UVB induces MVP Release in a PAF Dependent Manner in Skin-derived Epithelial Cell Line

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UVB induces MVP release in a PAF dependent manner in skin-derived epithelial cell line

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Abstract

Ultraviolet-B (UVB) irradiation of the skin can result in both acute inflammation and systemic immunosuppression, These effects are mediated by the release of bioactive molecules (e.g. cytokines and lipids) from resident skin epithelial and immune cells. Our lab has previously determined that UVB exposure stimulates the production of an inflammatory phospholipid activator, platelet-activating factor (PAF) and its oxidized analogs, which are involved in mediating the UVB responses. Due to the fact that UVB radiation is absorbed by the epidermis but causes systemic effects, we are interested in examining the PAF-mediated mechanisms of systemic intercellular communication. Microvesicle particle (MVPs) are small membrane-derived vesicles released from the plasma membrane that can facilitate intercellular transport of bioactive molecules. We have previously reported that UVB induces MVPs in a PAF-dependent manner, but the mechanism is not fully characterized. It has been reported that in other cell types non-UVB cell stresses result in the release of MVPs which are not associated with cell damage and are therefore not considered to be apoptotic. In this study, both UVB and CPAF (PAF receptor agonist) stimulated MVP release in various skin-derived epithelial cell lines. The PAF dependence of UVB-mediated MVP release was confirmed utilizing a PAF antagonist and PAFR +/- cell lines. In these experiments, the microvesicle release was observed to be UVB-dependent and not associated with cell death although the mechanisms underlying this effect are not fully understood.

Materials and Methods

Cell culture: NTERT cells were teleomerase-immortalized primary human keratinocytes and cultured in supplemented Epilite medium. Primary keratinocytes were obtained from surplus human skin from de-identified donors undergoing abdominoplasty surgery and cultured in supplemented Epilite medium. HaCaT cells are a spontaneously immortalized keratinocyte cell line. The human epidermoid cell line KB cells were either with or without transduction of the MSCV 2.1 LTR retrovirus encoding the human leukocyte PAFR and differentiated as KBM cells (without PAFR) and KB cells (with PAFR). KB and HaCaT cells were cultured in DME/F12 high glucose with 10% FBS.

MVP analysis: MVPs were collected from cell medium and isolated by differential centrifugation. MVP concentration was measured using a Nanosight NS300 instrument.

Results

Figure 1. Mechanisms of PAFR and MAPK associated MVP release under UVB exposure. UVB induced generation of PAF and other PAFR agonists which activates PAFR and induces MVP formation and release.

Figure 2. UVB and CPAF induced MVP release in HaCaT and NTERT cells. Cells were either treated with no treatment, 200 ng/ml CPAF and UVB and then incubated for 4 hours. For both treated groups (CPAF and UVB) there was a significant increase in MVP release compared to the no treatment group. The data depicted are mean ± SD for per ml per 100,000 cells. Groups were compared using one-way ANOVA. Differences in samples were considered significant with P value less than 0.05. P<0.05 (*), P<0.01 (**).

Figure 3. MVP release from primary keratinocytes. Cell culture plates either received no treatment, vehicle, CPAF (53.9 ng) or 3,600 J/m2 UVB and were incubated for 4 hours. For both UVB and CPAF treatment, there was a significant increase in MVP release compared to no treatment and vehicle groups. The data depicted are mean ± SD for MVP per ml per 100,000 cells. Groups were compared using one-way ANOVA. Differences in samples were considered significant with P value less than 0.05. P<0.05 (*).

Figure 4. MVP release in cells that express the PAFR (KB) and cells that do not express PAFR (KBM). KB and KBM cells either received no treatment, CPAF (53.9 ng) or 3,600 J/m2 UVB and then incubated for 4 hours. In KB cell line all treatment groups (CPAF, UVB, KB) with CPAF, UVB, and KB cells were cultured in DME/F12 high glucose with 10% FBS.

MVP analysis: MVPs were collected from cell medium and isolated by differential centrifugation. MVP concentration was measured using a Nanosight NS300 instrument.

Figure 5. Effect of CPAF-antagonist (WEB 2086) on CPAF-induced MVP release in HaCaT cells. Cell culture plates were pre-incubated with WEB 2086 or vehicle 30 minutes before treatments. Cells either received no treatment, CPAF (53.9 ng) or TRA (6.48 ng) and then incubated for 4 hours. As expected the CPAF treated group was blocked by the pre-incubation with the PAFR antagonist. The data depicted are mean ± SD for per ml per 100,000 cells. Groups were compared using one-way ANOVA. Differences in samples were considered significant with P value less than 0.05. P<0.001 (**). **

Figure 6. Effect of inhibitors on CPAF-induced MVP release in HaCaT cells. The various inhibitors [1.64 µg PDDC, 2.67 µg PDD8059, 0.22 µg SP600125, 3.77 µg SB203580, 0.52 µg Y-27632, or 11.22 µg Z-VAD-FMK] were added 3 hours before CPAF treatment (53.9 ng). Cells were then incubated for 4 hours. The addition of the NF-kB inhibitor PDDC, ERK kinase inhibitor PDD8059, JNK inhibitor SP600125, p38 MAPK inhibitor SB203580, and ROCK inhibitor Y-27632 all blocked MVP release after CPAF treatment (4 h). CPAF induced MVP release was not affected by the NF-kB inhibitor (PDDC), ERK kinase inhibitor (PDD8059, and a general caspase inhibitor Z-VAD-FMK (a, b, f). The data depicted are mean ± SD for MVP per ml per 100,000 cells. Groups were compared using one-way ANOVA. Differences in samples were considered significant with P value less than 0.05. P<0.05 (*), P<0.01 (**), and P<0.001 (**). **

Figure 7. Effect of inhibitors on UVB-induced MVP release in HaCaT cells. The various inhibitors were added 1 hour before UVB treatment (3,600 J/m2) and cells were then incubated for 4 hours. The addition of the JNK inhibitor (SP600125), p38 MAPK inhibitor SB203580, and ROCK1 inhibitor Y-27632 all blocked MVP release after UVB treatment (4 h) UVB induced MVP release was not affected by the NF-kB inhibitor (PDDC), ERK kinase inhibitor (PDD8059), and a general caspase inhibitor Z-VAD-FMK (a, b, f). The data depicted are mean ± SD for MVP per ml per 100,000 cells. Groups were compared using one-way ANOVA. Differences in samples were considered significant with P value less than 0.05. P<0.05 (*), P<0.01 (**), and P<0.001 (**). **

Figure 8. CPAF- and UVB-induced MVP release are inhibited by aMase inhibitor (EpiLife) in HaCaT cells. Cells were pre-incubated with EpiLife (6.34 µg) or vehicle 1 hour before treatments. Then, the cells either received no treatment, CPAF (53.9 ng) or 3,600 J/m2 UVB and incubated for 4 hours. For both UVB and CPAF treated groups, imipramine significantly inhibited the stimulus induced MVP release. The data depicted are mean ± SD for MVP per ml per 100,000 cells. Groups were compared using one-way ANOVA. Differences in samples were considered significant with P value less than 0.05. P<0.05 (*), P<0.01 (**), and P<0.001 (**). **

Summary

1. Both UVB and CPAF induce MVP release in epithelial cell lines, and UVB-mediated MVP release is PAF dependent.
2. MVP release induced by UVB and CPAF requires aMase activity.
3. UVB and CPAF mediated MVP release share common and distinct pathways via various MAPK signaling pathways.