Endometriosis is an estrogen-dependent gynecological condition characterized by chronic inflammation and clinically by chronic pelvic pain. Apart from the characteristic symptomatic picture, women with endometriosis frequently experience depression and anxiety. Scientific evidences suggest that dysregulation of inflammatory cells of the immune system, such as mast cells and microglia, could be the common denominator in the development of endometriosis and mood disorders. This review will focus on the role of mast cells and microglia as important players in the development of chronic inflammation and related pain in endometriosis as well as on their role on mood disorders. Mast cells and microglia will be considered as potential therapeutic targets for disease management. In particular, a promising therapy represented by palmitoylethanolamide, an endogenous fatty acid amide involved in the resolution of inflammation modulating mast cell and microglia activity will be described. We will summarize current knowledge from experimental studies and clinical trials demonstrating micronized palmitoylethanolamide efficacy and safety in chronic pelvic pain associated with endometriosis and mood disorders. Lastly, results from a clinical investigation evaluating the effect of co-micronized palmitoylethanolamide + polydatin on chronic pelvic pain and mood disorders in women affected by endometriosis will be described.

**KEY WORDS:** Endometriosis - Chronic pelvic pain - Mood disorders - Ultramicronized and micronized palmitoylethanolamide - Polydatin.

**Endometriosis - Dolore pelvicico cronico - Disturbi dell’umore - Palmitoiletanolamide ultramicronizzata e micronizzata - Polidatina.**

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**Introduction**

Endometriosis (EMS) is an estrogen-dependent disease, defined as the presence of endometrial-like tissue outside the uterus, which induces a chronic, inflammatory...
reaction (1, 2). EMS affects 10 to 15% of women of reproductive age and its clinical manifestations include chronic pelvic pain, severe dysmenorrhea, deep dyspareunia, dischezia and dysuria. Symptomatology is not directly related to disease severity, which in some cases is asymptomatic (2). Apart from the characteristic symptomatic picture, women with EMS frequently experience mood instability accompanied by varying degrees of depression and anxiety. In an epidemiological study on 104 women diagnosed with EMS, 64.4% presented moderate-to-severe depressive symptoms and 63.5% reported high levels of anxiety symptoms (3). Further, patients with EMS show higher frequency and levels of depression and anxiety disorders than patients with other gynecological diseases (4). The prevalence of depression is greater in women with EMS and chronic pelvic pain compared to women with asymptomatic EMS, in particular depressive features are mainly due to altered self-image, work inhibition, dissatisfaction, and sadness (5, 6). However, mood disorders appear also to be related to other consequences associated with EMS, such as infertility and sexual dysfunction (7, 8).

Current evidence suggests that dysregulation of immune system inflammatory cells, such as mast cells and microglia, may be the common denominator in EMS development and mood disorders. In particular, mast cells and microglia are considered coordinators of peripheral and central inflammatory processes, respectively, and might therefore trigger and sustain the persistent inflammatory process present in both the pathogenesis of EMS and mood disorders (9).

This review will focus on existing evidence for a primary role of mast cells and microglia in development of the complex EMS framework, as well as their role in mood disorders frequently associated. In addition, these cells will be evaluated as potential therapeutic targets for disease management. In particular, a promising therapy is represented by palmitoylethanolamide (PEA), an endogenous fatty acid amide signaling molecule involved in the resolution of inflammation and restoration of tissue homeostasis through modulation of mast cells and microglia inflammatory responses. We will summarize current knowledge from experimental studies and clinical trials demonstrating PEA (micronized, ultramicronized and co-micronized with polydatin) efficacy and safety in chronic pelvic pain associated with EMS and mood disorders. Lastly, results from an observational study evaluating the effect of ultramicronized PEA + polydatin (PLD), an antioxidant agent, on chronic pelvic pain and mood disorders in patients affected by EMS will be described.

Role of mast cells and microglia in the pathogenesis of EMS and in the development of pain

Persistent inflammatory processes associated with EMS is a constant feature of the disease, as confirmed by the observation of elevated serum and peritoneal fluid inflammatory markers (10-12). Moreover, pelvic pain associated with EMS is relieved by anti-inflammatory drugs, thus supporting the contribution of chronic inflammation to disease pathogenesis (13, 14).

Mast cells are principal players in orchestrating inflammatory responses after tissue injury, in response to certain metabolic conditions through hormonal signals, or by signals originating from the nervous system (15). Mast cell activation and degranulation results in the release of various mediators, including histamine, proteases, cytokines such as interleukin (IL)-1, IL-6, IL-8, granulocyte macrophage-colony stimulator factor, tumor necrosis factor-α (TNF-α) and transforming growth factor-β (16) that trigger an inflammatory reaction. By releasing cytokines and/or fostering cross-talk with other immune cells, mast cells mediate immunomodulatory functions (17, 18). Mast cells also play a primary role in the resolution of inflammation and maintenance of tissue homeostasis (19). However, dysregulation of mast cell function by chronic stimulation and/or defective control mechanisms can contribute to the onset of chronic inflammation and promote a pathological condition.

Mast cell numbers are reported to be increased at sites of chronic inflammation in different pelvic/gynecologic pathologies, such as bladder pain syndrome/interstitial cystitis, irritable bowel syndrome, and vulvar vestibulitis (20-25). Recent experimental (26-28) and clinical (29-34) studies have highlighted the importance of mast cell activity in the pathogenesis of EMS. In a rat EMS model, endometrial tissue that developed in the peritoneum shows proliferation and infiltration of inflammatory cells, namely mast cells, plasma cells, and macrophages (28). In woman, endometriotic lesions contain significantly more mast cells than unaffected tissue (30-35); the same pattern is observed for degranulated mast cells (30-33). Moreover, stem cell factor, the major growth, differentiation and chemoattractant factor for mast cells, is increased in EMS peritoneal fluid (36). Cytokines and growth factors secreted by the immune system and endometrial cells appear to promote invasion and growth of ectopic endometrium by inducing proliferation and angiogenesis (37).

Mast cell alterations correlate also with endometriotic lesion type and occur mainly at neuronal cell terminals.
Indeed, increased numbers of activated mast cells have been found in deep infiltrating endometriotic lesions (typically associated with more severe pelvic pain), proximal to nerves, suggesting that mast cells may contribute to endometriotic pain by a direct effect on nerve structures (31, 32, 35, 38-40). Deep-infiltrating endometriotic lesions contain significantly more activated mast cells in close proximity to nerves than peritoneal and ovarian EMS lesions and the presence of degranulated mast cells was also shown within the nerve structures, especially in the perineurium (31). Mast cell dysregulation is frequently associated with alterations in nerve terminal fiber function and density, a condition that facilitates the onset of chronic and neuropathic pain (40). Women with deep infiltrating EMS have significantly more nerve fibers in endometriotic lesions than those with superficial peritoneal EMS (40-42) and present hyperalgesia or allodynia (43), abnormal pain responses characteristic of neuropathic pain (44).

Persistent tissue inflammation induces pain that can include a neuropathic component over time, transforming the pain symptom into a disease itself (45). Initiation and maintenance of neuropathic pain involves communication between mast cells and neurons and between neurons and microglia. Mast cells mediators, including histamine, TNF-α, tryptase, prostaglandins, serotonin, IL-1, and nerve growth factor (NGF), can activate primary nociceptive neurons (46, 47), triggering spinal cord neuron responsiveness (48, 49) and microglia activation and causing the onset of neuroinflammation, with persistence and amplification of pain (50). Activation of mast cells may also contribute indirectly to the development of chronic pain by recruiting leukocytes that release algesic mediators (47). Mast cell and microglia role in inflammation, neuroinflammation and painful manifestation led to propose these cells as innovative targets in EMS.

Mood disorders: the role of inflammation

Growing evidence links inflammation with the risk of depression (51, 52). Patients with major depression exhibit essential features of inflammation, such as elevated peripheral blood and cerebrospinal fluid levels of the innate immune system-derived inflammatory cytokines TNF-α and IL-6, along with elevations in peripheral blood levels of other inflammatory mediators (52-54). Chronic stress induced in experimental models causes increased serum levels of IL-6 and TNF-α (55).

In addition, treatment with inflammatory cytokines (for example, IFN-α) or their inducers provokes symptoms of depression both in mammalian experimental models and man (56-58). In agreement with these observations, major depressive disorders are more prevalent in patients afflicted with conditions associated with chronic inflammation such as cardiovascular diseases, type 1 and 2 diabetes, rheumatoid arthritis, irritable bowel syndrome than in pathologies in general (59). There is evidence that not only chronic peripheral inflammation, but also induction of oxidative and nitrosative stress pathways and/or microglia activation underpin the pathophysiology of the above-mentioned disorders and depression (60).

Persistent peripheral inflammation, with its characteristic increase in cytokine levels may provoke longstanding neuroinflammation in the central nervous system (CNS) (61) in a “bottom-up” way. These inflammatory signals can diffuse through blood-brain barrier-compromised areas or be actively transported into the CNS by endothelial cell transporters to activate blood-brain barrier endothelial cells that elaborate, in turn, various factors capable of microglial cell activation. Inflammatory signals can also proceed from the periphery to the brain via the afferent vagus nerve, thereby alerting brain areas to the presence of systemic inflammation (62, 63). Also here, microglia are crucial players in the brain’s immune defense network and the first to be activated (17, 64). Microglia are especially responsive to harmful stimuli and may be primed to respond more vigorously to a subsequent disruption of the brain’s equilibrium, with production and release of the same initiating cytokines. Cytokines are involved in cross-talk between different CNS cell types, and overproduction of key pro-inflammatory cytokines like IL-1β, IL-6 and TNF-α have been reported in the brains of patients with major depression (65).

Brain mast cells are recognized as taking part in neuroinflammation (52). In fact, they are also critical regulators of CNS inflammatory processes that occur after traumatic or ischemic injury (66). In such settings, mast cells might act to peripherally and centrally coordinate inflammatory processes in neuropsychiatric diseases (9).

Mast cell-microglia axis: possible therapeutic target

Mast cells appear involved in chronic inflammatory processes contributing to the development of EMS, even
interacting directly or indirectly with microglia. Chronic mast cell deregulation contributes to events likely responsible for central sensitization mediated by microglia activation and onset of chronic pelvic pain. Anxiety and depression also have an underlying inflammatory basis, driven by an abnormal dysregulation of mast cells and microglia. Within this framework, mast cells and microglia represent critical targets for limiting the cascade of events implicated in chronic inflammatory processes, chronic pelvic pain and depression and anxiety associated with EMS.

A number of preclinical (47, 67-69) and clinical studies (70) carried out over the years have assessed agents capable of modulating or inhibiting mast cell activity. Among these, PEA is of particular interest. An endogenous fatty acid amide, PEA is a congener of the endocannabinoid anandamide and belongs to the N-acyl-lethanolamine family of lipid mediators (71). PEA plays a role in the resolution of inflammatory processes by down-modulating release of pro-inflammatory factors from mast cells (72-74) and by controlling microglia activation (75, 76). Oxidative stress is another important element in the pathophysiology of EMS (77). As PEA lacks antioxidant activity, it is often formulated in association with antioxidant compounds such as polydatin (PLD), a natural glucoside of resveratrol (78, 79).

**Exogenous PEA: effects in experimental models**

Synthetically produced PEA is used in formulations such as naïve, micronized (m-PEA) and ultramicronized (um-PEA) as well as co-micronized with PLD m(PEA/PLD). Micronization and ultra-micronization of PEA produce a crystalline structure with a higher energy content and lower particle size that contributes to better distribution and diffusion than the naïve molecule (75, 80). In a rat model of inflammatory pain, orally given um-PEA was more efficacious than the naïve form (80). In a standardized model of viscerovisceral hyperalgesia involving induction of EMS plus ureteral calculus, prolonged oral treatment with um-PEA during EMS cyst formation significantly reduced behavioral indices of both uterine and ureteral pain, in parallel with a reduction in cyst diameter. These effects were associated with lower mast cell numbers and reduced microglia algogenic markers chymase and NGF in cysts and dorsal root ganglia, suggesting that PEA modulation of mast cell activation translates into a reduced pain perception. Moreover, um-PEA-treated animals had a significant reduction of blood vessels in EMS cysts and down-regulation of vascular endothelial growth factor, the main pro-angiogenic mediator and its receptor. These last findings indicate a strong antiangiogenic role of um-PEA in this condition and account, at least in part, for the observed reduction in cyst diameter (68).

In an experimental model of surgically-induced EMS, treatment with m(PEA/PLD) significantly reduced matrix metalloproteinase 9 expression (responsible for fibrosis and consequent adherence and invasion of endometriotic cells and lymphocyte accumulation), while dampening up-regulation of intercellular adhesion molecule-1 (a mediator of interaction between neutrophils and endothelial cells at the adhesion phase). Further, m(PEA/PLD) significantly decreased nuclear factor-kB activation in endometriotic cysts of rats (81), confirming m(PEA/PLD) ability to control oxidant/antioxidant balance (82). This was accompanied by reductions in lipid peroxidation, nitrotyrosine formation, and poly(ADP)ribose polymerase activity (81).

PEA exerts anti-depressant and anxiolytic effects in experimental models (83, 84). In a mouse model of anxiety/depressive-like behavior induced with long-term exposure to corticosterone, a two-week treatment with co-ultramicronized PEA/luteolin relieved the depression evaluated using behavioral and biochemical approaches. In this study PEA has been co-ultramicronized with luteolin, a flavonoid with antioxidant and neuroprotective actions (83). In another study, the antidepressant-like effect was investigated using the tail suspension and forced swimming tests in mice. PEA significantly reduced immobility in both tests with results comparable to those produced by fluoxetine in a control group (84).

**Exogenous PEA: clinical evidence of efficacy in chronic pelvic pain**

m(PEA/PLD) has been tested in a number of clinical studies to evaluate its effectiveness in managing chronic pelvic pain related to EMS. In a pilot study, m(PEA/PLD) administration to 4 patients with EMS-related pain for 3 months reduced the severity of chronic pelvic pain and endometriotic nodule size. Patients showed also excellent tolerability to the therapy and reduced use of analgesics (85). In a double-blind study, 61 subjects with EMS laparoscopic diagnosis were randomized into 3 groups receiving m(PEA/PLD), placebo and celecoxib, respectively. After 3 months of treatment both m(PEA/PLD) and celecoxib brought about a statistically significant reduction in dysmenorrhea, dyspareunia and pelvic pain compared to placebo. Taking into account that m(PEA/PLD) showed optimal control of pain without adverse events, these
authors suggested that it may have utility especially in these patients unable to receive non-steroidal anti-inflammatory drugs, presenting with renal, gastric or hepatic diseases, those unable to receive hormonal therapy, or where long-term therapy is planned for pelvic pain related to EMS (86).

Characteristics of EMS pain vary as a function of lesion site depending on closeness of the lesion to nerve fibres (28). In view of these considerations, an open-label study was carried out to evaluate m(PEA/PLD) effectiveness in EMS patients selected according to disease location, in particular 19 patients with recto-vaginal septum lesion and 28 patients with ovary lesion (87). Patients with deep EMS of the posterior pelvic compartment presented mostly chronic pelvic pain, dyspareunia and dyschezia while patients with ovarian EMS reported mostly dysmenorrhea. In both groups m(PEA/PLD) induced a gradual reduction of all types of pain during the three months of treatment; a significant decline was already noted after the first month of treatment, confirming earlier results (87).

In an open-label study the effectiveness of m(PEA/PLD) in controlling the painful symptomatology associated with EMS was assessed also alongside hormonal drugs (estroprogestinics and/or gonadotropin-releasing hormone analogues). Thirty patients with a history of EMS-associated chronic pelvic pain were randomly assigned to three groups, and underwent a six-month treatment with: m(PEA/PLD), leuprorelin acetate or ethinylestradiol + drospirenone, respectively. Chronic pelvic pain, dysmenorrhea, and dyspareunia significantly decreased over time in all three groups, irrespective of the treatment applied, m(PEA/PLD) improved quality of life based on the SF12 assessment and, in particular, “physical activities” and “mental and emotional” components, unlike hormonal treatments which improved only the first component. These results demonstrate that pain relief elicited by m(PEA/PLD) is similar to that obtained with hormonal therapy. The occurrence of pregnancy in one patient under treatment with m(PEA/PLD) suggests that this therapy does not interfere with ovulation (88).

Preliminary results on the effect of um-PEA + m(PEA/PLD) in patients with chronic pelvic pain and mood alterations associated with EMS

Seventeen female patients suffering from EMS-associated pain for more than 6 months were selected for an observational study carried out at the endometriosis and pelvic pain outpatient clinic of the “San Giuseppe da Copertino” Hospital (Lecce - Italy) between March 2015 and July 2016. Diagnosis of EMS was performed by means of clinical symptom evaluation (chronic pelvic pain, dysmenorrhea, deep dyspareunia, dyschezia e dysuria), and laparoscopic examination. Patients who had suspended their previous therapies for at least 3 months before the study start were included and were allowed to continue taking analgesics/anti-inflammatory drugs for acute pain when needed. Patients to be included had a pain intensity score ≥ 5 for one or more symptoms at baseline, as evaluated by the Numeric Rating Scale (NRS). Patients with uncertain diagnosis of EMS, menopause, presence of concomitant pathological conditions unrelated to chronic pelvic pain due to EMS and pregnancy were excluded. A single sachet containing um-PEA 200 mg + m(PEA/PLD) 400 mg/40 mg [Pelvilen® DualAct, Epitech Group SpA, Saccolongo, Italy] was administered three times a day for 4 months. All patients were evaluated for: i) intensity of global pelvic pain, dyspareunia, dysmenorrhoa, dyschezia and dysuria by NRS, an 11-point grading scale (0-10), ii) quality of life, assessed with the Endometriosis Health Profile (EHP-30) questionnaire which consists of 30 items grouped in 5 sections (pain, control and powerlessness, emotional well-being, social support, and self-image). Each section was evaluated separately. All parameters cited were assessed at baseline (T0) and at the programmed follow-ups after 30 days (T1), 60 days (T2), 90 days (T3) and 120 days (T4 – end of treatment). Statistical analysis was performed using the generalized linear mixed model (GLMM) and the Tukey-Kramer adjusted test for multiple comparisons in post-hoc analysis. A p-value of less than 0.05 was considered significant. All parameter scores are presented as mean ± standard error (S.E.) unless specified otherwise. All patients provided written informed consent to participate. This study was carried out in accordance with the Helsinki Declaration of 1964 and its subsequent revisions and Good Clinical Practice (GCP) and with knowledge of the relevant health authority.

Mean patient age was 37±7 years (standard deviation). Based upon localization, extent and severity of the endometriotic lesions, EMS stage was classified as grade III (moderate) in 35.3% (6/17) and as grade IV (severe) in 64.7% (11/17) of the patients (American Fertility Society guidelines). Three patients withdrew from the study, 2 reported mild gastrointestinal disorders (diarrhea or epigastric discomfort), and 1 patient...
withdrew due to health reasons unrelated to the treatment.

Statistical analysis showed that global pelvic pain decreased significantly over time, from 7.8±0.37 at baseline (T0) to 3.3±0.74 after 4 months of treatment (T4; p<0.0002) (Figure 1). Also dysmenorrhea, dyschezia, and dyspareunia scores decreased from 8.1±0.66 to 3.7±0.85 (p<0.0002), from 6.2±0.77 to 2.3±0.63 (p<0.0001) and from 6.5±0.84 to 2.3±0.89 (p<0.0049), respectively. Dysuria, when present, showed a significant reduction (p<0.0215) from 3.6±0.91 to 1.2±0.53 (Figure 1). The answers obtained on questionnaire EPH-30 (Endometriosis Health Profile questionnaire) subdivided into 5 sections were analyzed by GLMM. “Emotional well-being” mean score decreased significantly over time (p<0.0079) from a value of 56.1±27.13 (T0) to 36.3±24.81 (T4); the Tukey-Kramer adjusted test between T0 and T4 confirmed this reduction as statistically significant (p<0.0168). The mean score of “pain” and “control and powerlessness” sections changed significantly over time (p<0.0254 and p<0.0301, respectively) because of a floating trend of the means; post-hoc analysis between T0 and T4 did not show significant difference (Table 1). “Social support” and “Self-image” mean scores did not show significant reductions (n.s.) (Table 1). Notably, one patient treated with um-PEA + m(PEA/PLD) was pregnant at study end, indicating that um-PEA + m(PEA/PLD) is devoid of anti-ovulatory effects.

Conclusions

Immune system cells, such as mast cells and microglia, play a key role in the development of a complex pathological framework of EMS characterized by chronic inflammation and pelvic pain, frequently associated with mood disorders. At the tissue level, persistent mast cell degranulation, together with cytokine mediator release, underlies the chronic inflammatory state in EMS. This phenomenon, which occurs predominantly at pelvic nerve terminals if prolonged over time contributes to the occurrence of events likely responsible for central sensitization. Subsequent activation of microglia initiates central neuroinflammation, the amplification of pain mechanisms and onset of chronic pain.

Further, mood disorders, such as anxiety and depression, have a solid inflammatory basis sustained by abnormal mast cell and microglia dysregulation.

<p>| Table 1 - Mean score and GLMM analysis for the 5 question groups of EPH-30 questionnaire. |
|-----------------------------------------------|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>EHP-30 question groups</th>
<th>Mean value T0</th>
<th>S.E.</th>
<th>Mean value T4</th>
<th>S.E.</th>
<th>GLMM Tukey Kramer adjusted test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>42.8</td>
<td>6.34</td>
<td>31.1</td>
<td>4.48</td>
<td>p&lt;0.0254</td>
</tr>
<tr>
<td>Control and powerlessness</td>
<td>49.0</td>
<td>7.46</td>
<td>37.2</td>
<td>6.40</td>
<td>p&lt;0.0301</td>
</tr>
<tr>
<td>Emotional well-being</td>
<td>56.1</td>
<td>6.58</td>
<td>36.3</td>
<td>6.63</td>
<td>p&lt;0.0090</td>
</tr>
<tr>
<td>Social Support</td>
<td>44.5</td>
<td>6.60</td>
<td>32.1</td>
<td>6.28</td>
<td>n.s.</td>
</tr>
<tr>
<td>Self-Image</td>
<td>35.8</td>
<td>5.71</td>
<td>27.4</td>
<td>7.71</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

*S.E. Standard error; n.s. not significant; GLMM generalized linear mixed model.*
Collectively this evidence supports the hypothesis of common pathways/mechanisms in the etiopathogenesis of such conditions. Targeting mast cells and microglia may represent a therapeutic strategy to contrast the symptomatology of both diseases. Pelvilen® dual Act represents an innovative approach to control the mast cell-microglia axis. PEA plays an important role in the inflammation process, modulating pro-inflammatory factor release from mast cells and controlling microglia activation; PLD is a natural glucoside of resveratrol with potent antioxidant effects. The present investigation and previous clinical studies confirm that the product significantly improve both EMS painful symptoms and the associated emotional component and consequently quality of life. Serious adverse events have never been detected. These results, albeit preliminary, together with previous studies suggest this treatment as a valid alternative to traditional therapies in EMS.

References


52. Skaper SD, Facci L, Giusti P. Neuroinflammation, microglia and mast cells in the pathophysiology of neurocognitive disorders: a review. CNS Neurol Disord Drug Targets. 2014;13(10):165-166.


61. Kubera M, Obuchowicz E, Goehler L, Brzeszcz J, Maes M. In animal models, psychosocial stress-induced (neuro)inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. Prog Neuropsychopharmacol Biol Psychiatry. 2011...

