Topical administration of an endocannabinoid protects MRL/lpr from developing cutaneous lesions

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Abstract

Background/Purpose:
Cutaneous lupus erythematosus (CLE) affects ~75% of SLE patients and has a profound impact on quality of life. While the morbidity from CLE is significant, effective therapeutic options, including topical therapies, are limited. There is growing evidence that the manipulation of the endocannabinoid system has immunomodulatory activity which could be clinically translated to a broad range of cutaneous and systemic inflammatory diseases, although data is limited especially with respect to topical administration. In this study, local application of anandamide (AEA), a highly lipophilic endocannabinoid, was evaluated in MRL/lpr lupus prone mice, which spontaneously develop skin lesions similar to chronic CLE both clinically and histologically. To overcome known issues with respect to topical delivery of cannabinoids, nanoparticle encapsulation was employed.

Methods:
Nanoparticles with 125 nm radius were loaded with 4% AEA (AEA-np) and mixed with coconut oil (25% by weight). Starting at 10 weeks of age, female MRL/lpr mice were treated twice a week with interscapular application of the AEA compound without hair removal. Control groups of mice were treated topically with empty nanoparticles ("control treated") or received only coconut oil ("untreated"). Mice were regularly scored using a validated modified CLASI tool to assess the effect of AEA on cutaneous disease. At 20 weeks of age, mice were sacrificed and the skin harvested for histological analysis.

Results:
AEA-np treated mice had significantly improved skin lesions. Interestingly, this improvement was noted both where the nanoparticles were directly applied, as well as the face/snout region. At the time of sacrifice, AEA treated mice had significantly less macroscopic lesions (Figure 1A), which were scored blindly ($p<0.0001$) (Figure 1B). Moreover, significant histologic improvement was seen as well ($p<0.05$; Figure 1C). No differences were seen in systemic disease parameters, namely circulating anti-dsDNA antibodies and proteinuria levels. Immunofluorescent staining of skin sections to characterize cellular infiltration and mechanistic studies in vitro are in progress.

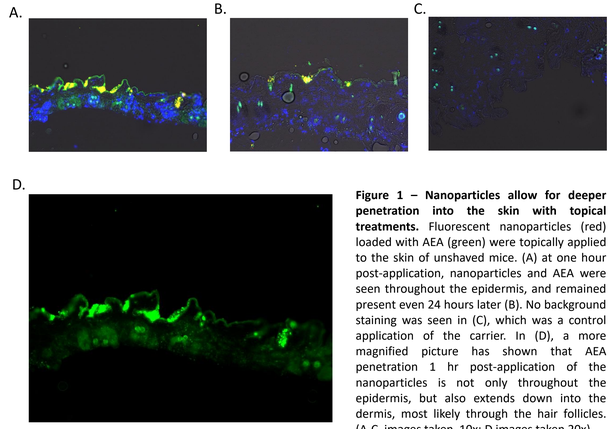

Figure 1 – Nanoparticles allow for deeper penetration into the skin with topical treatments. Fluorescent nanoparticles (red) loaded with AEA (green) were topically applied to the skin of unshaved mice. (A) at one hour post-application, nanoparticles and AEA were seen throughout the epidermis, and remained present even 24 hours later (B). No background staining was seen in (C), which was a control application of the carrier. In (D), a more magnified picture has shown that AEA penetration 1 hr post-application of the nanoparticles is not only throughout the epidermis, but also extends down into the dermis, most likely through the hair follicles. (A-C, images taken 10x; D images taken 20x)

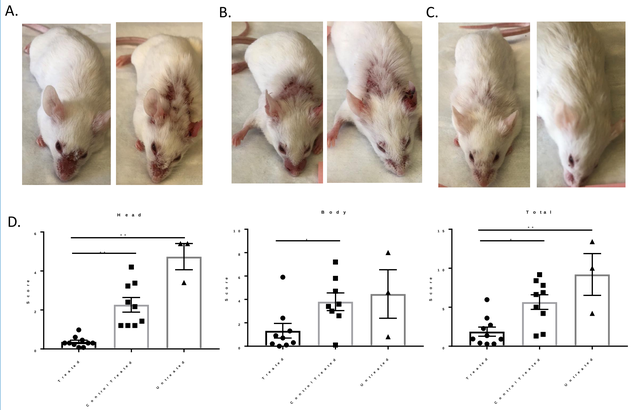

Figure 2 – AEA loaded nanoparticles prevent the formation of cutaneous lesions in MRL/lpr mice. (A) MRL/lpr mice develop spontaneous skin lesions both on their faces and their backs. (B) Twice weekly application of coconut oil and empty nanoparticles did not prevent the formation of skin lesions; however, (C) when the particles were loaded with AEA, we found significant improvement in the head and body lesions. (D) The lesions were scored for the different regions and totaled. Scores at sacrifice confirmed significant improvement with nanoparticle-AEA treatment. (untreated, n=3; control treated, n=10; treated, n=10)

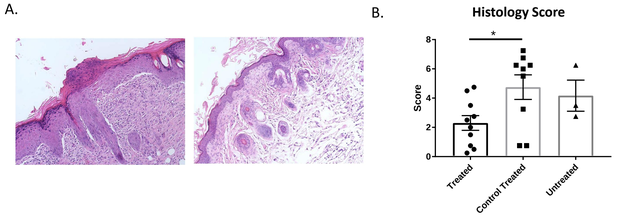

Figure 3 – Treatment with AEA-loaded nanoparticles improves skin histopathology. To confirm the results seen with improvements in macroscopic lesions, we had skin histology scored independently by two individuals blinded to the groupings. (A) Control treated mice developed histology characteristic of disease (A), including thickening of the epidermis and cellular infiltration, which was ameliorated with treatment (right). (C) The scoring confirmed a significant difference. (untreated, n=3; control treated, n=10; treated, n=10)

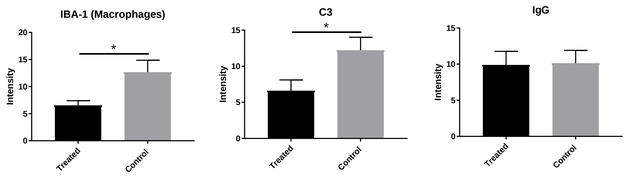

Figure 4 – Immunofluorescent staining of skin sections. Staining skin sections for macrophages (IBA-1), C3 and IgG, revealed that mice treated with AEA-loaded nanoparticles had significantly less IBA-1 cell accumulation, as well as significantly less C3 deposited in their skin when compared to control treated mice. There was no significant difference in IgG deposition. (untreated, n=3; control treated, n=10; treated, n=10)

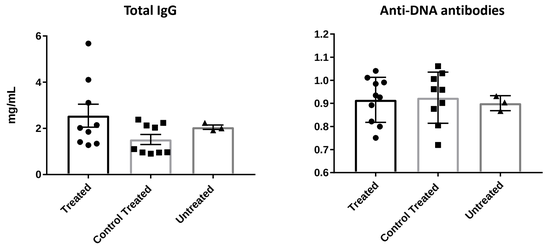

Figure 5 – Serum antibody levels. Terminal serum was analyzed by ELISA to determine levels of total IgG and anti-DNA antibodies. As shown, there was no significant difference between the various groups. (untreated, n=3; control treated, n=10; treated, n=10)

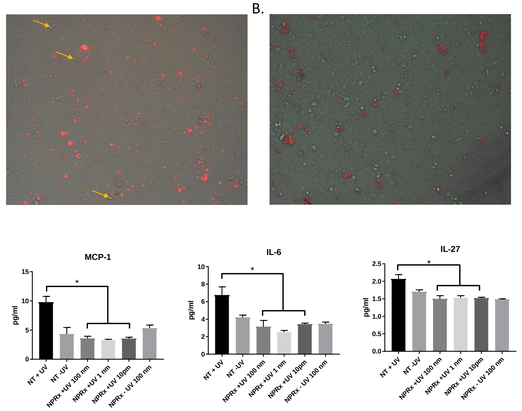

Figure 5 – *In vitro* effects of AEA-loaded nanoparticles. Immortilized keratinocytes (PAM212 cell line) were cultured to assess the effect of AEA-loaded nanoparticles on this cell type, which is the predominant cell of the epidermis. In lupus, UV injury to the keratinocytes is believed to be a major factor contributing to disease pathogenesis. We therefore exposed our cells to UV irradiation, and assessed the ability of AEA-loaded nanoparticles (NPRx) to prevent the release of inflammatory mediators. (A) First, we assessed the ability of the nanoparticles to target to the cells. As shown, within 1 hour of treatment, the nanoparticles (red) were seen colocalized with the cells (translight). (B) By 8 hours, we see that AEA (green) is now abundantly colocalized with the cells. (C) In a separate experiment, we pre-treated the cells with AEA-loaded nanoparticles and exposed them to UVB irradiation 1 hour later. After 24 hours, supes were collected. We assessed the supes for cytokines using Biolegend's Legendplex inflammatory assay. As shown there was significant reduction of several inflammatory cytokines relative to untreated cells. (All samples were run in triplicate)

Summary and Conclusions

Together, these data demonstrate that topical administration with AEA-loaded nanoparticles significantly prevents the development of CLE in an established animal model of lupus.

- AEA-loaded nanoparticles improves drug penetration
- AEA treatment prevents the development of skin lesions
- AEA treatment improved skin histopathology
- AEA treatment reduced macrophages and C3 accumulation
- Topical administration had no effect on antibody levels
- AEA reduces inflammatory cytokine secretion by keratinocytes

This work reinforces and highlights the utility of targeting the endocannabinoid system for autoimmune rheumatic diseases.