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Safety of oral alpha-lipoic acid treatment in pregnant women: a retrospective observational study

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Abstract. – OBJECTIVE: Alpha-lipoic acid is a natural molecule, which directly or by means of its reduced form, dihydrolipoic acid, exerts antioxidant, anti-inflammatory and immunomodulatory activities, very helpful also in preventing miscarriage and preterm delivery. Used as dietary supplement alpha-lipoic acid was demonstrated to be safe for living organisms even when administered at high doses. However, no study was made so far to verify the safety of its continuous administration on a substantial number of pregnant women. The present investigation was performed to answer this issue.

PATIENTS AND METHODS: An observational retrospective study was carried out analyzing 610 expectant mothers. They had been treated daily by oral route with 600 mg alpha-lipoic acid, for at least 7 weeks during gestation. The primary outcome was to verify alpha-lipoic acid safety in the mother and infant. Maternal safety was assessed by monitoring for adverse reactions, physical and clinical examination, including a morbidity assessment. Laboratory and clinical examinations were performed monthly. Neonatal safety was assessed by the evaluation of birth weight, gestational age, Apgar scores, neonatal death with the related cause of death. Data collected from the Birth Registry of Campania Region were used as control.

RESULTS: This study provided a very clear and reassuring picture about the safety of alpha-lipoic acid oral treatment during pregnancy. No adverse effect was noticed in mothers or newborns. The two sets of monitored data, from treated and controls, were completely superimposable or, in some cases, better in alpha-lipoic acid group.

CONCLUSIONS: Our results open a reassuring scenario regarding the administration of alpha-lipoic acid during pregnancy.

Key Words:
Adverse events, Alpha-lipoic acid, Dihydrolipoic acid, Newborns, Oral treatment, Pregnant women, Safety.

Abbreviations

ALA: alpha-lipoic acid; DHLA: dihydrolipoic acid; ROS: reactive oxygen species; RNS: reactive nitrogen species; Nrf2: transcription factor nuclear factor erythroid 2-related factor 2; NF-kB: nuclear factor kappa-light chain-enhancer of activated B cells.

Introduction

Alpha-lipoic acid (ALA), or 1,2-dithiolane-3-pentanoic acid, is an organosulfur compound (chemical formula: $C_8H_{14}O_2S_2$) with molecular weight (MW) 206.32 Da. It exerts antioxidant, anti-inflammatory and immunomodulatory activities. ALA synthesis occurs enzymatically from octanoic acid in the mitochondria. This natural molecule, chemically identified by Reed et al[1], is essential for life in many metabolic processes. ALA may exist as R- or S-enantiomeric form, however only the first one is found in living systems. Plants and animals normally can synthesize ALA, however human beings fail to produce consistent amounts of it; consequently, it must be taken from food[2]. Large quantities are present in foods such as potatoes, broccoli, spinach, tomatoes, Brussels sprouts, peas, brown rice and red meat[3].

After its absorption, ALA (the oxidized form of the molecule) may be transformed by specific enzymes (dihydrolipoamide dehydrogenase, thioredoxin reductase or glutathione reductase) into its reduced form, called dihydrolipoic acid (DHLA)[4,5]. The one or the other may be found in the living organisms, depending on the chemical features of the inner environment. Here we will often refer to the couple ALA/DHLA without making any distinction because in different contexts both forms may play the biological function. The

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following steps can affect the final concentrations of ALA/DHLA taken by systemic route: degradation process of the molecule, short plasma half-life, first-pass effect, and metabolic elimination[6,7]. In human plasma ALA and DHLA may be detected at 1-25 and 33-145 ng/ml, respectively[8]. In pharmacokinetic studies, different doses (50-600 mg) of orally administered ALA were shown to be completely absorbed within 30-60 min, with a plasma half-life (t ½) of 30 min[8-11]. The molecule is stored in tissues (mainly heart, kidney, and liver); however, it does not occur in relevant amount because ALA is rapidly metabolized[12]. Its bioavailability is about 30%, with a range between 20% and 38%, owing to a substantial pre-systemic metabolism in liver (first-pass effect)[6].

ALA/DHLA couple is believed by most scientists, though not by all[13], to directly scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS), either *in vitro* and *in vivo*[7]. Moreover, several data prompt to state that ALA/DHLA plays indirectly an antioxidant role[7]. Indeed, it regenerates other essential antioxidant molecules, i.e. coenzyme Q10, vitamin C, vitamin E, etc.[7,14-16], or it chelates many heavy metals, such as, iron, lead, cadmium, mercury, copper, and arsenic, involved in oxidative processes[7,17,18]. In addition, ALA/DHLA can also repair proteins, lipids, and DNA, when damaged due to oxidative phenomena[19]. The core and the origin of all these activities is thought to reside in the activation or repression of two essential nuclear factors, transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor kappa-light chain-enhancer of activated B cells (NF-kB). Several studies demonstrated that the chemoprotective action of ALA/DHLA is mediated by Nrf2 and NF-kB[7]. The wide and promising scientific documentation prompted to extensively test this dietary supplement in numerous diseases, showing a significant efficacy on many parameters[7], with growing interest on its therapeutic potential.

The administration of ALA in pregnancy is based on the following scientific evidence. It is well-known that the cytokine network induces positive or negative effects on the course of gestation. Its dysregulation, with high levels of proinflammatory (e.g. IL-1 and IL-6)[20,21], and/or low levels of anti-inflammatory molecules (IL-4 and IL-10)[22] can cause miscarriage and preterm delivery. Also, factors such as enzymes, adhesion molecules, and other endogen mediators are involved in inducing these disorders, as shown by several experimental researches and clinical studies. Therefore, the activation of COX2[23] and iNOS[24], as well as increased levels of ICAM-1[25], VCAM-1[25], PGE2[23], and NO[24], exert harmful effects, though contradictory activities can be found in some cases. The expression of matrix metallopeptidase 9 (MMP-9), involved in the degradation of extracellular matrix (ECM)[26] was shown to facilitate preterm birth.

On the other hand, ALA is a fine modulator of many pivotal pathways. It rebalances the target molecules concentration, without altering them when they are in the normal range; it decreases the levels of TNF-α, IL-1β and IL-6[27], whereas it stimulates the release of IL-10, an anti-inflammatory cytokine[28]. Furthermore, ALA reduces VCAM-1 and ICAM-1[29], increasing VEGF and alpha-SMA[30]. It can also decrease PGE2 and NO levels, by means of COX-2 and iNOS inhibition[27]. In addition, it counteracts TNF-induced and thrombin-induced weakening of human fetal membranes[31,32]. Other studies highlighted ALA efficacy in reducing the expression of MMP-9[33], and in countering TNF-induced and thrombin-induced weakening of human fetal membranes[31,32]. Ultimately, this dietary supplement shows many activities useful to prevent or positively solve important alterations that can occur during pregnancy. ALA activity by oral route in pregnant women was already investigated in reducing the incidence of spontaneous contractions[34]. Recently it was administered orally to ward of threatened miscarriage, speeding up the restoration process of physiological status[35]. Therefore, its use in pregnancy is very promising.

Normally the dietary supplements made from ALA contain a mixture of both R- and S-enantiomers. This is the best choice because the two stereoisomers are absorbed and metabolized differently depending on the site of uptake, which implicates the involvement of different enzymes[36].

A large number of researches and studies have demonstrated that ALA is not toxic for living organisms. This molecule is continuously introduced into our body with the diet, also during pregnancy. No relevant side effects were recorded when ALA is taken with food or as dietary supplement, even when administered at much higher doses respect to those used in the normal treatments. Furthermore, the Italian Ministry of Health has not established an upper limit for ALA intake. All these facts confirm its safety and are reputed reassuring for its administration to pregnant women, obviously under strict medical supervision.

Overall this molecule shows a reassuring profile[7], however no specific researches were made so far to verify the safety of its administration for several months on pregnant women. We carried out a retrospective observational study on a consistent sample size. We aimed to shed light on this unexplored field, with a first set of essential information on the safety of this dietary supplement during a very important period of woman life.

Patients and Methods

Patients

This is an observational retrospective study carried out at the clinic "Villa delle Querce" (Naples, Italy). It was based on the selection of data from a considerable amount of clinical records, regarding pregnant women treated with ALA over the last three years. Patients' information were obtained by means of the medical record review. The retrospectively collected data were retrieved from hospital delivery logbooks, with data entry, excluding unique identifying information, and presented in aggregate. Data were de-identified prior to access by the authors who did not have any contact with the mothers during the record review.

The inclusion criteria were: pregnant women representing the age range (15-45 years old) of occurred gestation in Italy; presence of clinical pregnancy; oral administration of 600 mg ALA for a period not less than four weeks, until the end of the 37th week of gestation.

The exclusion criteria were: presence of relevant pathologies in the mother or fetus, detected before the start of ALA supplementation (in mother: thyroid disorders, arterial hypertension, diabetes, PCOS, preeclampsia, maternal autoimmune diseases, antiphospholipid syndrome, lupus, hepatitis, thalassemia, HIV/AIDS, cancer, or a diagnosed alcohol or drug addiction; in fetus: malformations identified by amniocentesis or ultrasound examination), discontinuity in ALA treatment, lacking of some relevant clinical data in the medical register.

The dietary supplement was prescribed by the physicians for treating uterine contractions and threatened miscarriage, or for preparing the patients to undergo amniocentesis, or even as simple prevention.

A retrospective cohort of pregnant women comparable with those of our study was collected from the records of the Birth Registry of Campania Region[37], and used as control.

The primary outcome was to verify the maternal and neonatal safety. The first one was assessed by monitoring for adverse reactions, physical and clinical examination, including an assessment of

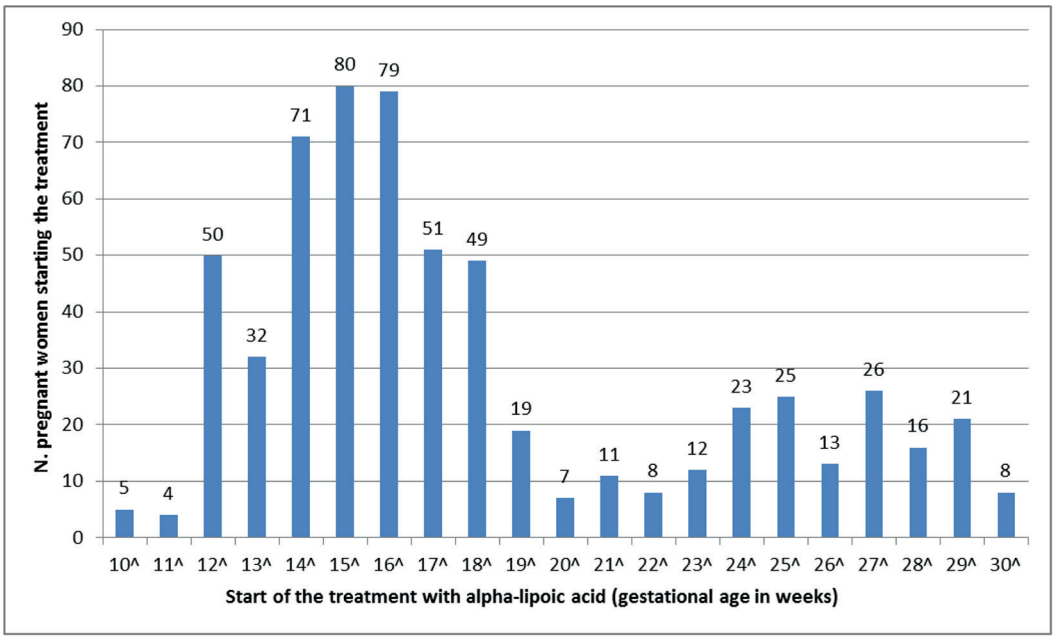

Figure 1. Number of pregnant women (n = 610) starting with ALA treatment at different time points of gestation. The administration lasted always until the end of the 37th week of gestation.

the present and previous morbidity. Laboratory examinations concerning the levels of blood glucose, red blood cells, platelets, serum iron, GOT, GPT, hemoglobin, azotemia, creatininemia, were performed monthly. Neonatal safety was assessed by evaluating birth weight, gestational age at delivery, Apgar scores at 1 and 5 minutes after birth, neonatal death with the related cause of death.

Statistical Analysis

The variables collected were summarized by appropriate descriptive statistics: mean, standard deviation, standard error, median, minimum and maximum for continuous variables; frequencies and % values for categorical variables. Comparison between ALA treatment variables and controls were performed using x^2-test for categorical variables. All statistical tests were carried out at a two-sided 5% significance level. Statistical analyses were performed using Stata™ 8.2 (StataCorp LLC, College Station, TX, USA).

Results

After the screening of the records, a sample of 610 pregnant women was identified for this observational retrospective study. The enrolled patients had received orally a therapeutically effective dose of ALA provided as a racemic mixture of both R and S enantiomers. The presence of S-ALA form avoids the polymerization of R-ALA[13] and increases the overall bioavailability of the dietary supplement. Regarding the dose, we decided to investigate ALA safe use in pregnancy analyzing the effects of 600 mg per day. It is the most common clinically investigated dosage prescribed to patients as total daily intake. Often this daily dosage was reached with two separate administration of 300 mg.

In the patient population here examined, ALA treatment started at different times of pregnancy, since 10th to 30th week and always lasted until the end of the 37th week of gestation (Figure 1).

All the pregnant women were administered daily without interruptions, under strict medical supervision, at least over a period of seven weeks; however, 420 patients took ALA for 20 weeks or more.

Mean age of the treated group was 27.6 ± 6.6 (mean ± SD) years old (range: 15-44 years old). Among the 610 patients were noticed 57 previous disorders. The most common pathologies were sickle cell anemia, rheumatoid arthritis, Crohn's disease, hepatitis C virus, psoriasis, recurrent headache, recurrent fetal loss, herpes simplex. In some cases, more than one was recorded in the same subject. Beside ALA, at times patients had received other single or combined treatments (Table I); according to our clinical experience this set of data is in line with that existing in the control population.

Patients were subjected to the usual laboratory examinations (glucose, red blood cells, platelets, serum iron, GOT, GPT, hemoglobin, azotemia, creatininemia), carried out over all period of pregnancy, and the results of these biochemical analysis were not found significantly different from the normal values (data not shown). It is very important to highlight that ALA treatment did not elicit any adverse event in the expectant mothers.

In the first trimester starting from the 10th week, 3 miscarriages were recorded in our sample. Moreover, 16 cases of preterm birth between the 33th and the 36th week occurred, and 2 therapeutic abortions due to genetic disorders of the fetus were found. All infants were healthy, without neonatal deaths. The mode of childbirth was spontaneous in 269 cases (44.4%) and caesarean in 336 cases (55.6%), with 6 twin births. Preterm (< 37 weeks), early term (37-38 w), full term (39-40 w), and late term (41-42 w) births, by gestational age, comprising both the spontaneous and caesarean deliveries, were 16 (2.6%), 103 (17.0%), 421 (69.7%) and 65 (10.7%), respectively. Referring only to spontaneous deliveries, they were 13 (5.2%), 35 (14.0%), 144 (57.9%) and 57 (22.9%), respectively (Figures 2-3).

The available Apgar scores at 1 and 5 min after birth were 6/7 in 1 newborn, 7/8 in 9 newborns, 8/9 in 410 newborns, 9/10 in 187 newborns. The mean for weight at birth was 3172.5 g ± 262.0 (range: 2320 - 4100 g) and for length was 49.8 cm ± 0.9 (range: 47-53 cm). As shown in Table II, all values were very similar or better than in controls.

Table I. Treatments administered in ALA patients (n = 610).

Drugs	No. of treatments in ALA group
Progestins	67
Antispasmodics	25
Antibiotics	16
Antihypertensives	13
NSAIDs	10
Corticosteroids	7
For hypothyroidism	5
Insulin	5
Analgesics/antipyretics.	3
Statins	1

Table II. Parameters (mean ± DS or percentage) monitored in ALA treated (610 pregnancies) and controls (retrospective cohort of pregnant women without ALA supplementation).

Parameter	ALA treated	Controls	p-value
Preterm birth	16 on 605 (2.6%)	3,162 on 52,179 (6.06%)	0.0004
Term birth	589 on 605 (97.4%)	49,017 on 52,179 (93.94%)	0.0004
Caesarean childbirth	336 cases on 605 (55.6%)	58.4%	0.1557
Spontaneous childbirth	269 cases on 605 (44.4%)	41.6%	0.1554
Neonatal death	0%	0.25%	N/A
Apgar < 7	0.20%	0.60%	0.1670
Weight (g)	< 2500 g n=1 (0.2%)	< 2500 g n=2,769 (5.3%)	<0.0001

Preterm birth: < 37 weeks gestational age - full term birth: ≥ 37 weeks gestational age.

Discussion

Our study was carried out with 610 expectant mothers, and showed that ALA oral treatment is completely safe also during pregnancy. ALA was administered without interruptions up to six months at the dose of 600 mg per day. It did not bring out any adverse effect both in mothers and infants. As reminded before, the Italian Ministry of Health has not established a maximum daily intake of this dietary supplement, meaning that the use of such molecule is safe, also at relevant doses. All the monitored parameters were not significantly different from the control population or, in some cases, were better in the treated group. These results agree with previously reported data, obtained in different kinds of experimental researches and studies.

In animal tests ALA acute and chronic doses provided very encouraging outcomes. In rats receiving orally 31.6 or 61.9 mg ALA/kg bw/day for a period of 4 weeks and 60 mg/kg/day for 2 years, ALA was found to be non-carcinogenic and adverse events were not recorded. The "No Observed Adverse Effect Level" (NOAEL) was fixed at 60 mg/kg bw/day[38]. Also, ALA by oral route in acute dose was not toxic to rats (LD50 > 2000 mg/kg bw)[39]. Moreover, ALA showed a protective effect on the fetus of diabetic, alcoholic, or exposed to toxic pollutants (dioxin) animals[40-43]. These results were confirmed in human beings, included elderly patients. Thus, the administration up to 1200 mg once a day i.v. over two 5-day periods[44] or at 600 mg once a day i.v. for 3 weeks, followed

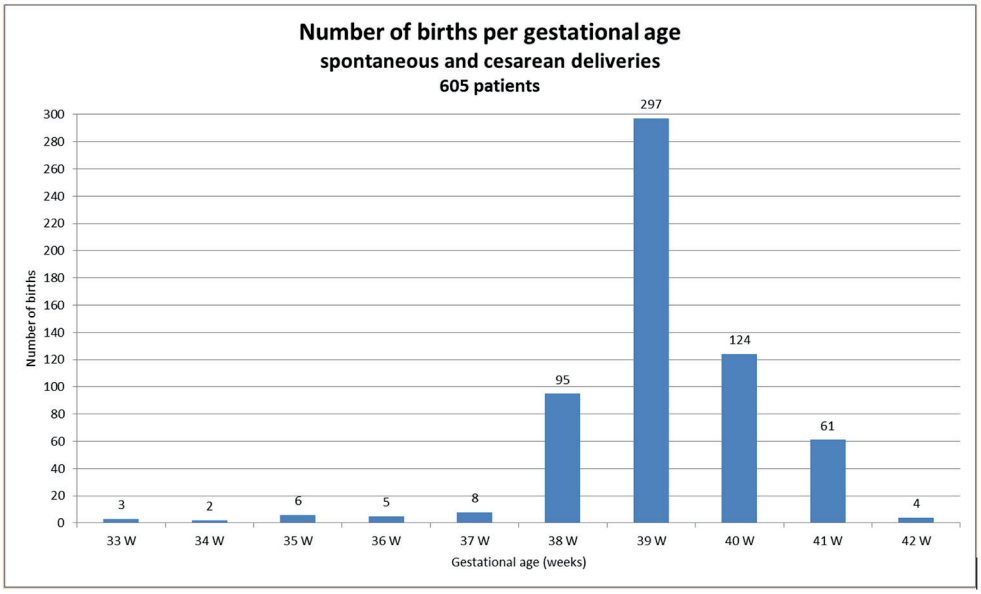

Figure 2. Number of births per gestational age (spontaneous and caesarean deliveries) in 605 women.

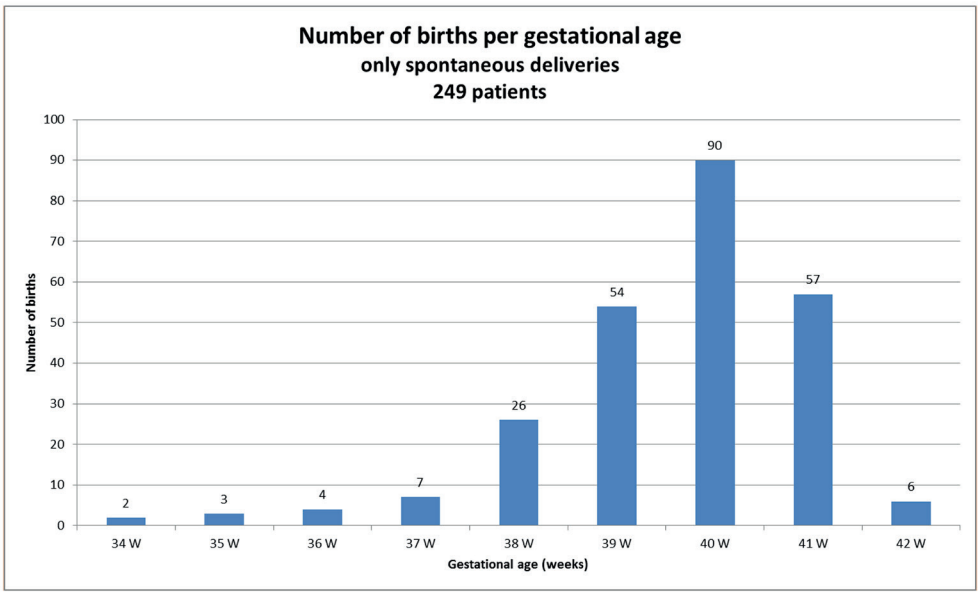

Figure 3. Number of births per gestational age (only spontaneous deliveries) in 249 women.

by 600 mg three times per day orally for 6 months[45], did not give rise to noteworthy adverse effects in comparison with controls. In a pilot study with elderly patients, the 600 mg dose of ALA was shown to be well tolerated[46]. Another significant support to ALA safety comes from the NATHAN 1 trial where the authors demonstrated that 4-year oral treatment with 600 mg ALA in mild to-moderate diabetic distal symmetric sensorimotor polyneuropathy was well tolerated[47]. Finally, we highlight also the meaningful experience made in Germany where ALA has been widely prescribed for decades. The post-marketing surveillance data showed low rates of adverse reactions, not different from those reported in the clinical trials. The same interesting profile of safety is confirmed by a great amount of other studies[48-52].

Also, our results are important for strengthening and supporting a new field of ALA use as therapeutic molecule to ward off threatened miscarriage and preterm delivery. In a double-blinded, randomized, placebo-controlled trial[34], it was demonstrated that ALA supplementation was effective in significantly reducing the incidence of spontaneous contractions with respect to controls. Among these patients, 52% of treated women reported no symptoms of uterine contractions throughout pregnancy, while persistent episodes of uterine contractions were significantly decreased compared with controls (20% *vs.* 60%, respectively). Furthermore, only 20% of supplemented subjects required hospitalization, while it was necessary for 40% in placebos. A recent randomized controlled clinical trial by Porcaro et al[35] provided the first demonstration that ALA oral supplementation contributes to significantly reduce threatened miscarriage. The study was aimed to test the efficacy of ALA supplementation (300 mg, twice a day, by oral route) in improving the standard treatment with progesterone vaginal suppositories. The parameters monitored were the healing of subchorionic hematomas and the reduction of subjective and objective signs of miscarriage. Controls received only vaginal suppositories containing progesterone. Both sets of patients improved, but those treated with progesterone plus ALA had a better and faster evolution during the first 20 weeks of gestation. The monitoring of the main signs of threatened miscarriage (chorioamniotic separation and uterine hematoma, vaginal bleeding, abdominal pain, and uterine contractions) clearly indicated that all symptoms decreased or disappeared in ALA group plus progesterone, faster than in the group treated with progesterone alone. Furthermore, we remind other two studies where ALA was administered by vaginal route. The first one was a randomized controlled study, carried out by Costantino et al[53], which provided similar results to those obtained by Porcaro et al[35], again in the therapy of threatened miscarriage. The second one was a pilot, prospective, randomized, placebo-controlled trial performed by Facchinetti et al[54], that investigated the effects of ALA after primary tocolysis. The treatment stimulated a significant increase of anti-inflammatory cytoki-

nes (IL-4 and IL-l0) in undelivered women after a preterm labor episode in comparison to placebo. Also, the stabilization of the cervix was found in ALA group, in this way avoiding its shortening.

Although preliminary, these results provided the background for a promising therapeutic activity of ALA in sustaining pregnancy length. Indeed, pregnancy is characterized by a cross talk among different immune cells, in a balanced but fragile network. ALA is likely to act as an accurate and selective regulator in this network[55,56]. It can re-establish the lost equilibrium, modulating the levels of various molecules involved in the maintenance of the physiological pregnancy.

Conclusions

The present results on ALA safety, stand next to the recently published studies on ALA efficacy in pregnancy (miscarriage and preterm birth). Promising opportunities can be envisaged for developing and testing long period of ALA treatments, with the aim to support its clinical indication.

Statement of Interest

The authors declare no conflicts of interest. This study was funded by Lo.Li.Pharma Srl, Rome, Italy.

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