New Insights in Mast Cell Modulation by Palmitoylethanolamide

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Abstract: Since its discovery palmitoylethanolamide was considered as an endogenous compound able to negatively modulate the inflammatory process. Its effects have been extensively investigated in *in vitro*, *in vivo* and in clinical studies. Notwithstanding some discrepancy, nowadays the efficacy of palmitoylethanolamide in controlling mast cell behaviour, which likely accounts for its many anti-inflammatory, anti-angiogenic and analgesic effects, is well recognized. In view of their strategic localization at sites directly interfacing with the external environment, mast cells act as surveillance antennae against different types of injury and can undergo activation, thereby regulating both innate and adaptive immune reactions through the release of several preformed and newly synthesized mediators. Mast cells are now viewed as key players in orchestrating several disorders including both acute and chronic inflammatory processes, and have a role in angiogenesis and hyperalgesia. Since mast cells exert also important physiological, homeostatic functions, the most recent goal for pharmacologists is to control, rather than block, mast cell degranulation in order to modulate the pathological scenario.

The aim of the present review is to summarise the evidence regarding the role played by palmitoylethanolamide in the control of mast cell activation, starting from *in vitro* studies, going through *in vivo* evidence in animal models of disease sustained by mast cell activation, and finally reviewing recent clinical studies using this molecule.

Keywords: Mast cells, palmitoylethanolamide, homeostasis, Autocoid Local Injury Antagonism, inflammation, pain, endocannabinoids.

1. PALMITOYLETHANOLAMIDE

Following the discovery of anandamide (AEA), an N-acylethanolamine (NAE), as a signalling lipid mediator of the endocannabinoid system there has been renewed research interest in other endogenous molecules, related for structure and function to AEA. Palmitoylethanolamide (PEA) a saturated NAE (C16:0) containing the palmitoyl moiety, which is structurally related to AEA, is another naturally occurring NAE. PEA is considerably more abundant than AEA in many tissues; however, unlike AEA or 2-arachidonoylglycerol (2-AG), PEA does not bind to cannabinoid CB1 / CB2 receptor sites in *in vitro* studies [1]. Notwithstanding this, PEA shares several important pharmacological effects with endocannabinoids, including potent anti-inflammatory and analgesic properties in several animal models of pathologies [2]. Therefore, it has been suggested that PEA constitutes a "parallel" endocannabinoid signalling system; this concept is supported by the evidence that PEA production and inactivation can occur independently from that of AEA and 2-AG, the latter mediated by fatty acid amide hydrolase (FAAH), or monoacylglycerol lipase, respectively [3]. In fact, the laboratory of Natsuo Ueda [4] discovered the existence of a unique enzyme able to hydrolyze PEA to a greater extent than AEA and 2-AG, named N-acylethanolamine-hydrolyzing acid amidase. Finally, while the hydrolysis of AEA and 2-AG by FAAH or monoacylglycerol lipase gives rise to new bioactive lipids (ie. arachidonic acid and eicosanoids), PEA hydrolysis gives rise to two relatively inactive products, palmitic acid and ethanolamine, suggesting that the role of N-acylethanolamine-hydrolyzing acid amidase is actually to stop biological responses initiated by increased PEA production [5].

Although PEA binding to CB1/CB2 receptor sites has not been demonstrated so far, some PEA pharmacological effects are mediated by endocannabinoid receptors [6-10] through a so-called "entourage" effect. According to this idea, PEA increases AEA tone that in turn could directly bind to CB_1/CB_2 receptors or to the transient receptor potential for vanilloid 1 [11]. On the other hand, actually at least two receptors responsible for some PEA biological properties are known, the peroxisome proliferator-activated receptor-α (EC_{50}=3μM) and GPR55 (EC_{50}=4nM); the binding of PEA to these two receptors explains some of its anti-inflammatory and analgesic effects [12-14] therefore suggesting a multi-target and pleiotropic mechanism for PEA effects. The last, but not the least, mechanism proposed to explain PEA action, first identified by Levi-Montalcini and co-workers in the 1990s, is the so called ALIA mechanism or the "Autacoid Local Inflammation Antagonism" [15], later modified into "Autacoid Local Injury Antagonism" [16]. This change in acronym was based on the observation that "the pharmacological effects of PEA appear to reflect the consequences of supplying the tissue with a sufficient quantity of its physiological regulators of cellular homeostasis" [16]; thus, PEA being viewed as a broad bioactive "protector" instead of limiting its field of action to the inflammation response. Specifically, PEA is considered the parent molecule of ALIAmides given its

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capability to negatively modulate mast cell (MC) activation [17].

2. MC ACTIVATION IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

MCs are immune-competent cells of hematopoietic lineage derived from $CD13^{+}CD34^{+}KIT$ $(CD117)^{+}$ bone marrow progenitors [18]. These progenitors leave the bone marrow, circulate in the blood and, depending upon the presence of specific chemotactic signals, migrate to the tissue where they become resident cells. MC precursors proliferate and differentiate into the tissue depending on the presence of local growth factors and cytokines, such as stem cell factor, interleukins-3, -4, and -9 and nerve growth factor (NGF) [19], secreted both by the MC precursors themselves and by other tissue cells. Despite having a common lineage, granulated morphology and functions, MC are highly heterogeneous and phenotypically malleable cells [20]. It is likely that this heterogeneity is produced by specific tissue localization or by a specific stimulus challenge. In fact, MCs change their phenotype under different conditions; moreover, the environment changes the MC protease profile [21]. MCs are divided in two different subsets depending on their localization and their content of chymases and tryptases: connective type MCs are preferentially located in the skin, while mucosal type MCs are located at mucosae of intestinal, bronchial and genito-urinary tracts (for an extensive review see [22]).

In view of their strategic localization at sites directly interfacing with the external environment, MCs act as surveillance antennae against different types of injury and can undergo activation, regulating both innate and adaptive immune reactions [23]. Moreover, MCs possess important physiological roles controlling tissue remodeling, wound healing and neuroimmune response to stress [24]. MC granules contain several biological mediators that are released following activation by both immunoglobulin (Ig)E-dependent (leading to an explosive degranulating (anaphylactic) release of mediators), and non-IgE-related stimuli (e.g. bacterial or viral infection, hormones, pH variation, drugs) leading to a more controlled mediator secretion [25]. MC mediators include: (i) granule-associated mediators, including histamine, serotonin (5-hydroxytryptamine), and a variety of proteases and peptidases which are pre-synthesized and released following fusion of secretory granules with the cytosolic membrane; (ii) eicosanoids such as prostaglandin D2 and leukotriene C4, which are generated and released following activation of cytosolic phospholipase A2; (iii) de novo synthesized mediators [26]. Because physiological and pharmacological compounds are able to activate MCs in a different manner, different mechanisms for MC activation are hypothesized to exist, ranging from the selective secretion of an individual class of cytokines or amines, as observed in anaphylaxis, to an exhaustive degranulation and acute secretion of a plethora of mediators during other pathologies [27].

The role of MCs in orchestrating both acute allergic reaction and chronic inflammatory processes is well recognized, playing a role in angiogenesis and hyperalgesia [28]. The most recent goal for pharmacologists is to control, rather than block, MC degranulation to modulate inflammatory disease, since MCs possess also physiological, homeostatic roles [22].

2.1. MC Pharmacology

Historically, clinical symptoms of MC hyper-activation during immune/allergic reactions were controlled by blocking MC degranulation mainly with chromones i.e. chromolyn; however, these drugs carry very severe side effects, including throat irritation and coughing, nausea and vomiting, fullness after eating, heartburn, or abdominal pain [29]. Another pharmacological strategy is to counteract the biological effect of the different mediators released by activated MC. In this contest, the use of anti-histaminic or anti-leukoriene drugs [30], together with glucocorticoids is effective in controlling the biological effects of MC mediators in many pathologies. In addition to a number of adverse side effects, the latter carry the risk of immune-suppression. At present, however, there is no specific drug affecting the whole content of MC granules released during activation. Given the importance of MC activation during both inflammatory and chronic degenerative disease, the discovery of new molecules able to control MC activation is of ever-increasing importance. Promising effects have been achieved by cannabinoid-based drugs, although their clinical use is limited by ethical problems. In this contest, PEA could represent a new molecule with a better pharmacological profile for the control of MC behaviour.

3. PEA EFFECTS IN MC ACTIVATION

3.1. *In Vitro* Studies

PEA has been considered as an endogenous modulator of MC activation, since it was showen to be effective in several MC-mediated experimental models of disease both *in vivo* and *in vitro*. The first evidence for PEA action in modulating MCs was presented in 1995 by Facci *et al*. [17], who showed that PEA, differently from AEA, prevented the immunological activation of the cognate mast cell line RHL-2H3, when stimulated with anti-dinitrophenol IgE/dinitrophenol human serum albumin. In this *in vitro* study the authors suggested that the PEA effect depended, at least in part, on the activation of CB_2 receptors. A second paper by the same group showed that PEA was able to protect neurons from injury triggered by MC activation, in mixed hippocampal cultures [31]. PEA selectively limited neuronal injury provoked by antigen- or myelin basic protein-stimulated peritoneal MCs, although PEA did not protect neurons from injury caused by astrocyte activation in the absence of MCs [31].

This pioneer study was later confirmed by several different papers, although with some discrepancy. First of all it was demonstrated that MCs are able to biosynthesize PEA when stimulated with ionomycin [32]. Also, immunogenic stimulation of RBL-2H3 cells leading to serotonin/histamine release caused PEA biosynthesis, albeit to a smaller extent [32]. In this study, the authors showed that MCs not only synthesize PEA but also have the complete biochemical machinery for PEA self-inactivation [32], since MCs are able to take up and degrade PEA. In MCs, PEA uptake is mediated partly by a facilitated transport mechanism, separate to that for AEA, and partly by passive diffusion

through the cell membrane [33]. Interestingly, it was reported that PEA, following its re-uptake is able to inhibit the hydrolysis of AEA and other fatty acid amides catalyzed by FAAH, acting as a competitive inhibitor of this enzyme [34]. These data should be given serious consideration, since they represent a promising pharmacological approach to increase both PEA and AEA tone, especially as it occurs during an inflammatory condition. In this paper, Maccarrone and colleagues [34] indicated for the first time that both AEA and PEA did not interfere with spontaneous or A23187-induced degranulation of human MCs. One possible explanation for this divergence could be that, unlike rat MCs, the human MC line (HMC-1) used does not express functional cannabinoid receptors on their surface in physiological conditions.

Similar results were reported also by others showing that PEA administration failed to reduce MC activation induced both by immunogenic and non-IgE stimulation [35]. In one of these studies, PEA did not affect spontaneous or anti-IgE-mediated histamine release from rat peritoneal MCs, whereas AEA induced histamine release from non-activated MCs; moreover, WIN 55,212-2 and HU-210 enhanced anti-IgE-induced histamine release [36]. In a previous study by Bueb and colleagues [37], both PEA and WIN 55,212-2 failed to induce histamine release from peritoneal MCs whereas a significant histamine release was induced by AEA only at a very high, supra-physiological, concentration. Also Granberg *et al*. [38] demonstrated that although there was a tendency for PEA to reduce the response of MCs to antigen stimulation, PEA itself did not exhibit a clear effect [38]. The discrepancy between all these observations, as suggested by the same authors, may be due to the different MC culture types used or to differences in experimental conditions.

On the other hand, a very recent publication demonstrated the ability of PEA to prevent MC degranulation [39]. According to this paper, the release of histamine, protaglandinD2 and tumour necrosis factor- , induced by IgE in isolated canine skin MCs were significantly and concentration-dependently inhibited in the presence of PEA [39]. Analogous results were achieved in human MCs challenged with phorbol-12-myristate-13-acetate where the authors showed that PEA was able to prevent NGF release from HMC-1 cells through the activation of GPR55 on the MC membrane. In fact, administration of PEA to GPR55-silenced MCs led to loss of its effect [14].

These discrepancies of PEA effects on MCs could be explained, at least in part, by the difficulty in studying MC functionality *in vitro*. The study of drug effects in isolated MCs is sometimes inappropriate, given the considerable degree of MC heterogeneity both with respect to their morphology, expression of proteins (such as tryptase and chymase) and according to different sensitivity to stimulants such as compound 48/80 and substance P [40]. In man for example, skin MCs are activated by both antigen, compound 48/80 and substance P, whereas sinus MCs are exclusively activated by an antigenic stimulus [41]. Also, different isolated MC types may have different sensitivities to anti-allergic agents [42]. Moreover, the reduction of antigen-induced -hexosaminidase and [^{3}H]serotonin release from RBL-2H3 cells, induced by sodium cromoglycate and by the 2-adrenoceptor agonist salbutamol [43], was rather modest, the latter in contrast to the results obtained in skin MCs [43]. Thus, a better way to test the effect of drugs on MC behavior is to study them in *in vivo* model of MC activation.

3.2. Animal Models

Given the aforementioned limitations inherent to *in vitro* studies, we hypothesize that studying the effect of PEA in animal models of disease sustained by MC will give more definitive information. The first *in vivo* evidence appeared in 1996 in a paper by Mazzari and collaborators [44] showing that PEA was able to control MC–derived inflammation in immunogenic and non-immunogenic animal models of disease. In this work, the authors showed that PEA administration dose-dependently reduced extravasation in the passive cutaneous anaphylaxis (PCA) test in mice. Moreover, the authors speculated that this effect on PCA-induced extravasation was mediated by a reduction of MC activation by the anaphylatoxins C3a and C5a released by the complement cascade, after immune complex formation. Results similar to those obtained with PEA on MCs were also obtained with cromolyn, a well-known MC stabilizer in the same model of PCA [44]. Moreover, in the same paper the authors showed that PEA was able to control also neurogenic inflammation induced by subcutaneous injection of substance P. Oral administration of PEA (0.1–10 mg/kg body weight) led to a dose-dependent reduction of the hind-paw oedema induced by substance P, as well as carrageenan or dextran and formalin, in laboratory animals [44]. Injection of substance P in the mouse ear *pinna* produced a highly significant increase in the number of locally degranulated MC. PEA prevented, in a dose-dependent manner, their degranulation induced by substance P already 10 min after the stimulus. Prevention of substance P-induced MC degranulation by oral administration of PEA resulted in the reduction of plasma extravasation induced by substance P [44]. Consistent with the work of Mazzari [44], another paper showed that PEA reduced also the oedema in response to compound 48/80, another type of degranulating agent different from substance P [45]. In contrast with the above *in vivo* data the authors were unable to show a PEA effect in controlling the effect of compound 48/80 in *ex vivo* experiments. In fact, PEA treatment was ineffective in controlling compound 48/80-induced -hexosaminidase release from mouse paw skin [45]. These data strongly confirm the difficulty to test the effect of PEA in *ex vivo* models and isolated MC cultures.

In line with this *in vivo* evidence, our group has demonstrated that PEA significantly reduces granuloma formation in a rat model of chronic inflammation actively sustained by MC activation [46]. First of all we showed that during granuloma formation there was a significant reduction of endogenous tissue PEA levels. Restoring PEA tone by exogenous PEA administration reduced the formation of granulomatous tissue 96 h after stimulus [47, 48]. Treatment of animals with PEA significantly reduced the number and degranulation of MCs in granulomatous tissues [48]. As a consequence of controlling MC degranulation, PEA treatment resulted also in the reduction of angiogenesis [47] and hyperalgesia [48]. In fact, as revealed by histological analysis of granulomatous tissues,

MCs are strategically located near blood vessels and nerves fibers [47, 48], where they are capable of differentially releasing a broad range of pro-inflammatory, pro-angiogenic and nerve-sensitising molecules such as chymase, vascular endothelial growth factor and NGF. According to this evidence, we found that the control of hyper-activated MCs by PEA not only reduced the inflammatory scenario, but also reduced the angiogenic parameters evaluated as number of blood vessels, haemoglobin content and CD31 expression in granuloma [47] Moreover, the ability of PEA to control MCs in close proximity to nerves fibers resulted in the reduction of mechanical allodynia evoked in these animals. The reduction of hyperalgesia was a consequence, at least in part, of PEA control of the pro-algogen mediators released by MCs, mainly NGF [48]. Similar results were obtained also with Ademidrol, a PEA analogue suitable for topical use that reduced MC activation as well inflammation and angiogensis when locally given to rats with granuloma [49].

The control exerted by PEA on MC activation is reinforced also by recent evidence showing that PEA significantly reduced the production and the release of several mediators by MCs, such as tumour necrosis factor- and neurotrophic factors like NGF in an *in vivo* model of neuropathic pain [6]. In this paper the authors, although not directly measuring MC activation, suggested that PEA exerted its effects mainly through the control of MC activation.

Finally, it was recently reported that PEA was able to modulate MC activation also in spinal cord injury, since a significantly lower MC density and degranulation after PEA treatment was observed in the injured spinal cord tissues [50]. Moreover, PEA administration reduced the expression of two well recognized MC markers, chymase and tryptase, during spinal cord injury [50].

3.3. Clinical Evidence

On the basis of encouraging *in vitro* and *in vivo* evidence, the effect of PEA was explored also in several clinical trials, both in human and in pet animals, for inflammatory and pain syndromes. It was reported that PEA-based drugs showed no notable side effects; overall, more than 2000 patients have been successfully treated with PEA, with no adverse effects reported in any of the trials [51].

The first clinical evidence appeared during 1970s; this clinical trial demonstrated the ability of PEA to control airway disease [52]. Following this pioneer work, and on the basis of PEA ability to control MC activation, a variety of clinical trials were initiated. A veterinary dermatological trial in 2001 reported that one month-treatment with PEA resulted in decreased pruritus, erythema and alopecia in cats affected with hypersensitivity skin disorders, i.e., eosiniphilic plaques and eosinophilic granuloma [53]. The clinical improvement of symptoms was clearly correlated with PEA control of MC activation; in fact, PEA, although failing to reduce the number of MCs in skin, significantly reduced MC degranulation in the skin biopsies obtained at study end as compared to biopsies at the study start [53]. Moreover, a recently published paper showed that a single oral dose of PEA (10 mg/kg) significantly reduced the wheal and flare reaction (i.e., a MC-driven response) in dogs with skin hypersensitivity [54]. The ability of PEA to control MC activation has been exploited in several human diseases, as well. Several studies demonstrated that PEA, alone or in combination with Polydatin, was able to control pain syndrome, above all in MC sustained pain. For example, a recent paper showed that administration of PEA and Polydatin appeared to be very useful in controlling chronic pelvic pain associated with endometriosis [55]. In agreement with the last report PEA was found effective in patients with chemotherapy-induced painful neuropathy [56]. In this study the effect of administration of PEA, for two months, in 20 patients undergoing Thalidomide and Bortezomib treatment for multiple myeloma was analyzed. It was reported that PEA restored nerve function in these patients, which most likely accounted for the reduction of pain perception [56].

Moreover, a recent pilot study was aimed to assess the efficacy and safety of twice daily application of a topical emulsion containing 2% Adelmidrol, an analogous of PEA, on 20 paediatric patients suffering of atopic dermatitis [57]. This study showed 80% of symptom resolution, through the inhibition of NGF release from cutaneous MCs. Actually a new drug containing PEA has been approved by the U.S. Food and Drug Administration for the treatment of dermatitis.

4. DISCUSSION AND CONCLUSION

Since its discovery and even before the understanding of its multi-target mechanism of action, PEA was considered as an endogenous compound able to negatively modulate the inflammatory process. As suggested for the first time by the Nobel Laureate Rita Levi Montalcini, there is a strong correlation between MC and PEA anti-inflammatory actions; for this reason, the term “ALIAmide” was coined to define PEA. An ALIAmide is an autocoid synthesized and released in response to injury or inflammation acting locally on MCs to counteract the pathological event [15]. This definition fits well the PEA mode of action. Since its discovery, the effects of PEA have been extensively investigated in *in vitro*, *in vivo* and in clinical studies. While discrepancies remain, the efficacy of PEA to control MC behaviour is now well-recognized by the scientific community.

The aim of the present review has been to reinforce the emerging role played by PEA in the control of MC activation. Starting from the milestone paper by Facci and colleagues [17], it is now evident that PEA is able to decrease the degranulation and/or number of MCs during chronic inflammatory and degenerative diseases. Strong evidence suggests that PEA significantly reduces the activation of MCs especially in animal models, whereas debate continues as concerns PEA effects reported for some isolated studies with MCs in culture. In any case, the growing body of data showing the beneficial effect of PEA in controlling MC behaviour has led to the assessment of PEA in clinical trials. The absence of reported side effects has facilitated PEA-based evaluation in various human diseases, above all those in which MC activation is believed to be a primary cause of pathology. Human studies on pain relief obtained with PEA have been extremely encouraging. As in the case of endometriosis or chemotherapy-neuropathy, the analgesic effect of PEA, is easily correlated

to the inhibition of MC activation and reduction of their degranulation.

In conclusion, the ability of PEA to modulate MC behaviour (MC number, activation state), more than the only blockage of MC degranulation, may provide new impetus to MC pharmacology, in view of the central role that these cells play in the development of the inflammatory process from its promotion to progression and chronicity. Interestingly, there is an emerging literature regarding the importance of MCs across a broad spectrum of disorders affecting society, ranging from atherosclerosis and male infertility [58], cardiovascular diseases and autoimmune pathologies [59], to renal diseases [60], cancer [24, 61], brain ischemia [62] and neuro-inflammatory disorders [63], and finally metabolic syndromes [64]. Although considerable efforts have been directed to studying the efficacy of PEA in *in vitro* and *in vivo* models, much work remains to be done to fully elucidate the PEA mechanism of action. In any case, it is most likely to depend on a pleiotropic mechanism rather than an effect on a single MC receptor.

ABBREVIATIONS

AEA = Anandamide

2-AG = 2-arachidonoylglycerol

FAAH = Fatty acid amide hydrolase

MC = Mast cell

NAE = N-acylethanolamine

NGF = Nerve growth factor

PCA = Passive cutaneous anaphylaxis

PEA = Palmitoylethanolamide

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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