SUPPRESSIVE EFFECTS OF TOPICAL NANOCRYSTALLINE SILVER IN ALLERGIC CONTACT DERMATITIS IN MICE

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Abstract

The objective of this study was to assess the anti-inflammatory effects of topical nanocrystalline silver using a mouse model of allergic contact dermatitis (ACD), and to study the possible mechanisms underlying this activity.

Dermatitis was induced on the ears of BALB/c mice using dinitrofluorobenzene. After the dermatitis was established, topical treatments including vehicles, 1% nanocrystalline silver in an emollient cream, tacrolimus ointment, and a high potency steroid cream were applied once a day for four days. Ear swelling was measured and the erythema was evaluated daily for four days. Ear samples were collected on the last day, for histological, immunohistochemical examination and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) staining.

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Abstract, continued

The ear swelling, erythema and histopathological inflammation in the no treatment group and vehicle-treated groups were not significantly reduced after four days of treatment. Compared to controls, significant reductions of ear swelling, erythema and histopathological inflammation were observed in the groups treated with 1% nanocrystalline silver, tacrolimus and high potency steroid after four days of treatment without any significant difference among them. TUNEL staining of the ear sections from the different groups demonstrated TUNEL-positive apoptotic cells. However, a significant increase in numbers of apoptotic cells was observed in the group treated with 1% nanocrystalline silver cream when compared to groups treated with vehicles, tacrolimus and a high potency steroid. The expression of IL-12 and TNF-α was significantly suppressed by 1% nanocrstalline silver, tacrolimus and high potency steroid.

This study suggests that nanocrystalline silver cream showed significant inhibitory activity against ACD in mice, similar to high potency steroid and tacrolimus. The nanocrystalline silver induces apoptosis of inflammatory cells, and suppresses IL-12 and TNF- α expression, which may be among the mechanisms by which nanocrystalline silver exerts its suppressive effects against inflammation in this model.

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Introduction

The incidence of inflammatory skin diseases, including atopic dermatitis and psoriasis, is increasing in adults and children. The mainstay of treatment for atopic dermatitis had been topical steroids. More recently, topical immunomodulatory drugs have been introduced. These available therapies often lack durable efficacy and/or are associated with adverse effects. Hence, there is a need for the development of a safe alternative treatment for inflammatory skin diseases. Nanocrystalline silver has been demonstrated to have exceptional antimicrobial and anti-inflammatory properties and has been successfully used in the treatment of burns and wound healing.

The objective of this study was to assess the anti-inflammatory effects of topical nanocrystalline silver using mice model of allergic contact dermatitis and to study the possible mechanisms underlying this activity.

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Materials and Methods

•Allergic contact dermatitis was induced in the ear of BALB/c mice using dinitrofluorobenzene.

•The dermatitis was treated once daily for four days, with topical nanocrystalline silver cream, high potency steroid (halobetasol propionate cream, 0.05%; Ultravate®), tacrolimus (0.1% Protopic® ointment) and appropriate placebo vehicles or no treatment.

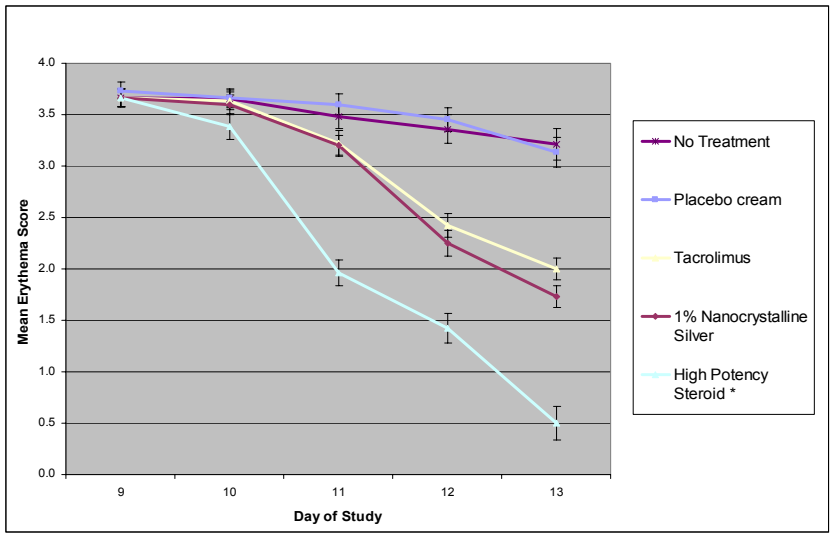
•Ear swelling and erythema was evaluated daily.

•After four days of treatment the mice were sacrificed and ear samples were collected for routine histology, immunohistochemistry, RT-PCR and TUNEL staining.

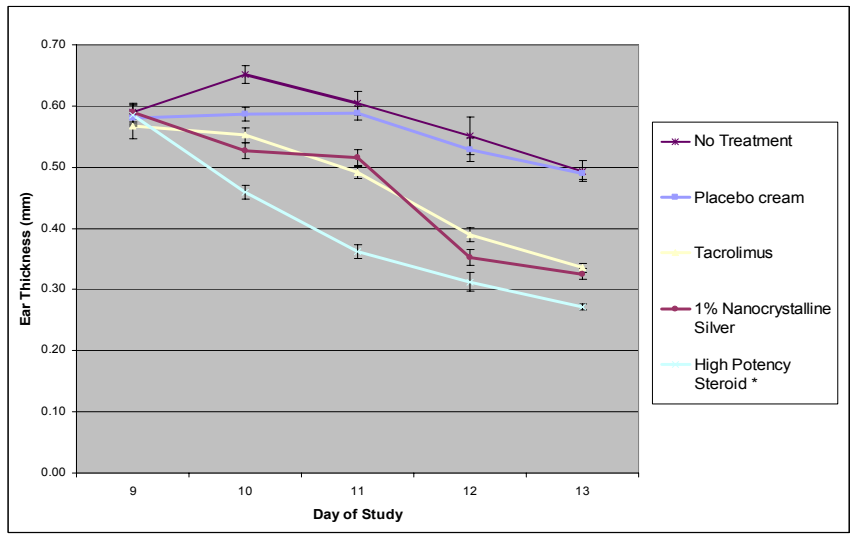
•Statistical significance of differences for edema, erythema, histopathological inflammation, cytokines expression, and presence of apoptotic cells, was assessed by Tukey-Kramer's multiple comparison test following ANOVA.

Results

Mean Ear Thickness (± SE; n=15-30) of Mice with Allergic Contact Dermatitis



Mean Erythema Score (± SE; n=15-30) of Mice with Allergic Contact Dermatitis



*Significant reduction ($p<0.05$) of body weight was observed in animals treated with high potency steroid, compared to other groups.

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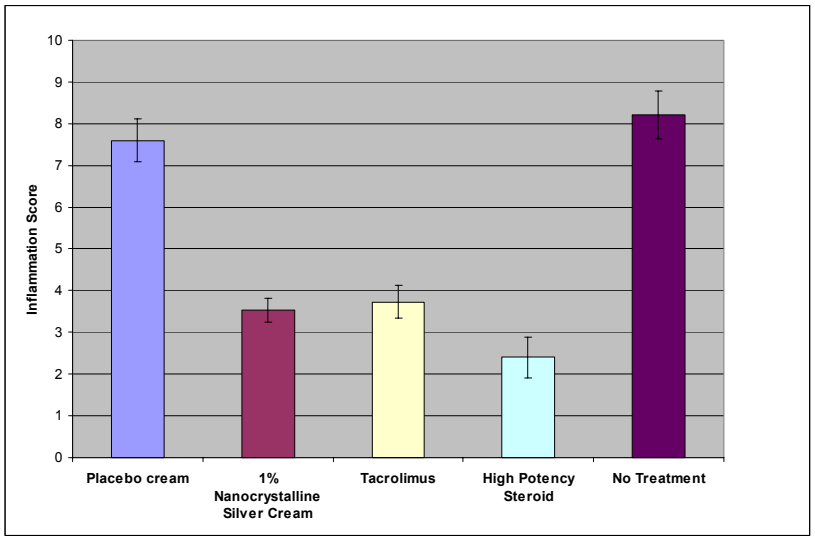
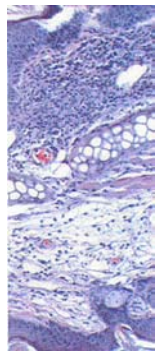
Mean Histopathological Scores (± SE, n=15) of Mice with Allergic Contact Dermatitis



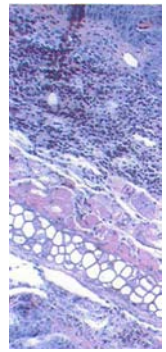
Inflammation in 3 distinct layers (Epidermis, Dermis and Subcutis), each evaluated on 0 – 4 scale.

H & E STAINING (X100)

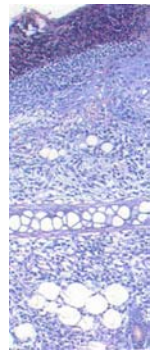
Placebo Cream



Placebo Ointment

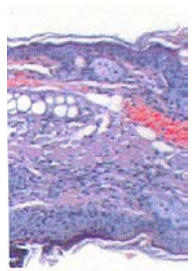


No Treatment

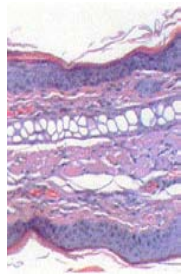


1% Nanocrystalline Silver

Tacrolimus



High Potency Steroid



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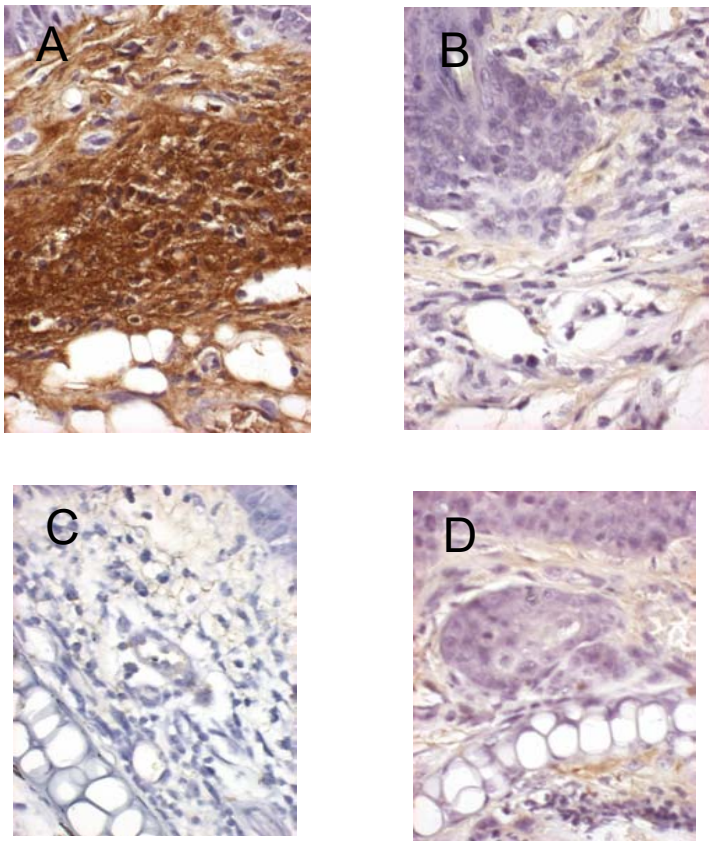
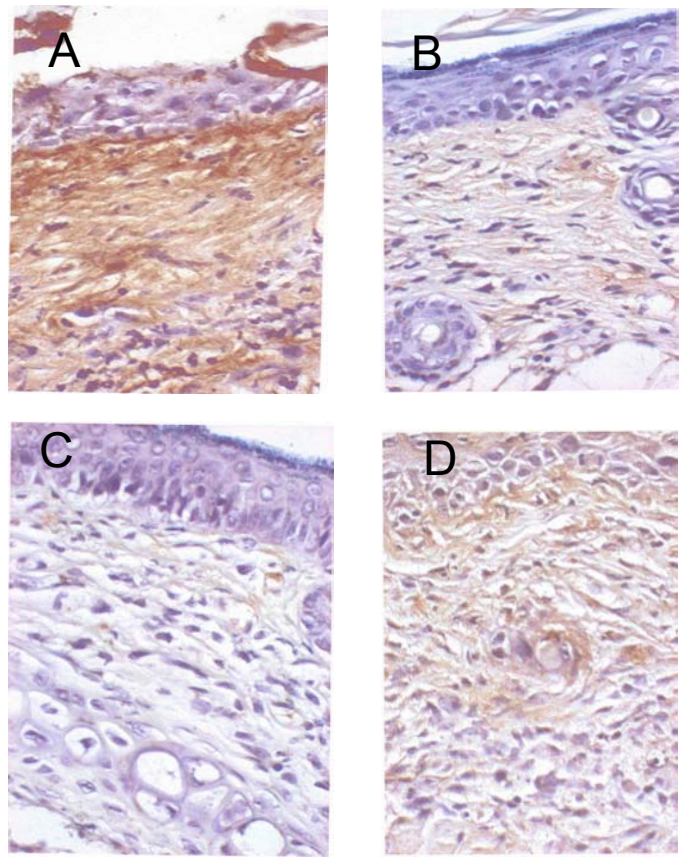
Immunohistochemistry

IL-12

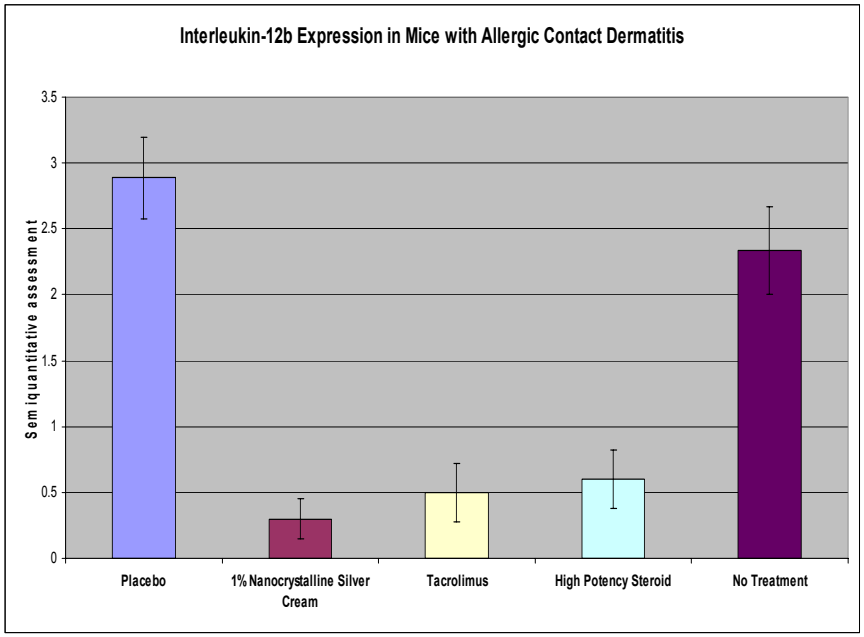


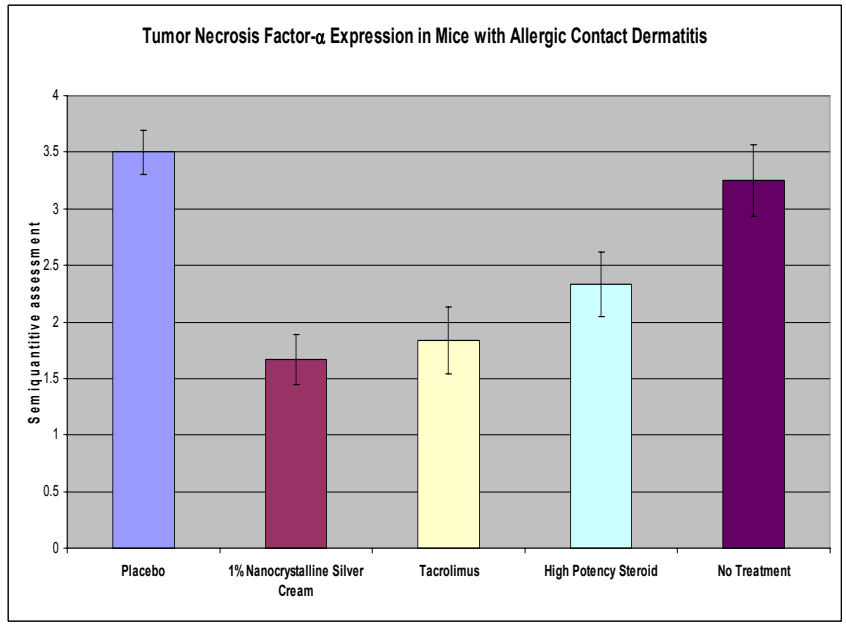
TNF-alpha



A, Placebo cream; B, 1% Nanocrystalline silver cream; C,Tacrolimus; D, High Potency Steroid. Note reduction of expression of both IL-12 and TNF-α by 1% Nanocrystalline silver, tacrolimus and high potency steroid.

Semi-quantitative Assessment of Immunohistochemical Staining





Semi quantitative assessment of staining of IL-12 and TNF-α was graded on a scale of 0 – 4. Mean ±SE; n = 15

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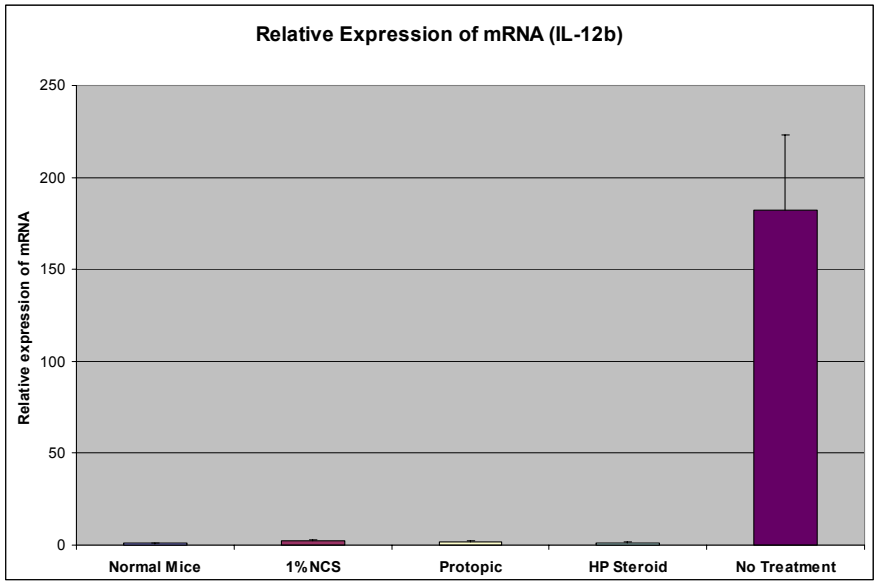
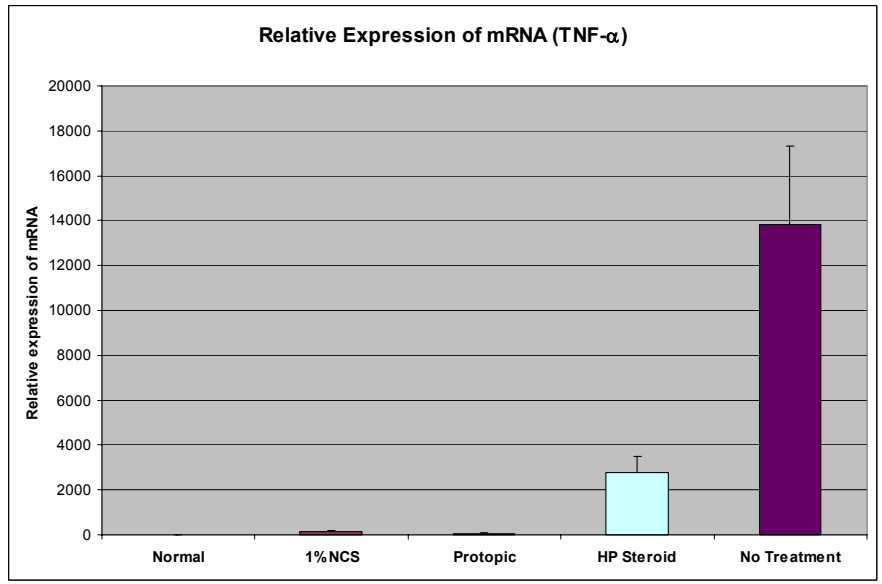
Relative Expression of m-RNA by RT-PCR; Mean ±SE; n = 5





The calculation of the gene expression was made using the corresponding C_T (Threshold cycle) values, according to the description in the TaqMan- Cytokine Gene Expression Protocol (Applied Biosystems)

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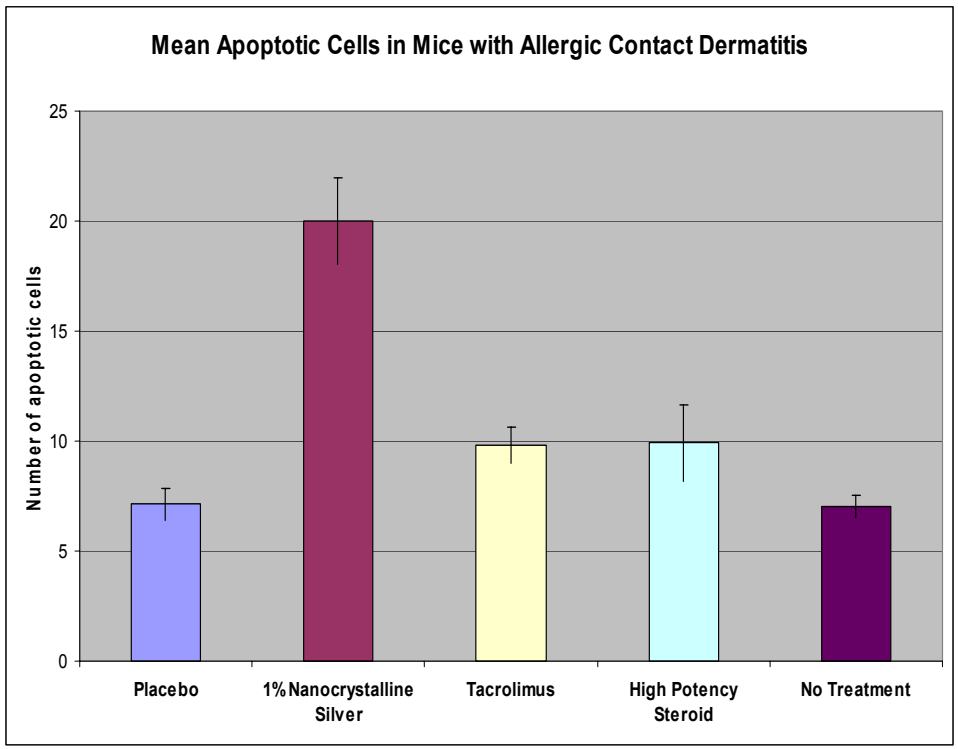
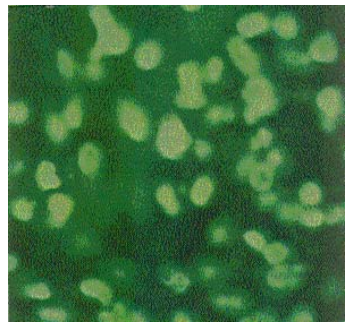
Mean ± SE, Number of apoptotic cells per microscopic field (X400)



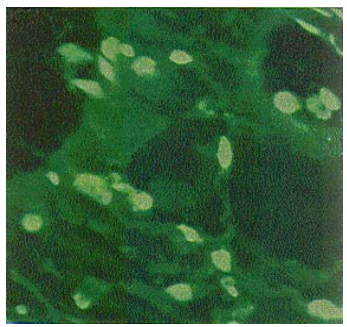
TUNEL Staining

Normal Tissue

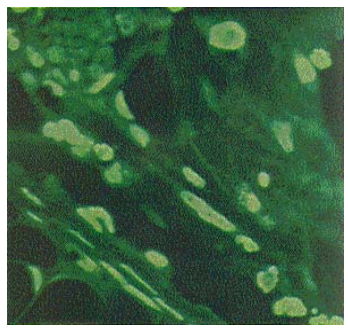
1 % NCS



Tacrolimus



High Potency Steroid



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Conclusion

- Topical nanocrystalline silver, tacrolimus and high potency steroid significantly suppressed the edema and erythema in mouse ears with allergic contact dermatitis.

- In this model, high potency steroid showed the anti-inflammatory effect more rapidly than nanocyrstalline silver and tacrolimus. However, high potency steroid significantly reduced body weight during the treatment period.

- This preliminary study demonstrates that nanocrystalline silver may induce apoptosis of inflammatory cells in the allergic contact dermatitis in mice.

- The expression of IL-12 and TNF-α was significantly suppressed by 1% nanocrystalline silver, tacrolimus and high potency steroid.

- This study suggests that the acceleration in resolution of ACD by nanocrystalline silver cream is associated with increased incidence of apoptosis of inflammatory cells as well as suppression of TNF-α and IL-12.

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