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UVB Generates Microvesicle Particles via Platelet-activating Factor-receptor Signaling: A Novel Pathway by which a Skin-specific Stimulus Exerts Systemic Effects

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UVB generates microvesicle particles via Platelet-activating Factor-receptor signaling: a novel pathway by which a skin-specific stimulus exerts systemic effects

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ABSTRACT

Though Ultraviolet B radiation (290-320 nm; UVB) exerts profound systemic effects, it is absorbed only in the epidermis. One important question in photobiology is how UVB signals are emanated from the epidermis. Microvesicle particles (MVP) are small (200-1000nm diameter) membrane-bound vesicles released by numerous cell types and can be found in the circulation. MVP can contain both nuclear and cytoplasmic components and are thought to provide a mechanism by which cells transmit signals systemically. Though various stressors have been demonstrated to generate MVP, the ability of UVB to exert this effect has not been demonstrated. Previous studies from ours and other groups have established that through its ability to act as a pro-oxidative stressor, UVB generates oxidized glycerophosphocholine (Ox-GPC) lipids with Platelet-activating Factor-receptor (PAF-R) agonist activity. Our first studies examined the ability of UVB to stimulate the release of MVP. Treatment of the human keratinocyte-derived cell line HaCaT with UVB resulted in the release of MVP. Treatment of HaCaT cells with the PAF-R agonist carbamoyl-PAF (CPAF) also generated MVP release. Preincubation of HaCaT cells with antioxidants N-acetyl cysteine and vitamin C blocked MVP release in response to UVB, but had no effect on MVP release in response to CPAF. To confirm that UVB generates MVP via PAF-R agonists, we used a human epithelial cell line with/without PAF-Rs. UVB generated MVP only in the PAF-R-expressing (KBP) not the PAF-R-negative (KBM) cells. Finally, we induced suction blisters on human skin explants and human skin in vivo and tested the ability of UVB irradiation of these blisters to induce MVP release into blister fluid. These experiments determined that UVB generated MVP in human skin. These studies describe a new pathway involving MVP generated following UVB-induced PAF agonist formation that could play an important role in transmitting systemic signals following this environmental pro-oxidative stressor.

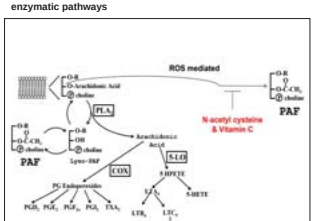
BACKGROUND

Platelet activating factor (PAF) is a lipid-derived 1-alkyl-2-acetly-glycerophosphocholine, produced in response to various stimuli that produces pronounced pro-inflammatory effects. These include marked vasodilatory effects, increased vascular permeability, broncho-constriction, and platelet activation. PAF has also been implicated in sepsis [1,2]. PAF acts by binding to a cellular heterotrimeric G-protein coupled receptor PAF-R. This receptor is found on many cell types including granulocytes, B-cells, epithelial cells (keratinocytes), and some mesenchymal cells. PAF-R activation is known to induce intracellular calcium mobilization and is expressed on keratinocytes. Recent studies have demonstrated that PAF can be produced both enzymatically and through non-enzymatic oxidation of membrane glycerophosphocholines (ox-GPCs) [3].

We and others have shown that in addition to its pro-inflammatory effects, PAF-R mediate pro-oxidative stressors including UVB-induced acute inflammation as well as delayed systemic immunosuppression [4-6]. Regarding the early acute effects of UVB, our previous studies have demonstrated that the PAF-R plays an important role in the pain associated with sunburn [6], and mediates the increased UVB sensitivity in the Xeroderma Pigmentosum Complementation group A (XPA) knock-out mouse [7].

Microvesicle particles (MVP) are small membrane-bound vesicles released by numerous cell types and can be found in the circulation. MVP can contain both nuclear and cytoplasmic constituents and are thought to provide a mechanism by which cells transmit cells systemically [8]. MVP are 200-1000nm in diameter and differ from smaller (40-200 nm) exosomes.

Figure 1- Biosynthesis of PAF through enzymatic and non-enzymatic pathways



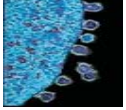


Figure 2. Picture of MVP released from a cell following stress

Given that PAF is involved in UVB-mediated acute effects, these studies were designed to define if MVP are released from keratinocytes following UVB, and if PAF was involved in this process.

MATERIALS & METHODS

For in vitro experiments, the human keratinocyte-derived cell line HaCaT was used. HaCaT cells were treated with UVB or the PAF-R agonist Carbamoyl-PAF (CPAF) or ethanol vehicle [3,5,7]. In some experiments HaCaT cells were preincubated for one hour with 0.5mM each of the antioxidants N-acetylcysteine and ascorbic acid before UVB/CPAF treatment. In some experiments the PAF-R-negative human epidermoid cell line KB cells transduced with functional PAF-Rs (KBP) or MSCV2.1 retroviral vector alone (KBM) were used [9]. At various times post-treatment supernatants were removed and MVP isolated using differential centrifugation and quantified using a NanoSight NS300 instrument using our previously published protocol [8,10].

For ex vivo experiments, we used skin discarded from human abdominoplasty experiments. The skin was warmed to ~37-38 degrees C and suction blisters separating epidermis from dermis were created using vacuum pumps and syringes (see Fig 5). Following blister formation, the blisters were treated with either sham or UVB or topical CPAF in ethanol:DMSO (1:9 v/v). Blister fluid was obtained and weighed in tared tubes. MVP were quantified as above.

For in vivo experiments, the volar forearm of human volunteers underwent suction blistering and blisters were treated either sham or UVB. Again, blister fluid was removed and weighed and MVP quantified as outlined above.

RESULTS

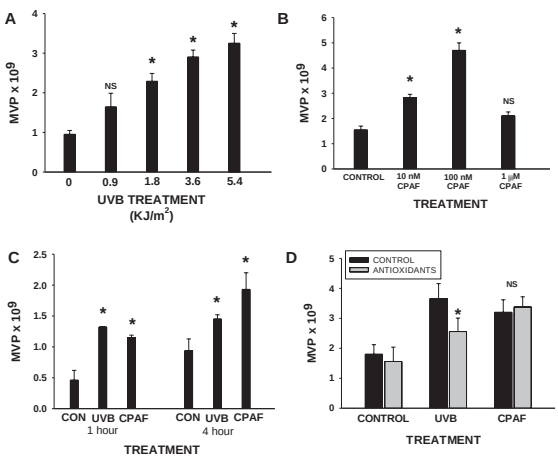


Figure 3. Effect of UVB and CPAF on MVP release in HaCaT cells.
HaCaT cells were either **A)** control (0) or UVB-irradiated at various fluences or **B)** vehicle control or various doses of CPAF. Four hours after treatment, the supernatants were removed and MVPs quantified as outlined in Methods. **C)** HaCaT cells were treated with 3.6 KJ/m^2 UVB or 100 nM CPAF or control (CON), and were harvested 1 or 4 hrs post treatment. **D)**. HaCaT cells were preincubated with 0.5 mM of N-aceytcysteine and 0.5 mM Vitamin C for one hour before treatment with 3.6 KJ/m2 UVB or 100 nM CPAF. The supernatants were removed and MVP measured 4 h later. The data presented are the mean ± SD. MVP numbers of duplicate values from a representative experiment of at least three performed. *Statistically (p<0.05) significant changes from control values. NS: not statistically significant from control values.

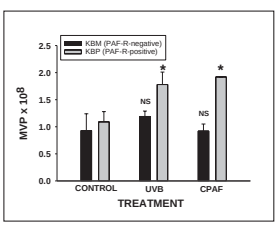


Figure 4. Effect of UVB and CPAF on MVP release in KBP vs KBM cells. KB cells stably transduced with functional PAF-Rs (KBP) or empty vector (KBM) were treated with 100 nM CPAF or irradiated with 3.6 KJ/m^2 UVB or control-treated. The supernatants were harvested at 4 hrs post-treatment. The data presented are the mean ± SD. MVP numbers of duplicate values from a representative experiment from at least three performed. *Statistically (p<0.05) significant changes from control values. NS: not statistically significant from control values.

RESULTS Cont'd

Figure 5. Suction blister formation ex vivo & in vivo

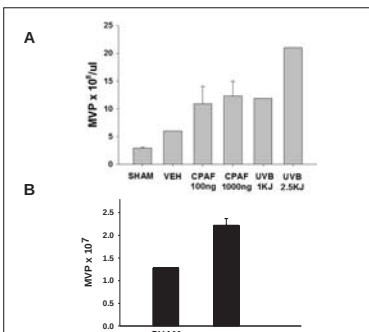
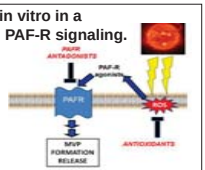


Figure 6. UVB generates MVP formation in skin.
A). Ex vivo blisters were treated with UVB (1 or 2.5 KJ/m2 or DMSO or CPAF (100 or 1000 ng). B). In vivo blisters were treated with sham or UVB (1KJ/m2). MVP tested at 2h.

SUMMARY

1. **UVB generates MVP in vitro in a process involving PAF-R signaling.**
2. **UVB also generates MVP in human skin.**
3. **As MVP are thought to be functional signaling agents, these studies could provide a mechanism whereby UVB can generate systemic effects.**



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