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Effectiveness of an antioxidant preparation with *N*-acetyl cysteine, alpha lipoic acid and bromelain in the treatment of endometriosis-associated pelvic pain: LEAP study

Iñaki Lete[a,b,*], Nicolás Mendoza[c,d], Esther de la Viuda[e,f], Francisco Carmona[g,h]

[a] Unidad de Gestión Clínica de Obstetricia y Ginecología, Hospital Universitario Araba, Vitoria, Spain
[b] Universidad del País Vasco, Vitoria, Spain
[c] Clínica Margen, Granada, Spain
[d] Universidad de Granada, Granada, Spain
[e] Hospital Universitario de Guadalajara, Guadalajara, Spain
[f] Universidad de Alcalá de Henares, Madrid, Spain
[g] Hospital Clínic, Barcelona, Spain
[h] Universidad Autónoma de Barcelona, Barcelona, Spain

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ABSTRACT

Objective: To assess the impact of an antioxidant preparation with *N*-acetyl cysteine, alpha lipoic acid and bromelain on endometriosis-associated pelvic pain.
Study design: Multicenter, open-label, non-comparative clinical trial in a representative sample of women with endometriosis-associated pelvic pain.
Results: In total, 398 patients with a mean age of 34.6 ± 7.2 years were treated with a combination of *N*-acetyl cysteine, alpha lipoic acid and bromelain for 6 months. At baseline, 92.7% of the patients had pain intensity > 4 on the visual analogue scale (VAS); at 3 months of treatment, this percentage decreased to 87.2% ($p = 0.074$) and at 6 months the percentage was 82.7% ($p < 0.05$).
Conclusions: Women with endometriosis who wish to become pregnant and are treated with a preparation containing *N*-acetyl cysteine, alpha lipoic acid and bromelain experienced a significant improvement in endometriosis-associated pelvic pain and required lower intake of rescue analgesics.

Introduction

Endometriosis is a benign disease characterized by the presence of endometrial tissue (glands and stroma) outside the uterine cavity [1]. Ectopic endometrium is able to grow, infiltrate and even disseminate in a manner similar to tumor tissue. Endometriosis is estimated to affect 5% of women [2].

Different processes may be implicated in endometriosis: (i) survival of ectopic tissue outside the uterine cavity; (ii) suppression of immune mechanisms; (iii) adherence to peritoneum and invasion of the extracellular matrix; (iv) angiogenesis and implant growth and (v) inflammation, cyclic bleeding and disease progression. Oxidative stress has been shown to be present in all of the above processes, with production of oxygen free radicals that favor disease persistence [3]. The typical clinical manifestation of endometriosis is pelvic pain in form of dysmenorrhea, dyspareunia or chronic pelvic pain [4]. Often, the pain is incapacitating for those affected, with a negative impact on quality of life.

The accepted treatments for patients with endometriosis include gonadotropin-releasing hormone (GnRH) analogues and hormonal contraceptives; however these treatments compromise the fertility of the women who use them during their use.[2] Currently, the only treatments available to patients diagnosed with endometriosis who wish to become pregnant are analgesics and/or anti-inflammatory agents that act through inhibition of the Cox-2 enzyme.

Recently, a combination of *N*-acetyl cysteine, alpha lipoic acid, bromelain and zinc has become available [1]. This combination has an antioxidant action upstream in the Cox-2 pathway [5] and has been shown, in clinical trials, to be effective in the control of endometriosis-associated pelvic pain (EAPP) [6,7], without impacting the patients' fertility and with a better side effect profile than nonsteroidal anti-inflammatory agents (NSAIDs). The present study aimed to analyze the efficacy of this preparation for controlling EAPP in a sample of patients with endometriosis.

* Corresponding author at: Unidad de Gestión Clínica de Obstetricia y Ginecología, Hospital Universitario Araba, C/ José Atxotegi s/n., 01009 Vitoria, Spain.
E-mail address: luisignacio.letelas.a@osakidetza.eus (I. Lete).

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Material and methods

The study was a multicenter, open-label, non-comparative clinical trial in a representative sample of women with endometriosis-associated pelvic pain. The study was approved by the Clinical Trial and Research Committee (CEIC) of the autonomous community of the Basque Country. All participants signed an informed consent prior to inclusion.

The study included patients over 18 years of age diagnosed with endometriosis either by prior surgery or imaging (ultrasonography or magnetic resonance imaging) with an EAPP of greater than 3 as measured by a visual analogue scale (VAS) who wished to become pregnant in the short term and so were not using hormonal contraceptives or hormonal treatments for endometriosis in the last cycle (use of combined hormonal contraceptives, including in the last cycle was an exclusion criterion).

The clinical criteria considered for diagnosis were endometriosis-associated pelvic pain in any of its presentations: dysmenorrhea, dyspareunia, dyschezia, or chronic pelvic pain. Given that one of the study inclusion criteria was the requirement for prior diagnosis of endometriosis and pain and that most patients included in the study had been diagnosed prior to entering the study, not all of them were assessed according to the ultrasonographic criteria proposed by the International Deep Endometriosis Analysis group (IDEA) [8].

Not all patients had undergone MRI. Only in cases of clinical suspicion of deep endometriosis and/or adenomyosis, in general, was MRI used to confirm diagnosis. The patients who presented adenomyosis alone were excluded from the study.

At the baseline visit, after assessing eligibility and signing informed consent, baseline sociodemographic data and clinical history of interest were recorded and EAPP intensity was measured using a VAS. EAPP was classed as mild when VAS was 4–5, moderate when VAS was 6–7, and severe when VAS was 8–10. Patients then began treatment with the study combination: *N*-acetyl cysteine, alpha lipoic acid and bromelain at a dose of 2 tablets per day (*N*-acetyl cysteine 600 mg, alpha lipoic acid 200 mg, bromelain 25 mg and zinc 10 mg) for 6 months.

Post-baseline pain was assessed as above at 2 time-points during treatment, first after 3 months and then after 6 months. At these visits, rescue analgesic medication and adverse events were also recorded.

Variables analyzed

The primary outcome measure was percentage of women with endometriosis and EAPP who reported an improvement in pain during the 6-month treatment period with *N*-acetyl cysteine, alpha lipoic acid and bromelain. Changes in VAS scores from baseline to 3 months follow-up and from baseline to 6 months follow-up were assessed as secondary outcome measures. Changes in rescue analgesic requirements were also assessed.

Sample size calculation

The Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) initiative established cutoffs to define when improvement in pain could be considered clinically relevant [9]. Improvements of at least 50% in pain intensity are considered a substantial improvement. There are no previous data on the tested combination or any of its components to determine the proportion of patients with endometriosis who experience at least a 50% decrease in their pain intensity score. Therefore, for the sample size calculation, we assumed maximum uncertainty, that is, the proportion would be 50%. With a margin of error of 5%, a 95% confidence interval, and to estimate a proportion of responders to pain (defined as those with at least a 50% decrease in pain compared with baseline) of 50%, we needed to recruit 377 patients.

Statistical analysis

Data were collected with an Excel spreadsheet designed specifically for the study. All analyses were performed on a single sample of evaluable patients. This sample included all patients who met the selection criteria and who also had information available on the primary outcome measure.

Categoric variables were described with absolute and relative frequencies, including 95% confidence intervals. Continuous variables were described using the mean, standard deviation, median, mode, minimum and maximum, including the total number of valid values.

For comparison of patient subgroups, parametric tests were used for quantitative variables (Student *t*-test or ANOVA).

Statistical procedures were performed using the SAS version 9.1 statistical package.

Results

In total, 398 patients with a mean age of 34.6 ± 7.2 years participated in the study. Of these, 55.5% were nulliparous or nulligravida and 92.7% reported moderate or intense EAPP. Endometriosis had been diagnosed clinically and by imaging in 271 of the 398 patients (68%) while 127 (32%) had undergone previous surgery that confirmed the presence of the disease: 11 had undergone surgery for resection of nodules from the recto-vaginal wall, 19 had undergone adnexectomy, and 97 had undergone cystectomy to remove an endometrioma. Prior to inclusion in the study, 74% of the patients had used hormonal contraceptives (Table 1).

At baseline, 92.7% of the patients had a pain intensity > 4 on the visual analogue scale (VAS); at 3 months of treatment, this percentage decreased to 87.2% ($p = 0.074$) and at 6 months the percentage was 82.7% ($p < 0.05$).

At baseline, the mean VAS score for pain was 6.68 ± 1.97 and 344 of the 398 patients (86.4%) needed NSAIDs to control pain. Of the patients who took NSAIDs, 55.8% were taking them every 8 h for 4–5 days per cycle and 13.6% needed to take an NSAID continuously each day.

In the intermediate visit at 3 months, data were obtained from 373 patients. The mean VAS score for pain was 4.55 ± 1.97 and 57.4% were taking NSAIDs, of whom 4.3% were required this medication continually.

Table 1
Characteristics of Patients Included in the Study.

Characteristic	N = 398
Age, years	34.6 ± 7.2a
Parity	
Nulligravida	221 (55.5%)
Pregnancies	177 (44.5%)
EAPP	
Mild (4 and 5 on VAS)	29 (7.3%)
Moderate (6 and 7 on VAS)	312 (78.5%)
Severe (8–10 on VAS)	57 (14.2%)
Diagnosis of endometriosis	
Clinical + imaging	271 (68%)
Surgery	127 (32%)
Prior use of HC	295 (74%)

Data expressed as n (%) unless otherwise stated.
aMean ± SD.
Abbreviations: EAPP: endometriosis-associated pelvic pain; HC: hormonal contraceptive; VAS: visual analogue scale.

At the visit at 6 months, data were collected from 346 patients. The mean VAS score for pain was 3.52 ± 1.91 and 37.4% were taking NSAIDs, of whom 1.3% were required this medication continually.

The changes from baseline in the VAS score for pain at the 3-month and 6-month visit were statistically significant ($p < 0.0001$) (see Table 2).

Table 3 shows the changes in proportions of patients classified according to pain intensity by study visit. These data show a decrease of 91% in the proportion with severe pain and of 67% in the proportion with moderate pain occurred. At the 6-month visit, 52% of patients reported pain equal to or less than 3.

Fig. 1 shows the change in percentage of women using NSAIDs to control pain.

During the study, 52 patients withdrew from treatment. In 27 of these, the reason for withdrawal was pregnancy. The remaining 25 withdrawals were due to uncontrolled pain (12) and presence of side effects (13), mainly nausea and vomiting.

Comment

Our study shows that women with endometriosis who wish to become pregnant and are treated with a preparation containing *N*-acetyl cysteine, alpha lipoic acid and bromelain show a significant improvement in endometriosis associated pain and require lower intake of rescue analgesics. There was a significant decrease in the percentage of patients with EAPP (92.7% at baseline, 82.7% at 6 months) as well as a significant reduction in the VAS score. The largest decrease in VAS occurred in women with most severe pain (40.2% had severe pain at baseline to 3.6% at 6 months, corresponding to a 91% reduction). The proportion of patients with moderate pain decreased by 68% (35.1% at baseline to 11.4% at 6 months). The sample size calculation was based on a 50% reduction in pain. These results confirm that the sample of women included was sufficient to obtain evaluable results.

We decided to include patients who wanted to become pregnant in the short term to avoid including patients treated with hormonal contraceptives, as these patients would confound the results of EAPP.

Oxidative stress has been postulated to play a fundamental role in the pathogenesis of endometriosis [10]. Oxidative stress is defined as an imbalance between production and neutralization of reactive oxygen species (ROS), either as a result of increased production or deficiency in antioxidant mechanisms [11]. The role of oxidative stress in the persistence and clinical symptoms of endometriosis was suggested in a laboratory study in which cultures of endometrial cells from women with and without endometriosis were used to assess endometrial cell proliferation by a thymidine uptake assay [12]. Endometrial stromal cells were cultivated in presence of antioxidants or oxidative-stress inducing agents and it was found that antioxidants induced a dose-dependent inhibition of thymidine uptake whereas cultures exposed to oxidative-stress inducing agents led to endometrial stromal growth.

Among the mechanisms to counteract oxidative stress, of particular note is the antioxidant system that eliminates free radicals and is based, essentially, on the action of the superoxide dismutase, which eliminates the superoxide anion, and glutathione peroxidase, which eliminates hydrogen peroxide. In patients with endometriosis, there is decreased antioxidant activity [13]. The intracellular antioxidant system based on glutathione plays a major role in endometrial detoxification reactions and this system has been suggested to play a role in the pathogenesis of endometriosis [14].

In a case-control study that analyzed blood samples and peritoneal fluid from 30 women without endometriosis and 32 with endometriosis obtained immediately before surgery in the case of blood samples and during the surgical procedure in the case of peritoneal fluid, it was observed that women with endometriosis had diminished peritoneal T helper type 1 immune response with altered pro-inflammatory, chemiotactic, angiogenic and oxidative stress markers in the peritoneal area of women with endometriosis [15]. In another study performed in women with and without endometriosis who followed a diet rich in antioxidant substances (vitamins A, C and E), women with endometriosis had, at baseline, lower antioxidant uptake and increased antioxidant markers after receiving this diet [16].

The mechanism by which this preparation with antioxidant activity may be effective in the treatment of EAPP is linked, essentially, with the use of *N*-acetyl cysteine. Cysteine is a precursor of reduced glutathione, one of the main intracellular antioxidants [17] and its mechanism of action includes the following effects: antioxidant action through the production of reduced glutathione and elimination of ROS, hydrogen peroxide and the hydroxyl radical [18]; anti-inflammatory action, through inhibition of cytokine (IL-8 and IL-6) activity and tumor necrosis factor alfa [19] and antiangiogenic action mediated through inhibition of vascular endothelial growth factor (VEGF) and inhibition of metalloproteinase (MMP) expression [20]. In view of the above, it is plausible that administration of this agent has a beneficial effect on oxidative stress and, as a result, on EAPP.

In an in vitro study in which the authors produced 4 cell lines of epithelial cells and ovarian stroma from 14 patients with endometriosis and 14 patients without endometriosis and analyzed the antiproliferative capacity of endometriosis of *N*-acetyl cysteine, danazol and mifepristone, the authors observed that in presence of *N*-acetyl cysteine there was a decrease in hydrogen peroxidase production and endometrial cell proliferation [21]. Studies have also been performed in animal models and these have

Table 2
Variation in VAS score for pain between baseline and 6 months.

	Mean VAS score	Difference	95% CI	*P*
Baseline Visit	6.68			
Visit 1	4.55	−2.13	1.92–2.26	<0.0001
Visit 2	3.52	−1.03	0.88–1.19	<0.0001

Abbreviations CI: confidence interval; VAS: visual analogue scale.

Table 3
Changes in VAS according to pain intensity (percentage of women in each group).

VAS score	≤ 3	4–5	6–7	8–10
Baseline visit		24.7	35.1	40.2
Visit 1	29.6	40.0	24.6	5.8
Visit 2	52.0	33.0	11.4	3.6

Mild pain: VAS score 4–5; Moderate pain: VAS score 6–7; Severe pain: VAS score 8–10.

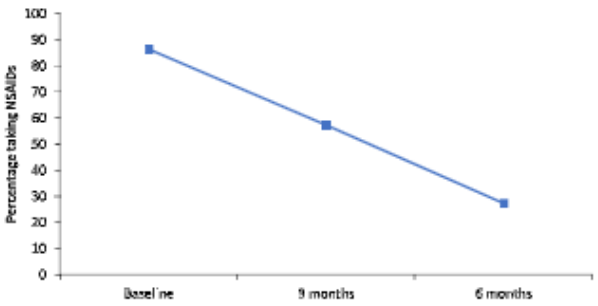

Fig. 1. Change in non-steroidal anti-inflammatory drug (NSAID) use as rescue analgesic during the study period.

shown that *N*-acetyl cysteine is effective at reducing Cox-2 and MMP expression [22], and at decreasing the surface area of endometriosis implants and levels of TNF-alfa in serum and peritoneal fluid [23].

In a comparative study of 92 patients with endometriomas, of whom 47 were treated with *N*-acetyl cysteine, alpha lipoic acid and bromelain and 45, in the control group, received no treatment, it was observed that administration of the study preparation led to a significant reduction in the size of endometriomas in the treatment group [24].

¨Our study is subject to certain limitations. The most important is probably that we do not know the phenotype of endometriosis of the women included in the study; we do not know how many had deep endometriosis or endometriosis of the rectovaginal wall or adenomyosis. Moreover, this was an observational study in which the preparation analyzed was not compared with any other type of treatment. Nevertheless, a strength of our study is that it analyzed the impact of treatment on EAPP. To the best of our knowledge, this is the first study to assess this outcome with the preparation comprising *N*-acetyl cysteine, alpha lipoic acid and bromelain. Moreover, the fact that we recorded rescue analgesic use during the study period enables us to eliminate an important source of bias.

Conclusion

Our study shows that women with endometriosis who wish to become pregnant and are treated with a preparation containing *N*-acetyl cysteine, alpha lipoic acid and bromelain experience a significant improvement in pain associated with endometriosis and require lower intake of rescue analgesics.

Conflicts of interest

The authors declare they have no conflict of interest

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References

[1] Maia H., Haddad C, Casoy J. Correlation between aromatase expression in the eutopic endometrium of symptomatic patients and the presence of endometriosis. Int J Womens Health 2012;4:61–5.
[2] The American College of obstetricians and gynecologists. Practice bulletin: management of endometriosis. Obstet Gynecol 2010;116:223–36.
[3] Gilabert J. Nuevas perspectivas en la endometriosis: desde la fisiopatología al tratamiento médico de la enfermedad. Rev Iberoam Fertil 2011;28:15–31.
[4] Ray K, Fahrmann J, Mitchell B, et al. Oxidation-sensitive nociception involved in endometriosis-associated pain. Pain 2015;156:528–39.
[5] Santanam N, Kavtaradze N, Murphy A, Dominguez C, Parthasarathy S. Antioxidant supplementation reduces endometriosis-related pelvic pain in humans. Transl Res 2013;161:189–95.
[6] Onalan G, Gulumser C, Mulayim B, Dagdeviren A, Zeyneloglu H. Effects of amifostine on endometriosis, comparison with N-acetyl cysteine, and leuprolide as a new treatment alternative: a randomized contolled trial. Arch Gynecol Obstet 2014;289:193–200.
[7] Agostinis C, Zorzet S, De Leo R, Zauli G, De Seta F, Bulla R. The combination of N-acetyl cysteine, alpha-lipoic acid, and bromelain shows high anti-inflammatory properties in novel in vivo and in vitro models of endometriosis. Mediat Inflamm 2015 ID 918089.
[8] Guerriero S, Condous G, Van den Bosch T, et al. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International deep endometriosis analysis (IDEA) group. Ultrasound Obstet Gynecol 2016;48:318–32.
[9] Turk DC, Dworkin RH, Burke LB, et al. Developing patient-reported outcome measures for pain clinical trials: IMMPACT recommendations. Pain 2006;125:208–15.
[10] Taylor RN, Yu J, Torres PB, et al. Mechanistic and therapeutic implications of angiogenesis in endometriosis. Reprod Sci 2009;16:140–6.
[11] Diplock AT. Antioxidants and free radical scavengers. New Compr Biochem 1994;28:113–30.
[12] Foyouzi N, Berkkanoglu M, Arici A, Kwintkiewicz J, Izquierdo D, Duleba AJ. Effects of oxidants and antioxidants on proliferation of endometrial stromal cells. Fertil Steril 2004;82(Suppl. 3):S1019–22.
[13] Szczepańska M, Kożlik J, Skrzypczak J, Mikołajczyk M. Oxidative stress may be a piece in the endometriosis puzzle. Fertil Steril 2003;79:1288–93.
[14] Lee SR, Kim SH, Lee HW, et al. Increased expression of glutathione by estradiol, tumor necrosis factor-alpha, and interleukin 1-beta in endometrial stromal cells. Am J Reprod Immunol 2009;62:352–6.
[15] Mier-Cabrera J, Jiménez-Zamudio L, García-Latorre E, Cruz-Orozco O, Hernández-Guerrero C. Quantitative and qualitative peritoneal immune profiles, T-cell apoptosis and oxidative stress-associated characteristics in women with minimal and mild endometriosis. BJOG 2011;118:6–16.
[16] Mier-Cabrera J, Aburto-Soto T, Burrola-Méndez S, et al. Women with endometriosis improved their peripheral antioxidant markers after the application of a high antioxidant diet. Reprod Biol Endocrinol 2009;7:54.
[17] Sadowska AM, Verbraecken J, Darquennes K, De Backer WA. Role of N-acetylcysteine in the management of COPD. Int J Chron Obstruct Pulm Dis 2006;1:425–34.
[18] Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. Cel Mol Life Sci 2003;60:6–20.
[19] Radomska-Leśniewska DM, Hevelke A, Skopiński P, et al. Reactive oxygen species and synthetic antioxidants as angiogenesis modulators: clinical implications. Pharm Rep 2016;68:462–71.
[20] Masood R, Cai J, Zheng T, Smith DL, Hinton DR, Gill PS. Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor–positive human tumors. Blood 2001;98:1904–13.
[21] Ngô C, Chéreau C, Nicco C, Weill B, Chapron C, Batteux F. Reactive oxygen species controls endometriosis progression. Am J Pathol 2009;175:225–34.
[22] Pittaluga E, Costa G, Krasnowska E, et al. More than antioxidant: N-acetyl-L-cysteine in a murine model of endometriosis. Fertil Steril 2010;94:2905–8.
[23] Onalan G, Gulumser C, Mulayim B, Dagdeviren A, Zeyneloglu H. Effects of amifostine on endometriosis, comparison with N-acetyl cysteine, and leuprolide as a new treatment alternative: a randomized controlled trial. Arch Gynecol Obstet 2014;289:193–200.
[24] Porpora MG, Brunelli R, Costa G, et al. A promise in the treatment of endometriosis: an observational cohort study on ovarian endometrioma reduction by N-acetylcysteine. Evid Based Complement Alternat Med 2013;2013:240702.