Palmitoylethanolamide shows anti-angiogenic effect in the late phase of DNFB-induced dermatitis in mice

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Contact allergic dermatitis (CAD), a commonly observed skin disease, is one of the principal causes for professional diseases in adults. Repeated applications of haptens induce a chronic allergic skin lesions leading to erythema, edema and itching. Moreover, the occurrence of angiogenesis has also been demonstrated in dermatitis [1] since oxygen supply and cell-trafficking are necessary to chronicity. CAD develops in two steps: *priming* with allergen, followed by the re-exposure after 7 to 21 days (*challenge)*. The early re-exposure to DNFB causes an irritative reaction sustained by activated keratinocytes, while late phase of CAD is accompanied to an allergic/inflammatory reaction sustained by activated mast cells (MCs) which release pro-inflammatory mediators and cytokines, as VEGF and NGF, sustaining CAD signs. We have already demonstrated that palmitoylethanolamide (PEA), an endogenous lipid mediator exhibiting anti-inflammatory, anti-nociceptive and anti-angiogenic properties [2], was able to reduce both the early [3] and the late phase of CAD reducing MC number and degranulation [4]. Here we investigated the effect of PEA on DNFB-dependent angiogenesis in the late phase of CAD. Moreover, since some PEA actions are mediated at $CB_2$and/or PPAR-a receptor site, the involvement of these signaling pathway in PEA effect has been studied.

CAD was induced in 8 weeks female C57BL/6J mice by painting 10ml of 0.3% DNFB on mice's right ear, as previously described [5]. Ear swelling was calculated as the difference in ear thickness between the unchallenged and the challenged ear measured by an engineer's micrometer. After the 21^{st}day challenged animals were euthanized, the ears were removed and processed for biochemical and histological analysis. In tissue homogenates the expression of VEGF and its receptor TLK1, was detected by western blot analysis. In histological section vessel number was evaluated by staining with H&E. PEA (5mg/kg), AM630 (2mg/kg), a CB_2antagonist and GW6471 (2mg/kg), a PPAR-a antagonist, and their association were intra-peritoneal administrated for three days after the challenge.

PEA was able to reduce ear thickness of DNFB challenged mice, an effect that was reversed by the co-treatment with AM630 (CB_2antagonist) but not by GW6471 (PPAR-a antagonist). Moreover, we studied the occurrence of angiogenesis in late phase of CAD evaluated as both vessel number and VEGF and FLK1 protein levels in ear tissue. Number of vessel per $mm^2$was increased in CAD, as well the expression of VEGF, the main pro-angiogenic cytokine, and its receptor FLK1. PEA was able to significantly reduce both of vessel number, VEGF and FLK1 levels, while AM630 reversed these effects. GW was ineffective.

We demonstrate that the late phase of CAD was accompanied to angiogenesis and exogenous PEA administration was able reduce number of vessel per area of tissue of DNFB-challenged mice. Moreover PEA was able to reduce the main pro-angiogenic pathway, VEGF/FLK1 in ear of CAD mice. PEA anti-angiogenic activity was dependent on CB_2receptor activation, since the co-administration of CB_2receptor antagonist, AM630, but not by the PPAR-a antagonist, reversed PEA effect. All these results, taken together to the previously reported, let us to hypothesize that PEA by inhibiting MC degranulation, reduces VEGF levels and angiogenesis in CAD. Whether PEA directly binds to CB_2receptor or acts with the so called 'entourage effect' at this level is not clear; however, this study consolidate the efficacy of PEA [6] in the control of all those chronic inflammatory diseases, accompanied to angiogenesis, mainly sustained by MC activation as the late phase of CAD.

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