

Review Article

Palmitoyl Ethanol Amide in Prophylaxis and Treatment of Viral Infections

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Abstract

There is currently a pressing need to develop new therapeutic alternatives to the annual influenza vaccines and the existing antiviral agents such as oseltamivir and zanamivir. Palmitoyl Ethanol Amide (PEA), an endogenous anti-inflammatory compound and PPAR alpha and gamma agonist, available as food supplement, might be such an innovation. PEA has been tested in a variety of animal models and reduced mortality after inoculation with various microorganisms. PEA may also modulate 'cytokine storm', and reduces the secretion of pro-inflammatory proteins such as NGF, CXCL1, IL-1 β , IL-6 and TNF-alpha. PEA inhibits iNOS expression and nuclear NF- κ B translocation. PEA further inhibits overactive mast cells, which play a role in the pathogenesis of the 'cytokine storm'. Furthermore, PEA has been clinically evaluated in 6 randomized double blind placebo controlled trials in over 3000 patients and was found to be effective and safe in the prophylaxis and treatment of influenza and respiratory tract infections. We will review data supporting PEA's role as an adjunct to antiviral treatment and discuss some of its supposed mechanism of action.

Keywords: Cytokine Storm; Flu; Infection; Inflammation; Mast Cells; PPAR

Introduction

Hyper induction of proinflammatory cytokine production, the 'cytokine storm', is correlated directly with tissue injury and with unfavorable prognosis of severe infections [1]. Induced by an influenza infection, various signaling pathways within the infected cell are activated, and this activation is meant to be an adequate biological response [2]. The pathways lead to the activation of our innate immune system among which mast cells, and interferons, proinflammatory cytokines and chemokines levels rise. This innate immune response is activated in a short period of time, minutes to hours after infection, and a cascade of secreted proteins are activated and targeted against the virus, resulting in a state of inflammation. However, excessive and uncontrolled stimulation of the activated innate immune response proved to be quite harmful, especially in influenza and sepsis. 'Cytokine storm' modulators, such as IL-10, most promising in the beginning of this century, have not been successful in the clinic [3]. Other modulators,

such sphingosine-1-phosphate analog therapy, have been tested in a number of preclinical models, but have not yet been tested in clinical trials [4]. Peroxisome Proliferators-Activated Receptor (PPAR) agonists have also been mentioned as a putative new class of anti-cytokine storm agents [5-6].

Budd et al. point out that for instance gemfibrozil, an old molecule and PPAR-alpha agonist, could reduce mortality in a mouse model of severe influenza due to its ability to inhibit a number of pro-inflammatory cytokines [7]. However, this effect could not replicate in a different model, and fib rates such as gemfibrozil and fenofibrate might have an additional negative impact, for instance impairment of mitochondrial functions [8].

PEA is combined alpha-and gamma-PPAR agonists [9]. Such mixed PPAR-agonists might hold a promise for treating 'cytokine storm'. A natural PPAR-alpha and PPAR-gamma agonist, biochanin A, has been documented to reduce production of the pro-inflammatory cytokines TNF- α and IL-8 in an LPS-induced inflammation paradigm [10].

PEA is without any documented clinical relevant side-effects

and we will review the evidence for PEA as a putative ‘cytokine storm’ modulating agent. Since one of the classical mechanisms of action discovered for PEA is via the modulation of the mast cell, it seems relevant to point out that very recently it was demonstrated that mast cells play a significant role in the pathogenesis of such ‘cytokine storm’ [11]. During the ‘cytokine storm’ synthesis and secretion is stimulated of great number of chemo tactic (RANTES, GM-CSF, MIP-1 alpha, MCP 1, MCP-3, and IP-10), pro-inflammatory (IL-1 beta, IL-6, IL-18, NGF and TNF-alpha), and antiviral (IFN alpha/beta) cytokines. In response to the initial increase of pro-inflammatory proteins, immune competent cells are activated, and recruited to sites of infection. This excess activity contributes further to the pathology induced by influenza viruses, and cause an excessive infiltration of the tissues by immune cells and the micro environment becomes flooded with pro-inflammatory molecules [12]. One of the reasons why the 1918 flu was so aggressive has been postulated to be based on the fact that the 1918 virus may have selectively attenuated the expression of specific innate-response genes [13]. This altered expression lead to extensive damage to the lungs with acute, focal bronchitis and alveoli is, massive pulmonary edema, hemorrhage and rapid destruction of the respiratory epithelium [14]. One of the reasons why people were so susceptible to the 2009 new mutate of H1N1 influenza also related to such a lack of appropriate immunity. The influenza H1N1 2009 infection triggered a massive inflammatory response leading to fever, or other tissue damage, eventually leading to organ failure and death [15]. Such pathology is induced by the inflammatory mechanisms initiated by such infections spinning rapidly out of control.

Initial attempts to modulate pathological cytokine triggered immune cascades utilizing corticosteroids were disappointing, with an overall increase in mortality [16]. New immune modulators not compromising the host immunity while modulating the excess reactions of the innate immune system are therefore greatly needed [11]. PEA can act as such an immune modulator, tempering the overactive inflammatory cascade and thus reducing collateral damage, and his may leads to quicker recovery and less severity of symptoms [17]. We will discuss and review the data related to PEA’s role as an anti-inflammatory agent and its putative relevance in the treatment of severe influenza and other infections, especially there were cytokine storm is to be expected, and in inadequate inflammatory responses.

PEA’s Activity in Various Inflammatory Paradigms: Initial Findings

After several investigators had indicated that extracts of egg yolk, peanut oil and soybean lecithin have inflammatory activity, purification of these substances was achieved and identified as N (2-hydroxyethyl) palmitamide, now called Palmitoyl Ethanol Amide (PEA) [18]. In 1957 Kuhl et al tested this PEA in a pas-

sive joint anaphylaxis assay in the guinea pig [18]. This passively sensitizing the guinea pig knee joints leads to inflammation and swelling, and PEA could counteract the swelling. This was the reason for the authors to state that PEA is a natural occurring anti-inflammatory agent [18]. In 1959, more specific details regarding the anti inflammatory action of PEA were discussed by Ganley et al from the Merck Institute for Therapeutic Research [19]. They assessed the mortality in mice after an anaphylaxis test with intra peritoneally injected killed smooth Bordetella pertussis cells, and found PEA, even in low dose, to increase survival from 20% (untreated) to 70% in the high dose group. The effect was comparable to the rescue data with high dose hydrocortisone [19].

In the beginning of the 70s the modifying effects of PEA on immunological reactions were well established [20]. Perlik et al. summarized: “It has been shown that N-(2-hydroxyethyl)-palmitamide (PEA) can decrease the intensity of several inflammatory and immunological processes.” [21]. This clearly was a premonition, as ‘cytokine storm’ was only first described in 1981 [22].

In the period between 1972 and 1977 in total 3627 patients and volunteers completed 6 different placebo-controlled double-blind trials of which 1937 received PEA up to 1800 mg/day. Relevant side effects were not reported and especially the trials conducted during the flu season demonstrated a treatment, as well as a prophylactic effect. The last study in children was not significant due to the fact that during the study period no influenza epidemic occurred. The results can be seen in Table 1 [24].

Study (year)	PEA (n)	Placebo (n)	% Protection	Significance (p)
Masek (1972a)	223	221	45	<0.05
Masek (1972b)	436	463	32	<0.0005
Kahlich (1973)	436	465	34	<0.0002
Kahlich (1974)	411	199	52	<0.002
Kahlich (1975)	235	118	59	<0.004
Plesnik (1977)	196	224	16	NS

Table 1: Incidence of endpoints between both the PEA and the placebo groups [23].

However, the mechanism of action of PEA remained unknown, and in spite of much speculations, it remained an enigma till in the 90s of last century Nobel laureate Rita Levi-Montalcini and her co-workers proved PEA to be an anti-inflammatory agent. This was based on its properties to inhibit overactive mast cells and reduce the secretion of pro-inflammatory proteins such as NGF and TNF-alpha [24,25].

PEA: A Pleiotropic Agent, Multiple Mechanisms Relevant for Treating Infections?

Following the initial discovery by Levi-Montalcini that PEA acted as an autacoids mast cell modulator in 1993 [26], many ex-

periments were conducted and her findings were duplicated in a variety of in vitro and in vivo paradigms [27-31]. Furthermore, activated mast cells have been described since as important pathogenetical factors in a number of inflammatory and auto-immune disorders [32]. Mast cells amplify immune responses via a number of different mechanisms [33]. Mast cells also seem to contribute to lung pathology during infections [34]. Moreover, in nearly all infection these potent immuno modulatory cells play an important pathogenetical role [32]. This also holds true for viral lung infections: human H1N1, H3N2, and influenza B virus isolates activate mast cells in vitro [35]. Mast cells enhance lung injury that results from H5N1 infection by releasing proinflammatory mediators, including histamine, tryptase, and gamma interferon (IFN- γ) [36].

Already in 1996 it was demonstrated that PEA can inhibit excitotoxic neuronal injury, and such injury is intimately connected to enhanced markers of inflammation [25]. Levi-Montalcini and her group published the results of a series of experiments with pure PEA and concluded that by providing the cells with exogenous PEA: "one might be making available quantities of its physiological modulator sufficient to restore cellular homeostasis in the face of an excitotoxic challenge [37]." Since the discovery of PEA as a mast cell modulator and an inhibitor of injury-induced damage, many new mechanisms have been discovered (Figure 1).

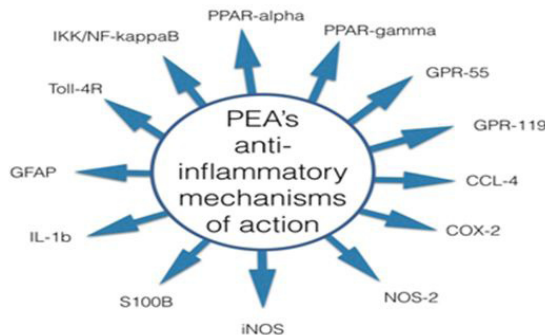


Figure 1: Main and downstream targets of Palmitoyl ethanol amide: CCL4: Chemokines (C-C motif) Ligand 4; COX2: CycloOxygenase-2; GFAP: Glial Fibrillary Acidic Protein; GPR: G Protein-coupled Receptor ; IKK/NF-kappa B: I kappa B kinase/ NF-kappa B signaling pathways; IL-1b: Interleukin-1 beta; iNOS: inducible Nitric Oxide Syntheses; NOS: Nitric Oxide Syntheses; PPAR: Peroxisome Proliferators Activated Receptor; S100B: S100 calcium binding protein B; TLR4: Toll-Like Receptor 4; TNF- α : Tumor Necrosis Factor- α .

We will discuss briefly some of these, in relation to influenza and cytokine storm and to the mechanisms of action of PEA: the Toll like receptors and the PPAR receptors.

PEA, Toll-Like Receptors and PPAR Receptors

Toll Like Receptors (TLRs) are key molecules that alert the

immune system to the presence of microbial infections [38]. TLRs are single, membrane-spanning receptors expressed in immune cell such as macrophages, dendritic cells, mast cells and T-cells, that recognize structurally conserved molecules derived from micro-organisms. The acute lung injury caused by infections is secondary to the generation of host-derived, oxidized phospholipids such as oxidized 1-palmitoyl-2-arachidonoyl-phosphatidylcholine potentially stimulating Toll-Like Receptor 4 (TLR4)-dependent inflammation [39]. Furthermore, TLR4-/- mice are highly refractory to influenza-induced lethality [40]. The Toll Like Receptor 4 (TLR4) ligand fimbriae H protein (Fim H) has been associated with the induction of the innate antiviral responses in the lung leading to protection against lethal influenza infection in mice [1]. New chemical entities and TLR-4 antagonists Eritoran (E5564) (Eisai, Inc.), an extremely potent TLR-4 antagonist, is highly protective when administered therapeutically to mice infected with a lethal dose of influenza [41].

In a model for chronic colon inflammation PEA treatment dose-dependently via the TLR4/PPAR-alpha mechanism improved all macroscopic signs of colitis ulcerous and decreases significantly the expression and release of all the proinflammatory markers tested: iNOS, COX2, S100B and GFAP protein expression [42]. In that study PEA, dose-dependently, ameliorated colitis in wild-type mice and PEA decreases enteric activation and inflammatory markers expression and release in both the colitis model in mice as well as in human colitis samples. PEA also reduces macrophage and neutrophil infiltration in both experimental mouse colitis model as well as human colitis samples. The authors defined PEA as a new pharmaceutical tool in inflammation, due to fact that PEA could counteract mucosal immune cells infiltration, enteric abnormal activation and inhibit the release of proinflammatory mediators during colitis. As PEA is a pleiotropic molecule, its antiviral and immune-modulating properties might be related to other mechanisms of action [43]. For instance influenza virus activates the cellular IKK/NF-kappa B [44] signaling pathway for replication, and recently it has been suggested that this pathway may be a suitable target for antiviral intervention. PPARs cross-talk and PEA as a PPAR-alpha agonist can amelioration oxidative/nitrosative stress induced by NF-kappa B [45] and inhibit many proinflammatory genes [46].

Conclusion

Over 600 papers have been referenced in Pub Med in the last 50 years describing PEA's anti-inflammatory profile. PEA has been tested in a variety of animal models for infections and inflammation since 1957, and the results are concordant. PEA was also tested positive for the treatment and the prophylaxis of influenza and common cold in 5 clinical placebo controlled clinical trials. PEA is an endogenous compound, available as a food supplement and has a dual action in infections. It may modulate hyperactive

immune reactions and mitigates 'cytokine storm' most probably via its role as a PPAR-alpha agonist.

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