

New AN0128 Formulations Improve *In Vitro* Skin Penetration and *In Vivo* Efficacy

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ABSTRACT

The purpose of this investigation was to determine if new AN0128 formulations could improve *in vitro* skin penetration and *in vivo* efficacy in an animal model of inflammation. AN0128, a novel compound with antibacterial and anti-inflammatory activity, is in development for the topical treatment of acne, atopic dermatitis, and psoriasis. The original cream formulations (Cream, 1% and Cream, 5%) contain AN0128 at 1% and 5% w/w. New ointments and creams were manufactured with AN0128 at 5% w/w. A percutaneous absorption study was performed using human cadaver skin mounted in Franz diffusion cells. Samples were collected at 5 time points from the receiver well and at 48 h from tape strip, epidermis, dermis, and surface wash following a 5 $\mu\text{L}/\text{cm}^2$ dose. The ONT-0001 and CRM-0005 formulations demonstrated high penetration (4.7-6.3% of applied dose) through the skin and good deposition in the dermis and epidermis (0.03-0.44% and 2.0-2.7%, respectively). Male CD-1 mice were treated on the anterior and posterior surfaces of the ear (20 mg/ear) at 30 min before and 15 min after topical challenge with phorbol 12-myristate 13-acetate. Ear swelling was measured with a micrometer at 6 h after challenge. Whereas the original 1% and 5% cream formulations reduced ear edema by 2-10%, the ONT-0001 and CRM-0005 formulations reduced ear edema by 50% and 35%, respectively. AN0128 original cream formulation has demonstrated efficacy in early phase acne and atopic dermatitis clinical trials. ONT-0001 and CRM-0005 will be evaluated in a psoriasis microplaque assay to determine if these new formulations with greater skin penetration and *in vivo* efficacy demonstrate improved clinical efficacy. In conclusion, new AN0128 formulations demonstrated increased flux and increased dermal, epidermal, and tape strip concentrations and also demonstrated good efficacy in a mouse inflammation model.

METHODS

Skin Penetration. Cryopreserved, split-thickness (~0.25 mm) human cadaver trunk skin was cut to fit 1.0 cm^2 Franz diffusion cells. The dermal chamber was filled with phosphate-buffered saline, and the epidermal cell was left open to ambient laboratory conditions. The dermal bathing solution was stirred magnetically at approximately 600 RPM and the skin surface temperature maintained at $32.0 \text{ }^\circ\text{C} \pm 1.0 \text{ }^\circ\text{C}$. The integrity of each skin section was verified by determining its permeability to tritiated water. All formulations were applied to the skin sections at 5 $\mu\text{L}/\text{cm}^2$. Receptor solution samples were collected at 4, 8, 12, 24, and 48 h after dosing. In Figure 1, flux values are plotted at time points reflecting the midpoint between collection times. After the last sample was collected, the surface was washed twice with acetonitrile. Following the washes, the skin was tape stripped up to 10 times. The skin section was then removed from the chamber, split into epidermis and dermis, and extracted with acetonitrile. All samples were analyzed by LC/MS/MS.

Phorbol-Ester (PMA)-Induced Ear Edema. Ear swelling was induced by topical application of phorbol ester 12-myristate 13-acetate (PMA) to the right ears of male CD-1 mice. The left ears were untreated controls. Vehicle and test substances were applied 30 min before and 15 min after PMA challenge at a volume of 20 $\mu\text{L}/\text{ear}$ or 20 mg/ear. Five mice were used for each treatment group. Dexamethasone, in a vehicle of ethanol:acetone (1:1), was used as the positive internal reference control. AN0128 was tested in nine different formulations. PMA (4 $\mu\text{g}/20 \mu\text{L}$ of acetone) was topically applied to the anterior and posterior surfaces of the right ear of each animal. A control group was treated with PMA only. Six h after the PMA challenge, the thickness of the right and left ears was measured using a micrometer gauge. Inhibition of $\geq 30\%$ was considered significant anti-inflammatory activity.

CONCLUSIONS

- New ointment and cream formulations of AN0128 demonstrate increased skin penetration and increased *in vivo* anti-inflammatory activity.
- AN0128 is currently being evaluated in a clinical trial for efficacy in a psoriasis microplaque assay using two of the new formulations (ONT-0001 and CRM-0005).

FIGURE 1. Percutaneous absorption of AN0128 through human cadaver skin over 48 h from a single application (n=3 donors).

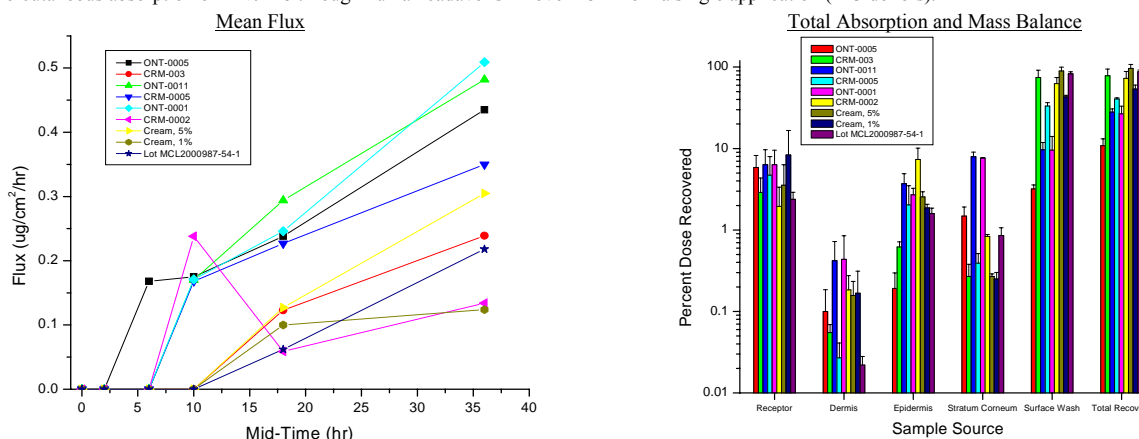


FIGURE 2. Effect of AN0128 on inhibition of PMA-induced ear edema in male CD-1 mice. Values (mean \pm SEM, n=5) shown are ear thickness minus baseline thickness (ear without PMA treatment). Percent inhibition of induced edema shown above bars in red.

