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

UVB-induced MVP release via various skin-derived epithelial cell lines has been reported to be mediated by the release of bioactive molecules (e.g. cytokines and lipids) from resident skin epithelial and immune cells. Our lab has previously demonstrated that UVB exposure stimulates the production of an inflammatory phospholipid activator, platelet-activating factor (PAF), and the oxidized analogues, 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. Microvesicles (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 stressors require sphingomyelin-catalysed activity of acidic sphingomyelinase (aSMase) and kinase-mediated signaling cascades for MVP release. In this study, both UVB and CMF (PAF 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 vitro, immunoprecipitation of aSMase and release of methyl-PAF were utilized to determine if UVB-induced MVP release suggests that aSMase activity is required for these responses. Interestingly, CMF-mediated MVP release was suppressed by NF-kB, ERK 1/2, JNK, p38 MAPK, and ROCK1 small molecule inhibitors, while UVB-MVP release was only suppressed by the JNK, p38 MAPK, and ROCK1 inhibitors. These findings suggest common and distinct pathways between the two stimuli. Subsequently, this study has confirmed the role of PAF, lipid remodeling, and robust kinase signaling in UVB and PAF-MVP formation. The targeting of such mechanisms of UVB-mediated MVP release could have potential therapeutic benefits in mitigating UVB-induced acute inflammation and systemic immunosuppression.

Materials and Methods

Cell culture: NTER cells were telomerase-immortalized primary human keratinocytes and cultured in supplemented EpiLife medium. Primary keratinocytes were obtained from surplus human skin from de-identified donors undergoing abdominoplasty surgery and cultured in supplemented EpiLife medium. HaCaT cells are a spontaneously immortalized keratinocyte cell line. The human epidermal cell line KB cells were either with or without transduction of the MCV 2.1 vector expressing the human leukocyte PAFR and differentiated as KBM cells (without PAFR) and KB cells (with PAFR). KB and HaCaT cells were cultured in DMEM 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.

Objective

To study the potential mechanisms involved in UVB and CMF mediated MVP release in skin derived epithelial cell line.

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 CMF induced MVP release in HaCaT and NTERT cells. Cells were either treated with no treatment, CMF (53.9 ng) or 3.600 mJ/m2 UVB and then incubated for 4 hours. For both treated groups (CMF and UVB) there was a significant increase in MVP release compared to the no treatment group. The data depicted are mean ± SE 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 3. MVP release from primary keratinocytes. Cell culture plates either received no treatment, vehicle, CMF (53.9 ng) or 3.600 mJ/m2 UVB and were incubated for 4 hours. For both UVB and CMF treatment, there was a significant increase in MVP release compared to no treatment and vehicle groups. The data depicted are mean ± SE 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). KBP and KBM cells either received no treatment, PMA (61.68 ng), CMF (53.9 ng) or 3.600 mJ/m2 UVB and then incubated for 4 hours. In KBP cell line all treatment groups (PMA, CMF, UVB) had significant increase in MVP release compared to the no treatment group. However, in KBM cells only PMA treated group had significant MVP release. There was noted to be significant increase in MVP release in the absence of the PAFR for the CMF and UVB. The data depicted are mean ± SE 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 5. Effect of PAFR antagonist (WEB 2086) on CMF-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, CMF (53.9 ng) or TRA (61.68 ng) and then incubated for 4 hours. As expected the CMF treated group was blocked by the pre-incubation with the PAFR antagonist. The data depicted are mean ± SE MVP per ml per 100,000 cells. Groups were compared in using one-way ANOVA. Differences in samples were considered significant with P value less than 0.05. 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 mJ/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 (f). UVB induced MVP release was not affected by the NF-kB inhibitor (P208, ERK 1/2 inhibitor (PD98059), and a general caspase inhibitor (Z-VAD-FMK) (a, b, & g). The data depicted are mean ± SE 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<0.001 (***)

Figure 8. CMF- and UVB-induced MVP release are inhibited by aSMase inhibitor (imipramine) in HaCaT cells. Cells were pre-incubated with imipramine (6.34 µg) or vehicle 1 hour before treatments. Then, the cells either received no treatment, CMF (53.9 ng) or 3.600 mJ/m2 UVB and incubated for 4 hours. For both UVB and CMF treated groups, imipramine significantly inhibited the stimulus induced MVP release. The data depicted are mean ± SE 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<0.001 (***)

Summary

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