a natural and safe molecule with an anti-inflammatory and antioxidant profile[22-24], can improve efficaciously the therapy with progesterone. The reason why ALA was used depends on its capability to counteract the pathologic changes of complex networks of cytokines, chemokines and growth factors, restoring their physiological state, and in consideration that such factors are involved also in inducing threatened miscarriage.

The clinical trial has evaluated the innovative therapeutic effects of ALA, by oral route, and Progesterone vaginal suppositories, compared with Progesterone alone, in reducing significantly the subchorionic hematomas and other symptoms linked to spontaneous abortion.

Patients and Methods

Pregnant women with threatened miscarriage were assessed for eligibility from March 2013 to February 2014 at the Division of Obstetrics and Gynecology, University of Perugia, Italy. We applied the following inclusion criteria: the subjects had to be between 20 and 40 years old, between 6th and 13th week of physiological pregnancy, with pelvic pain and/or vaginal bleeding, and subchorionic hematomas, observed by ultrasound examination. The exclusion criteria were the presence of multiple pregnancy, pre-pregnancy or gestation pathologies (arterial hypertension, maternal autoimmune diseases, antiphospholipid syndrome), treatment with anti-hypertensive and anti-coagulant drugs, that could be confusing factors. The study was approved by the Bioethics Committee SIFIOG (Italian Society of Phytotherapy and Dietary Supplements in Obstetrics and Gynecology). All pregnant women were informed about the nature of the study and verbally acceded to participate to it. The clinical trial was a randomized controlled study, with 1:1 allocation ratio. The study had two groups: controls, with 400 mg Progesterone (200 mg 2 times per day), given by vaginal suppositories, and treated (case study) with the same Progesterone dosage, plus ALA (DAV®, Lo.Li. Pharma srl, Rome, Italy), given orally at the dose of 600 mg (300 mg 2 times per day, in the morning and in the evening,). In our study we used only these two groups of treatment, because it was impossible to add a third arm without treatment, owing to the Bioethics Committee refusal to accept this possibility. The protocol prescribed to administer all compounds until complete resolution of the clinical picture.

Primary outcome: reduction/disappearance of subchorionic hematoma detected by sonography, vaginal bleedings, pelvic pain and uterine contractions. The size of the hematoma was compared with the size of the gestational sac during the examination and classified as small (< 20% of the gestational sac), medium (20%-50% of the gestational sac), or large (> 50% of the gestational sac)[6]. In the case study the hematoma was medium-sized in five patients, and large in four cases, whereas three controls had a large hematoma and four a medium-sized one. Progressive hematoma resorption during treatment was calculated as percentage between two subsequent time points for each patient and the medium value for each group has been obtained and compared. With the aim of recording systematically the changes in pain symptomatology for the subsequent analysis and evaluation, patients received a sheet where they had to note the daily number of contractions during the time period between two medical examinations.

Secondary outcomes: placental abruption, decrease of miscarriage incidence and preterm delivery.

The improvements have been monitored during all study, and evaluated respect to the baseline (t = 0).

Statistical Analysis

Data analysis was performed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). The M-estimator of regression curve, used for the statistical analysis of significance, was calculated, for each patient, considering only the first 100% value in the healing. The Mann-Whitney U-test was applied for determining the significant difference between the two groups. The level of statistical significance was set below $p = 0.05$.

Results

Among twenty-nine assessed subjects, with threatened miscarriage, a total number of nineteen pregnant women were included in the study. The age range was from 26 to 39 years, with a mean value of 32.5 ± 3.9 years, and a mean body weight of 58.94 ± 7.01 kg. The mean age in control group was 31.7 ± 2.56 years, and in treated group was 33.1 ± 4.75 years. The mean body weight was, respectively, of 58.7 ± 7.2 kg and 59.11 ± 7.3 kg. The average of gestational age was 8.7 ± 1.4 weeks in the case study and 8.5 ± 0.4 weeks in the control group. There was no statistically significant difference between the case study and the control group in terms of mothers' ages and gestational ages. There were three dropouts: two patients, in the Progesterone group, failed to complete the study owing to the increased vaginal bleeding and admission to hospital at the beginning of the trial, whereas one patient in the case study underwent termination of pregnancy because of fetal trisomy 21. Among the sixteen patients, who completed the study, there were eight nulliparous women in the case study, and one multigravida (three subjects had a previous miscarriage), while in the control group, there were six nulliparous, and one multigravida (two subjects had previously a miscarriage). Nobody had had caesarean section. Patients were controlled after a week from the baseline, and later every fifteen days until the resolution of the clinical picture. In no case, symptoms not noticed during the 1st medical examination, later afflicted the patient. Treatments did not cause any adverse effect on mother or foetus.

At ultrasounds hematoma appears such as an anechoic area (i.e., echo free on a sonographic image) of variable size, located between the uterine wall and the chorionic membrane (Figures 1A, B and C).

The improvement was general, but with a very different time trend in the healing, which was clearly much faster in patients treated with ALA plus Prog (Figure 2A).
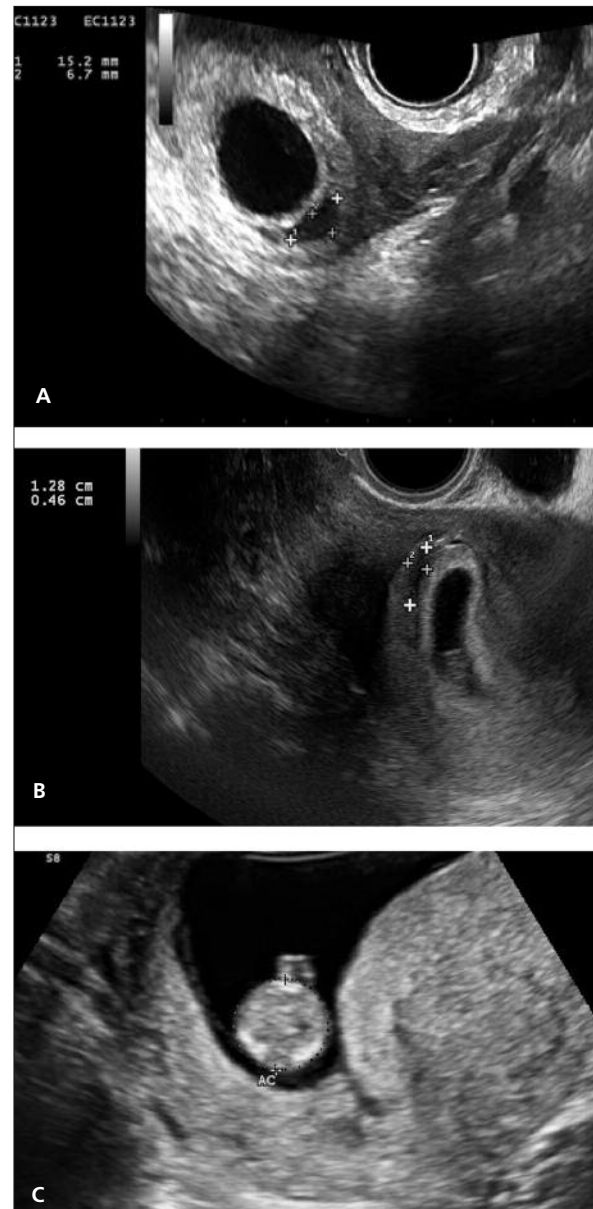
Figure 1. *A,* Subchorionic hematoma (9th week of pregnancy) in ALA plus Progesterone group. *B,* Subchorionic hematoma reduction (10th week of pregnancy) in ALA plus Progesterone group. *C,* Complete subchorionic hematoma resorption (12th week of pregnancy) in ALA plus Progesterone group.

The two groups were found significantly different (Mann-Whitney U-Test: The U-value is 8. The critical value of U at $p \leq 0.05$ is 12. Therefore, the result is significant at $p \leq 0.05$) (Figure 2B).

Concerning the symptoms such as pelvic pain, uterine contractions, vaginal bleeding and soft uterus, the changes of these parameters, from the baseline (t_0) to the following medical controls (t_1 t_3), are shown in Table I.

Only in soft uterus the changes have been the same in both groups, whereas evident differences, but not statistically significant, can be observed in parameters such as pelvic pain, uterine contractions and vaginal bleeding. In both groups, episodes of threatened miscarriage, preterm deliveries, placental abruption, were not recorded.

Discussion

Our preliminary results indicate that patients treated with ALA plus Progesterone have had a better and faster evolution of the clinical signs and symptoms related to threatened miscarriage.

The monitoring of subchorionic hematoma, vaginal bleeding, pelvic pain, and uterine contractions showed that all signs and symptoms decreased or disappeared in the group treated with ALA plus Progesterone, before than in controls (Progesterone alone). Indeed, despite the treatments show comparable results in the objective resolution of uterine contractions (soft uterus), the results related to the subjective symptoms (i.e. uterine contractions) highlighted that patients in the case study showed a faster resolution of the symptoms, directly improving the life quality of pregnant women.

In particular, the results obtained for the hematoma resorption, detected by scan, are of particular interest. In fact, the ALA plus Progesterone group showed a clear improvement, statistically significant, whereas the other effects (on vaginal bleeding, pelvic pain and uterine contractions) were not statistically significant.

The use of progesterone in the management of threatened miscarriage is prescribed by therapeutic protocols, but the real extent of its efficacy in reducing miscarriages is debated[21,25-29]. Much more interesting and innovative is the therapeutic activity of ALA, a safe natural molecule. The biological systems synthetize this compound, but it can be also assimilated in food or nutritional supplements. ALA exerts a powerful anti-inflammatory and antioxidant activity[22-24]. It is important to highlight that ALA substantially decreases the secretion of inflammatory cytokines, such as TNF-alpha, IL-1beta and IL-17, whereas it stimulates the release of the anti-inflammatory cytokine IL-10; it significantly suppresses the number and percentage of encephalitogenic Th1 and Th17 cells, increases splenic Treg-cells, inhibits
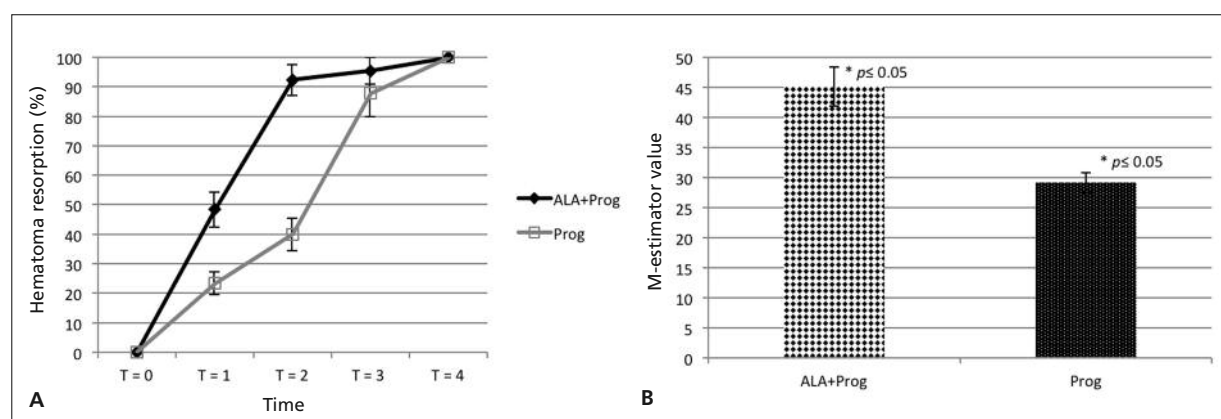
Figure 2. *A,* Progress of subchorionic hematoma resorption (Δ percentage of Mean ± SEM), detected by sonography at different time points of treatment, in ALA plus Prog. group (n = 9) and Prog. group (n = 7). The size of the hematoma was compared (%) with the size of the gestational sac during the examination. 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. *B,* Subchorionic hematoma resorption: statistical significance of the regression slopes (ALA plus Prog. group vs. Prog. group). Mean ± SEM of M-estimator for ALA plus Prog. Group (n = 9) and Prog. Group (n = 7). The M-estimator of regression curve, used for the statistical analysis of significance of hematoma resorption, was calculated, for each patient, considering only the first 100% value in the healing. The two groups were found significantly different (Mann-Whitney U-Test: The U-value is 8. The critical value of U at $p \leq 0.05$ is 12. Therefore the result is significant at $p \leq 0.05*$).

Table I. Effects of the treatments (ALA + Progesterone or Progesterone) on symptoms of threatened miscarriage.

Symptoms	Baseline med. exam. t_0		1st medical control t_1		2nd medical control t_2		3rd medical control t_3	
	ALA+Pr (n = 9)	Pr. (n = 7)	ALA+Pr. (n = 9)	Pr. (n = 7)	ALA+Pr. (n = 9)	Pr. (n = 7)	ALA+Pr. (n = 9)	Pr. (n = 7)
Vag. bleed.	5 (55%)	4 (57%)	1 (11%)	2 (29%)	0	0	0	0
Abd. pain	9 (100%)	7 (100%)	2 (22%)	4 (57%)	0	1 (14%)	0	0
Soft uterus	3 (33%)	4 (57%)	9 (100%)	7 (100%)	9 (100%)	7 (100%)	9 (100%)	7 (100%)
Uter. contr.	8 (89%)	7 (100%)	4 (44%)	7 (100%)	0	5 (71%)	0	1 (14%)*

Data are given as sample size (number and percentage) of each group at different time points: t_0: baseline medical examination; t_1: 1 week after baseline medical examination; t_2: 3 weeks after baseline medical examination; t_3: 5 weeks after baseline medical examination. *The symptom disappeared at the subsequent medical examination.

the production of vascular and intracellular adhesion molecules (VCAM-1 and ICAM-1)[30-35]. Moreover, ALA treatment also inhibits IL-6 production and decreases T cell proliferation and activation[31]. These data suggest that ALA can enhance systemic Treg-cells which counteract the inflammatory response. Acute treatments with ALA have been shown to directly decrease the expression of NF-κB and matrix metalloproteinase-9 (MMP-9)[20]. In an animal model, ALA was able in reducing the time of wound healing in uterine full thickness injury, increasing the Vascular endothelial growth factor (VEGF) and alpha-Smooth Muscle Actin (alpha-SMA) distribution in tissues[36]. ALA supplementation decreases prostaglandin E2 (PGE2) secretion[33], and reduces pain and paraesthesia in patients with sciatica, carpal tunnel syndrome and diabetic neuropathy[37-40]. In addition, other studies highlighted its efficacy in contrasting TNF-induced weakening of human fetal membranes[41,42], and the involvement of its deficiency in damages to foetus development[43-45]. The therapeutic use of ALA is safe and tolerable: in humans the oral ALA supplementation at doses up to 2400 mg/day does not show any adverse effect[23]. Summarizing, our results could be explained by several physiological actions above mentioned, and mainly due to ALA activity in decreasing TNF, IL-1, IL-6, IL-17, and increasing splenic Treg-cells and IL-10. Furthermore, ALA may speed up the process of 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. All these effects have also as result the acceleration of tissue repair and angiogenesis.

Conclusions

Our preliminary results suggest that ALA supplementation may play a pivotal role in significantly ameliorating the medical conditions of both the mothers and the foetus, reducing the complications linked to threatened miscarriage, and improving the pregnancy outcome.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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