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Injury-Induced Microvesicle Particle Release in Human Skin Explant Tissue

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Abstract

Microvesicle particles (MVP) are 100-1000 nm vesicles that bud from the cell membrane and transmit intercellular signals locally and systemically via their bioactive cargo (e.g. cytokines). We have previously shown that thermal burn injury or UVB radiation result in the formation Platelet Activating Factor (PAF), and UVB or a PAF agonist (CPAF) can promote MVP formation in keratinocytes. Thermal burn and UVB radiation can result in systemic effects, even though their injury is localized to the skin. We therefore propose that PAF-producing injuries, UVB irradiation or thermal burn injury, result in the formation of MVPs that can potentially transport cytokines through the blood stream and induce systemic effects. This study, in-part, addresses that proposed mechanism by demonstrating increased MVP release after thermal burn injury in vivo and ex vivo. In vivo there was a significant increase in MVP concentration in mouse skin that received thermal burn injury compared to untreated skin. This demonstrates that there is an increase in MVP release after injury in living models. Human skin explants that received suction blisters to separate the epidermis from the dermis exhibited increased MVP release into the blister fluid in response to CPAF, UVB or thermal burn injury as compared to controls, suggesting epidermal MVPs are mobile and can exit the epidermis. Acid Sphingomyelinase (aSMase), an enzyme that breaks sphingomyelin into ceramide and phosphorylcholine, has been found to be involved in MVP release in other cell types. Utilizing an FDA approved aSMase inhibitor, Imipramine, we found that UVB, CPAF and thermal burn-mediated MVP release is completely dependent on aSMase in keratinocyte cell lines and human explant skin. These results suggest that PAF-producing skin injuries (thermal burn and UVB) can induce MVP release, and the release can be therapeutically inhibited by imipramine.

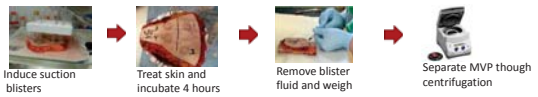
Methods

Mouse Model: C57BL/6 EGFP Wild type mice

Human Explant Tissue Model : Abdominoplasty, brechioplasty, and gynecomastia skin were graciously donated from Sycamore Medical Center (Miamisburg, OH). Patients (n=25) were between the ages of (23-65).
Treatments: Ø, Vehicle, .29 ng topical CPAF, 2,800 J/m² UVB, or 5 seconds thermal burn with 90° C water.

- **Punch Biopsies Method:**

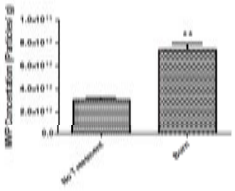
- **Blister Method:**



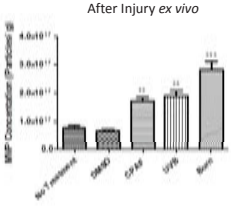
- **Inhibitors:** Inhibitors were applied topically in 90% DMSO/ 10% EtOH (1.64 µg PDTC, 2.67 µg PD98,059, 0.22 µg SP600125, 3.77 µg SB203580, 0.32 µg Y27632, 11.22 µg Z-VAD-FMK, or 1.58 µg imipramine). The inhibitors were incubated for 1 hour before proceeding to treatments.

Results: Punch Biopsy Method

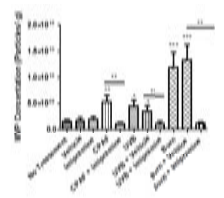
MVP Release Increases After Thermal Burn injury *in vivo*



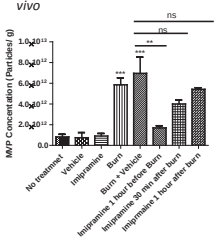
MVP Release Increases After Injury *ex vivo*



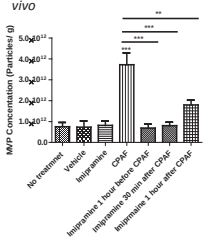
ASMase Inhibitor Blocks MVP Release *ex vivo*



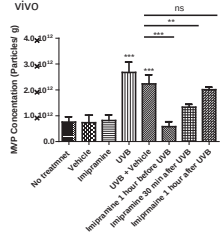
Post-Injury ASMase Inhibitor Effect on Burn Induced MVP Release *ex vivo*



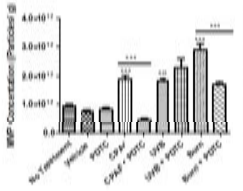
Post Injury ASMase Inhibitor Effect on CPAF Induced MVP Release *ex vivo*



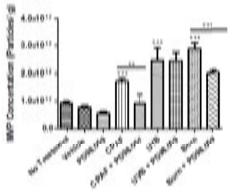
Post-Injury ASMase Inhibitor Effect on UVB Induced MVP Release ex vivo



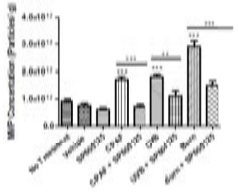
NF-kB Inhibitor Effect on Injury Induced MVP release ex vivo



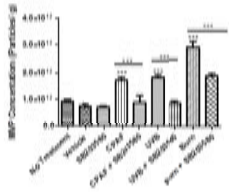
ERK1/2 Inhibitor Effect on Injury Induced MVP release ex vivo



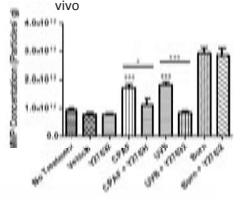
JNK kinase Inhibitor Effect on Injury Induced MVP release ex vivo



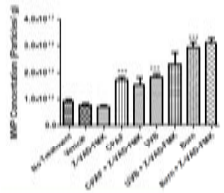
P38 Inhibitor Effect on Injury Induced MVP release ex vivo



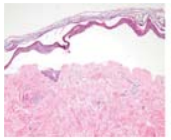
Rho kinase Inhibitor Effect on Injury Induced MVP release ex vivo



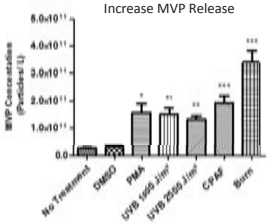
Caspase Inhibitor Effect on Injury Induced MVP release ex vivo



Results: Blister Method



Various Treatments Increase MVP Release



Conclusions

- There was a significant increase in MVP concentration in mouse skin after thermal burn injury.
  - This demonstrates that there is an increase in MVP release after injury in living models.
- CPAF, UVB and thermal burn injury on human ex vivo skin does increase the amount of MVP release compared to untreated skin.
- When the roof of a blister is damaged with thermal burn injury there is increased MVP in the blister fluid compared to untreated blisters.
  - This demonstrates that MVP were derived from the epidermis and are traveling to the dermis.
- Thermal Burn, UVB and CPAF seem to involve different mechanisms in MVP release.
  - For burn treatment this mechanism seems to involve NF-kB, ERK1/2, Jun-kinase, and p38.
  - For UVB treatment this mechanism seems to involve Jun-kinase, p38, and Rho kinases.
  - For CPAF treatment this mechanism seems to involve NF-kB, ERK1/2, Jun-kinase, p38, and Rho kinases.
- For all three treatments (CPAF, UVB, and thermal burn), the increase in MVP release seem to be completely dependent on aSMase.
- As these MVP carry bioactive substances that may be regulating systemic effects of UVB and thermal burn, aSMase inhibitors like imipramine may be a potential therapeutic agent to prevent these effects.

Acknowledgments

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