Topical Application of MS-275 Decreases the Imiquimod-Induced Hyperproliferative Epidermis and Interleukin-23 Expression in the Upper Dermis of BALB/c Mouse

Matthew H. Friedland, Emily A. Mann, Daniela N. Frankel, Hye Jin Chung, Jean S. McGee

Department of Dermatology, Boston University School of Medicine, Boston, MA, United States

Dear Editor:

Epigenetics refers to the study of genetic controls by factors other than DNA sequence\(^1\). Epigenetic regulation is accomplished by DNA methylation, histone modification, and non-coding RNA regulation\(^2\). Recently, epigenetic modifications have been explored extensively for their potential therapeutic applications\(^3\). In dermatology, vorinostat and romidepsin are the two histone deacetylase inhibitors (HDACs) currently available to treat cutaneous T-cell lymphoma\(^4\). However, given the systemic routes of administration, these compounds cannot be targeted to specific organs, resulting in unintended side effects. MS-275 is an epigenetic compound that inhibits class I HDAC1 and HDAC3. In fact, HDAC-1 is overexpressed in psoriatic skin compared to that in healthy skin\(^5\). Here, we aim to assess therapeutic potentials of MS-275 by characterizing its effects as a topical agent in the setting of imiquimod (IMQ)-induced hyperproliferative epidermis and interleukin (IL)-23 driven inflammation.

The animal study protocol was approved by the IACUC at Boston University under the protocol number, AN-15609. Given that IMQ induction of acanthosis and parakeratosis, hyperproliferative epidermis, and dermal IL-23 expression peaks at day 4 and 5 and starts to normalize despite continued IMQ application (unpublished work), we devised a co-treatment protocol as follows. At the start of each experiment, the backs of BALB/c female mice between the ages of 6 ∼ 7 weeks (The Jackson Laboratory, Bar Harbor, ME, USA) were prepared by shaving a small area (approximately 1 cm\(^2\)). Then, either vehicle or approximately 2.5 g equivalent of active IMQ 5% cream (SKU 050726; Henry Schein, Melville, NY, USA) was applied topically once daily for 5 days on the shaved area (Fig. 1A ∼ C and Fig. 1D ∼ L, respectively). Starting day 3, acetone (#534064; Sigma Aldrich, St. Louis, MO, USA), 0.1 μmole of Clobetasol dissolved in acetone (#C8037; Sigma Aldrich), or 0.1 μmole of MS-275 dissolved in acetone (in-house) was co-treated on the skin and continued for one additional day without co-treatment with IMQ (Fig. 1A ∼ F, Fig. 1G ∼ I, and Fig. 1J ∼ L, respectively). On day 7, the treated skin was harvested and processed for H&E (performed by the Skin Pathology Laboratory at Boston University) and immunostaining with anti-Ki67 antibody to detect actively proliferating cells and anti-IL-23 antibody. As compared to the control skin (Fig. 1A), the skin co-treated with IMQ and acetone (Fig. 1D) demonstrated significant acanthosis and parakeratosis. As compared to the control skin (Fig. 1B), the skin co-treated with IMQ and acetone (Fig. 1D) exhibited a significantly higher level of Ki67 staining in the epidermis. Lastly, using immunohistochemistry, a level of IL-23 expression was assessed with the IL-23 antibody. As compared to the control skin (Fig. 1C), the skin...
Fig. 1. Topical MS-275 attenuates imiquimod (IMQ)-induced acanthosis, hyperproliferative epidermis, and interleukin (IL)-23 dermal expression in BALB/c mouse. Either vehicle (A∼C) or IMQ (D∼L) was applied topically once daily for 5 days on the mouse skin. Starting day 3, acetone (A∼F), clobetasol dissolved in acetone (G∼I), or MS-275 dissolved in acetone (J∼L) was co-treated on the skin. On day 7, the skin was harvested for hematoxylin and eosin (H&E) stain (A, D, G, J), Ki67 immunostain (B, E, H, K), and IL-23 immunostain (C, F, I, L) (10×).

Co-treated with IMQ and acetone (Fig. 1F) demonstrated a significantly elevated level of IL-23 expression in the upper dermis.

Using H&E stain, we assessed the topical effects of MS-275 on acanthosis. The Clobetasol co-treatment significantly countered the IMQ induction of epidermal acanthosis (Fig. 1G vs. Fig. 1D). In comparison, the MS-275 co-treatment counteracted the IMQ induction of acanthosis to a much lesser degree (Fig. 1J vs. Fig. 1D). Using immunohistochemistry with the Ki67 antibody, we assessed the topical effect of MS-275 on the actively proliferating cells in the epidermis. The Clobetasol co-treatment almost completely reversed the IMQ-induced Ki67 staining (Fig. 1H vs. Fig. 1E). Similarly, the MS-275 co-treatment significantly countered the IMQ-induced Ki67 staining (Fig. 1K vs. Fig. 1E). Lastly, using immunohistochemistry with the IL-23 antibody, we assessed the topical effect of MS-275 on the IMQ-induced dermal expression of IL-23. The Clobetasol co-treatment significantly countered the IMQ-induced IL-23 expression in the upper dermis (Fig. 1J vs. Fig. 1F). Similarly, the MS-275 co-treatment countered the IMQ-induced IL-23 expression in the upper dermis (Fig. 1L vs. Fig. 1F).

To our knowledge, our study is the first to characterize the potential therapeutic effects of MS-275 as a topical agent in psoriasis-like dermatitis in the BALB/c mouse. In particular, MS-275 has been demonstrated to inhibit proliferation of human cutaneous squamous cell carcinoma cell lines. In addition to the anti-proliferative properties, pan HDAC inhibition can repress the production of IL-6, IL-10, IL-12p70, IL-23, and TNF-α. Therefore, topical MS-275 may have therapeutic implications in the hyperproliferative epidermis of and IL-23 driven inflammation in psoriasis. In recent years, the focus of drug development has gravitated towards introducing new biologics. However, given their rising costs and possible systemic side effects, topical medications can still offer unique benefits in areas where biologics fail. Even better, if we can develop topical medications that target specific pathways and prevent systemic side effects, we can widen the therapeutic options for our patients. Our study can serve as a basis for future investigation into developing novel topical epigenetic therapy.

ACKNOWLEDGMENT

We would like to thank Dr. Rhoda Alani and Dr. Vincent Falanga for mentoring this project. We would also like to thank the Skin Pathology Laboratory at Boston University School of Medicine for processing H&E stains.
CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

FUNDING SOURCE

We would like to acknowledge the Women’s Dermatologic Society for funding this research.

DATA SHARING STATEMENT

Research data are not shared.

ORCID

Matthew H. Friedland, https://orcid.org/0000-0001-9343-0314
Emily A. Mann, https://orcid.org/0000-0002-2293-5603
Daniela N. Frankel, https://orcid.org/0000-0002-1049-3342
Hye Jin Chung, https://orcid.org/0000-0003-0204-4085
Jean S. McGee, https://orcid.org/0000-0001-8490-0169

REFERENCES

1. Rodríguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. Nat Med 2011;17:330-339.
2. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. Circulation 2011;123:2145-2156.
3. Mervis JS, McGee JS. Epigenetic therapy and dermatologic disease: moving beyond CTCL. J Dermatolog Treat 2019;30:68-73.
4. Lee JJ, Murphy GF, Lian CG. Melanoma epigenetics: novel mechanisms, markers, and medicines. Lab Invest 2014;94:822-838.
5. Tovar-Castillo LE, Cancino-Díaz JC, García-Vázquez F, Cancino-Gómez FG, León-Dorantes G, Blancas-González F, et al. Under-expression of VHL and over-expression of HDAC-1, HIF-1alpha, LL-37, and IAP-2 in affected skin biopsies of patients with psoriasis. Int J Dermatol 2007;46:239-246.
6. Kalin JH, Eroglu A, Liu H, Holtzclaw WD, Leigh I, Proby CM, et al. Investigation into the use of histone deacetylase inhibitor MS-275 as a topical agent for the prevention and treatment of cutaneous squamous cell carcinoma in an SKH-1 hairless mouse model. PLoS One 2019;14:e0213095.
7. Song W, Tai YT, Tian Z, Hideshima T, Chauhan D, Nanjappa P, et al. HDAC inhibition by LBH589 affects the phenotype and function of human myeloid dendritic cells. Leukemia 2011;25:161-168.