

Nail Penetration and Nail Concentration of AN2690, a Novel Broad-spectrum Antifungal Agent in Development for the Topical Treatment of Onychomycosis

K.M. Hold¹, V. Sanders¹, W. Bu¹, S.J. Baker¹, J.J. Plattner¹, K.R. Maples¹, C. Wheeler¹, T. Jones², K. Beutner¹

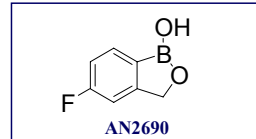
1. Anacor Pharmaceuticals, Inc., 1060 East Meadow Circle, Palo Alto, CA 94303, USA; 2. J&S Studies, 3201 University Dr. East, Suite 475, Bryan, TX 77802, USA

OBJECTIVES

- Determine the effect of formulation strength on *in vitro* nail penetration of AN2690
- Define systemic exposure when AN2690, 7.5%, was applied to all 10 toenails of subjects with moderate to severe onychomycosis (absorption study)
- Determine AN2690 nail retention three months after dosing in the subjects from the absorption study

1. INTRODUCTION

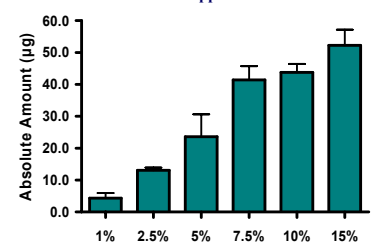
Onychomycosis, a common fungal infection of the toenails, remains difficult to treat,¹ probably because current therapies have poor penetration throughout the nail unit.² AN2690, a novel boron-containing small molecule designed to penetrate nails, has broad spectrum antifungal activity and is in clinical trials to treat onychomycosis topically.



2. EFFECT OF AN2690 FORMULATION STRENGTH VERSUS *IN VITRO* PENETRATION

Human cadaver fingernails were rehydrated overnight on dampened gauze at 32 °C. AN2690 was formulated in a nail lacquer at concentrations of 1, 2.5, 5, 7.5, 10 and 15% w/v. A single dose of 2µL was applied to the top of human cadaver finger nails mounted on poloxomer gel supports and left for 24 hours. At study end, the poloxomer gels were analyzed by LC/MS/MS for AN2690. The results are shown in Figure 1. We found the amount of AN2690 penetrating into the poloxomer support increased linearly as strength increased to 7.5% w/v. Above this strength, the amount of AN2690 found leveled off, indicating that 7.5% w/v is the optimal strength *in vitro*. This 7.5% formulation was selected for topical dosing in the clinical absorption study.

FIGURE 1. Amounts of AN2690 found under the nail plate versus strength of applied dose



3. EXTRACTION OF AN2690 FROM NAIL CLIPPINGS

AN2690 (20 µg) was spiked onto 10 mg of nail clippings. Samples were digested with 2 mL of 1N NaOH overnight at 45 °C after which the pH was adjusted to 3.0. Samples were extracted with methylenechloride and contained an internal standard. After extraction, the organic layer was evaporated and samples were reconstituted in acetonitrile for LC/MS/MS analysis. The limit of detection for AN2690 from the nail clippings was 25 ng/mg of nail. Recovery from spiked nail clippings for AN2690 was 89 ± 3% (n=3)

4. ABSORPTION TRIAL DESIGN: 7.5% AN2690, DAILY DOSING FOR 28 DAYS

- Fifteen patients enrolled with moderate to severe onychomycosis
 - Onychomycosis involving > 80% of both great toenails, as determined by visual inspection after the nail has been trimmed
 - Great toenail possess a combined thickness of the nail plate and nail bed > 3 mm
- All ten toenails were treated daily in the clinic
- At least 200 µl (for both feet)/patient/day of 7.5% AN2690 solution was applied for 28 consecutive days
- Blood PK Samples were taken on day 1, 14 and 28 before dose and 0.25, 0.5, 1, 2, 3, 4, 6 and 8 hours after dosing
- Nail clippings were taken starting with right foot before dosing, left foot at day 1, subsequently alternating at days 14, 28, 42, 84, 120 and will continue at 5, 6, 8, 10 and 12 months
- KOH and culture samples were taken from each foot before, day 14, 28, 42, 84, 120 and will continue at 5, 6, 8, 10 and 12 months
- Twelve of the fifteen patients elected to participate in the follow-up beyond day 84

5. ABSORPTION TRIAL: PLASMA AND NAIL ANALYSIS

- Plasma and nail samples were analyzed for AN2690 by LC/MS/MS
 - Blood samples were analyzed by a GLP validated method
 - LLOQ (lower limit of quantitation) in human plasma is 25 ng/mL
- Nail clippings were weighted, dissolved in 1N NaOH, followed by liquid-liquid extraction
 - LLOQ in nail clippings 25 ng/mg based on 10 mg of sample
- Mycology
 - Scrapings were taken from left and right great toenail
 - KOH was determined by the Calcofluor test or regular KOH test
 - Cultures were grown for dermatophytes

6. AN2690 IS UNDETECTABLE IN HUMAN PLASMA AFTER TOPICAL DOSING OF ALL TOENAILS

Drug levels were below the LLOQ at all timepoints, on all three days, in all fifteen patients
No adverse events occurred during the 28 day treatment period

FIGURE 2: AN2690 is Retained in the Nail in all Twelve Subjects Three Months after Dosing (Other timepoints are being collected)

- One Month of Dosing**
 - Day 28 concentration
 - 28.8 ± 22.0 µg/mg
 - This concentration results in
 - ~4,793x MIC₉₀ *T. rubrum*
 - ~600x MFC *T. rubrum*
- Three Months After Dosing**
 - Day 120 concentration
 - 0.97 ± 0.96 µg/mg (range 0-2.5 µg/mg)
 - This concentration results in
 - ~160x MIC₉₀ *T. rubrum*
 - ~20x MFC *T. rubrum*

At day 120, 10 out of 12 subjects had nail levels above the MFC *T. rubrum*

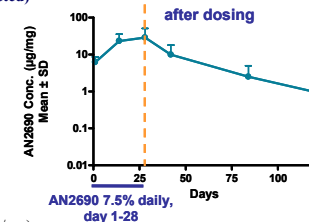


FIGURE 3: Ten out of Twelve Patients had AN2690 Nail Concentrations above the MFC *T. rubrum* Three Months after Dosing

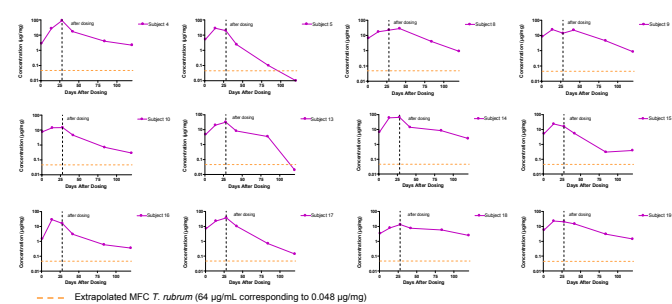


FIGURE 4. AN2690 Nail Concentrations Compared with Terbinafine and Ciclopirox

AN2690 Levels in Toe Nail Clippings Eight Weeks After Dosing Are 2,000X Higher than Terbinafine Levels

AN2690, Topical 7.5% daily, 4 weeks

Terbinafine, Oral 250 mg daily, 12 weeks
Schatz F. (1995) Clin Exp Derm 20; 377-383

AN2690 Levels in Toe Nail Clippings 14 Days After Dosing Are >10X Higher than Ciclopirox Levels

AN2690, Topical 7.5% daily, 28 days

Ciclopirox, 8% daily, 45 days
Summary basis of approval

- At Day 28/30 AN2690 Conc. in nails ~5x higher compared to Ciclopirox
- Fourteen Days after last dose, AN2690 levels >10x Higher than Ciclopirox

CONCLUSIONS

- In Vitro* penetration of AN2690 increases in a linear manner with formulation strength, up to 7.5%
- Absorption Study
 - There is no detectable systemic exposure after maximal dosing (LLOQ 25 ng/mL)
 - High levels of drug are achieved in the nail plate
 - No adverse events occurred during the 28 day treatment period
 - Therapeutic levels of drug remain in the nail plate at least twelve weeks after the last dose (more time points are being collected)
 - All cultures were negative at the end of the 28 days of treatment (more time points are being collected)