Development of pharmaceutical products to treat diseases of the nail: an industrial perspective

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Outline

• Anatomy and physiology of the nail
• Nail disease
• Interactions between formulations and the nail unit
• Principal treatments for onychomycosis
• Formulation development for pharmaceutical topical products to treat fungal infections of the nail
  – Formulation selection and development processes
  – Nail lacquers: composition development
  – Performance testing
    • Application of techniques to support formulation characterization
    • Application of techniques to support formulation development and optimization
• Conclusion
  – Formulation selection and development process for onychomycosis treatments
ANATOMY AND PHYSIOLOGY OF THE NAIL

**Nail anatomy**
- The nail unit can be divided into several parts including the following:
  - Nail plate
  - Nail folds
  - Nail bed
  - Nail matrix

**Nail physiology**
- Nails are flattened, elastic structures of a horny texture
- Nail plate is the largest component of the nail apparatus.
- It is composed of up to 901 layers of dead, keratinized, flattened cells, and is 0.25-0.6 mm thick on the fingers and up to 1.3 mm on the toes.
- Nails can be divided into three layers:
  - A thin, outer dorsal layer,
  - A thicker, highly fibrous intermediate layer (accounts for most of the nail’s thickness)
  - A ventral layer that connects the nail plate to the nail bed.
- The keratinized cells are tightly bound to each other by numerous intercellular desmosomes
- The nail plate consists predominantly of proteins organized into 10 nm filaments with an inter-filamentous matrix plus 10-30% water and ≤1% lipid

NAIL DISEASE

- **Onychomycosis (tinea unguium),** a fungal disease of the nail unit caused by yeasts, dermatophytes, or other molds (Trichophyton rubrum or T. mentagrophytes, rarely other trichophyton species or Epidermophyton floccosum).
  - Accounts for approximately 50% of all nail disorders in humans.
  - In about 80% of onychomycosis cases, the toenails are infected, whereas in the remaining 20%, the fingernails are infected.
  - The signs and symptoms of this disease include split, thickened, hardened, and rough nail plates, and partial separation of the nail plate from the nail bed creating an air gap in some areas.

- **Treatment options**
  - Oral medications which, rarely, can cause severe side effects including liver failure.
  - For advanced onychomycosis, especially if more than one nail is infected, systemic medication is preferred.
  - Mild onychomycosis sometimes responds to a combination of topical antifungal medication, sometimes applied as special medicinal nail lacquer, and periodic filing of the nail surface.

- **Principal challenge for topical therapies**
  - Inherent low permeability of keratinized nail plates*
INTERACTIONS BETWEEN FORMULATIONS AND THE NAIL UNIT

• The nail and surrounding structures are complex
• Common elements that are evaluated in support of formulation development include:
  – Nail plate
    – Possesses significant barrier properties related to the thickness and organization of the tightly bound keratinocytes within its three layers
    – Understanding of how topically applied actives permeate through, and interact with these layers can facilitate formulation design and support enhanced product performance.
    – Key factors include: API physicochemistry, Formulation, Disease state
  – Nail folds
    – Interaction of actives and formulations with the skin associated with these tissues should also be evaluated e.g., permeability and local tolerance.

PRINCIPAL PRODUCTS FOR THE TREATMENT OF ONYCHOMYCOSIS

• Loceryl Nail Lacquer (RoW ex US)
  – Amorolfine 5%
  – Genericized
• Penlac Nail Lacquer (US)
  – Ciclopirox, 8%
  – Genericized
• Jublia Topical Solution (US/Canada)
  – Efinaconazole, 10%
  – FDA Approval: June 9, 2014
  – Scheduled for launch Q3 2014
  – US Patents 8,039,494; 7,214,506
• Kerydin Topical Solution,
  – Tavaborole, 5%
  – FDA Approval: July 8, 2014
  – US Patents 7,767,657; 7,582,621
FORMULATION DEVELOPMENT FOR PHARMACEUTICAL TOPICAL PRODUCTS TO TREAT FUNGAL INFECTIONS OF THE NAIL:

Formulation selection and development processes

FORMULATION SELECTION: Decision Tree

Defined Formulation Space and TPP

Preformulation and Formulation Development

API released / delivered?

Tolerance assessed?

Target Defined?

Formula optimization?

Pharm. Criteria met?

Acceptable delivery data

Formulation well tolerated

API active at target

YES

NO

Acceptable data generated

YES

NO

YES

NO

YES

Reformulation

Optimization

Formulation selection for Non-clinical and Clinical studies

FORMULATION DESIGN / DEVELOPMENT

SCREENING

Release / Penetration (PK)

Tolerance

Pharmacodynamic (PD)

OPTIMIZATION
FORMULATION DEVELOPMENT PROCESS

• **TPP**
  - Desired Product requirements and performance characteristics (Medical and marketing)
  - Feasibility assessment
  - Bibliography
  - Evaluate challenges – solubility, stability, delivery, etc.

• **Preformulation**
  - Emphasis on support and development of the drug product
  - Saturated solubility
    - Single solvents
    - Mixtures
  - Impact of stabilizers
    - pH adjusters / Buffers
    - Antioxidants
    - Chelating agents
  - Compatibility and Stability
    - Chemical and physical analysis
      - Up to 3M: 5, 25, 40°C
      - F/T 5/40°C cycling
    - Degradation profiling and structural analysis (as necessary)

• **Formulation development - Screening**
  - Multiple concepts with varying components to reduce risk of potential unexpected incompatibilities
  - Formulation characterization
    - Macro and microscopic analysis
    - Drying test
    - Film quality: Hardness / Gloss
    - Surface tension and spreadability on to the nail plate
  - Stability
    - Chemical and physical analysis
    - Centrifugation, F/T, 5/40°C cycling
    - Up to 3M: 5, 25, 40°C
  - Selection for performance testing after 1M stability data
  - Preliminary Performance testing (optional)
    - Keratin binding
    - Nail Penetration

• **Formulation development – Optimization**
  - Performance testing
    - Keratin binding
    - Nail Penetration
    - Contact angle measurement
    - Microbiological efficacy
    - Confocal Raman
    - Tolerance

• **Formulation selection**
  - Lead and back-up formulations for Non-clinical and Clinical studies

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NAIL LACQUERS:

Composition development

NAIL LACQUER COMPOSITIONS

<table>
<thead>
<tr>
<th>Function</th>
<th>Trade Name</th>
<th>INCI Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>ETHANOL</td>
<td>Ethanol</td>
</tr>
<tr>
<td></td>
<td>ETHYL ACETATE</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td></td>
<td>BUTYL ACETATE</td>
<td>Butyl acetate</td>
</tr>
<tr>
<td>Film former</td>
<td>EUDRACIT RL 100</td>
<td>Acrylates / Ammonium Methacrylate Copolymer (E. P. Ammonio methacrylate copolymer type A)</td>
</tr>
<tr>
<td>Plasticizer</td>
<td>TRIACETIN</td>
<td>Glycerol triacetate</td>
</tr>
<tr>
<td>API</td>
<td>AMOROLFINE HCL</td>
<td></td>
</tr>
</tbody>
</table>

AMOROLFINE HCL
NAIL LACQUERS: COMPOSITION DEVELOPMENT

**Preformulation**

**SOLVENTS**
- API Solvents (volatiles vs. non-volatiles)
- Effect of stabilizers (Antioxidants, chelating agents etc.)

**Film Formers + Plasticizers**
- Acrylic derivatives
- Malic acid derivatives
- Glycols
- Esters

**Penetration Enhancers**
- Solubility enhancement
  - NMP, DMSO
- Surfactants
  - SLS, Non-ionic, Anphoteres
- Keratin disruption
  - Keratolytics: Urea, Salicylic acid
  - Reducing agents (Disulfide bonds): Thioglycolates, N-Acetyl cysteine, Mercaptoethanol
  - Oxidizing agents: H₂O₂

**Performability Testing:**
Application of techniques to support formulation characterization
**PRELIMINARY FORMULATION SCREENING**

Macro and microscopic evaluation

- Application of formulations onto glass slides followed by macroscopic and microscopic assessment: Film evaluation after drying (20 minutes)

### TERBINAINE SOLUTION 10%

<table>
<thead>
<tr>
<th>Function</th>
<th>INCI Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvents</td>
<td>Water/Aqua</td>
</tr>
<tr>
<td></td>
<td>Propylene glycol</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td>Viscosity enhancer</td>
<td>Hydroxyethylcellulose</td>
</tr>
<tr>
<td>Stabilizers</td>
<td>Butyl hydroxytoluene</td>
</tr>
<tr>
<td></td>
<td>Disodium EDTA</td>
</tr>
<tr>
<td>API</td>
<td>Terbinafine HCl</td>
</tr>
</tbody>
</table>

**FORMULATION CARACTERIZATION**

Drying test

- Evaluate formulation drying time at 32°C
  - IR radiation facilitates evaporation of volatiles
  - Formulation quantity = 48 µl (~17 µl/cm²)
  - Application surface = 2.8 cm²

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating program</td>
<td>Standard dessication</td>
</tr>
<tr>
<td>End of analysis</td>
<td>Duration or Constant weight</td>
</tr>
<tr>
<td>Weighing value resolution</td>
<td>1 mg</td>
</tr>
<tr>
<td>Results</td>
<td>Dry extracts (%R)</td>
</tr>
</tbody>
</table>

**Sartorius moisture analyzer Type MA100**

- EVAPORATION at 32°C
- Formulation: control 1

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Film hardness

- Nail lacquer films can be characterized by evaluation of hardness using Persoz pendulum test.
  - Film Hardness: resistance of a material to permanent deformation
  - Indication if lacquer has fully hardened following application (glass slide)
  - Test methods: French standard AFNOR NF T 30-016 or ISO1522

Film Gloss

- The gloss of nail lacquer films can also be characterized following application onto a contrast card.
- Predominantly a test for cosmetic nail lacquers
  - Test method: NF T 30-064

Performance Testing:

Application of techniques to support formulation development and optimization
### IN VITRO KERATIN BINDING STUDIES

**EFFECT OF AN AMPHOTERIC SURFACANT ON THE KERATIN BINDING OF FORMULATIONS CONTAINING 10% TERBINAFINE**

**Terbinafine hydrochloride**

Cocobetaine (Cocoyl sarcosine): a zwitterionic surfactant where R represents the alkyl groups derived from coconut oil.

<table>
<thead>
<tr>
<th>Ingredient (INCI Name)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Solvent</td>
</tr>
<tr>
<td>Disodium EDTA</td>
<td>Chelating agent</td>
</tr>
<tr>
<td>Hydroxyethylcellulose</td>
<td>Polymer</td>
</tr>
<tr>
<td>Cocobetaine</td>
<td>Surfactant</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Solvent</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Solvent</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Terbinafine HCl</td>
<td>API</td>
</tr>
</tbody>
</table>

- Formulation pH 3.6 due to Terbinafine hydrochloride, 10%.
- Cocobetaine utilized at a concentration between 1-10%.
- Cocobetaine was protonated under these conditions and anticipated to preferentially compete with the cationic active for negatively charged keratin binding sites.
- Identical compositions with and without Cocobetaine were compared.

### EFFECT OF AN AMPHOTERIC SURFACANT ON THE KERATIN BINDING OF FORMULATIONS CONTAINING 10% TERBINAFINE: RESULTS AND CONCLUSION

**IN VITRO KERATIN BINDING STUDIES**

**EFFECT OF AN AMPHOTERIC SURFACANT ON THE KERATIN BINDING OF FORMULATIONS CONTAINING 10% TERBINAFINE: RESULTS AND CONCLUSION**

- Addition of amphoteric surfactant, under the pH conditions of the formulation, enhances the free concentration of Terbinafine HCl, when incubated with powdered keratin.
- The presence of Cocobetaine was hypothesized to competitively inhibit the binding of Terbinafine HCl to keratins of the nail.
- Enhanced delivery of Terbinafine HCl to the nail bed when formulated with Cocobetaine were anticipated.
IN VITRO NAIL PENETRATION MODEL

• Parameters that can be evaluated:
  – Nail lacquer solvent screening
  – Film former screening
  – Penetration enhancer screening
  – Dose effects

Franz-type diffusion cell

Human nail plate

Receptor fluid

IN VITRO NAIL PENETRATION MODEL
STUDY DESIGN

Day 1
Day 2
Day 3
Day 4
Day 5
Day 1
Day 2
Day 3
Day 4

Sectioning (100 µm): Analyte distribution within nail

Dose: 10 µL of Loceryl nail lacquer per cm² containing [14C] Amorolfine HCl

Removal of residual formulation: Acetone soaked Cotton swab
In Vitro Human Nail Penetration of Amorolfine

Single application

Multiple application

No Amorolfine detected in receptor with 10µl dose and described experimental set up

DOSE RANGE STUDIES: AMOROLFINE

Methods

- Full thickness of nail measured and then mounted
- The receptor compartment was filled with previously sonicated PBS (0.172M, pH 7.3) containing 0.25% w/w Tween 80 at 32°C
- 100 µl of formulation was applied directly to the surface of the nail, exposed to the air for 10 minutes (to allow the lacquer to dry) before covering the cells with Parafilm (n=5 per formulation upon initiation).
- Samples (1 ml) of receptor fluid were removed at 1 h, 24 h, 48 h, 72 h, 96 h, 120 h, 168 h, 216 h and 264 h (11 days).

Conditions modified to facilitate screening process and formulation discrimination: Increased dose + duration
Objectives
- Evaluate the properties of the two layers of the nail, back and middle in terms of penetration and diffusion of Terbinafine HCl
- Assess the impact of an amphoteric surfactant (Cocobetaine) on the penetration and deposition of Terbinafine HCl in various nail layers

Methods
- Nail sourcing
  - Human cadaver nails free from damage and mycosis
- Thickness modification
  - Dremel sanding tool remove portions of either ventral or dorsal surfaces corresponding to 40% of the nail thickness
    - Ventral + intermediate (dorsal layer removed / modified)
    - Intermediate + dorsal (ventral layer removed, modified)
- Penetration studies
  - Duration: 5 days
  - Dose: 10 µl/cm² (once per day)
  - Receptor medium: 0.1% Volpo 20 in PBS
  - Surface wipe performed prior to re-dosing
    - Cotton swab soaked in 1% SLS in PBS
  - Nails microtomed into 300 µm sections prior to extraction

RESULTS

<table>
<thead>
<tr>
<th>Amount penetrated</th>
<th>Amount deposited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative amount Terbinafine-HCl (ng/cm²/mm)</td>
<td>Cumulative amount Terbinafine-HCl (ng/mg)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>10% Solution with amphoteric surfactant</td>
<td>10% Solution without amphoteric surfactant</td>
</tr>
<tr>
<td>285.24</td>
<td>232.24</td>
</tr>
<tr>
<td>107.98</td>
<td>92.54</td>
</tr>
<tr>
<td>10% Solution without amphoteric surfactant</td>
<td>10% Solution without amphoteric surfactant</td>
</tr>
<tr>
<td>325.54</td>
<td>303.50</td>
</tr>
</tbody>
</table>

Conclusion
- Ventral + intermediate layers (Intermediate) more permeable than Intermediate + dorsal (dorsal) layer.
- Lower deposition in the intermediate layer than the dorsal layer.
- Data indicates the denser dorsal layer represents a greater barrier for the Terbinafine HCl when applied in a solution formulation at 10% w/w.
- Amphoteric surfactant (Cocobetaine) enhanced penetration and deposition of Terbinafine HCl, potentially by reducing keratin binding.
Models of skin and nail infection using C. albicans, A. niger T. rubrum and T. mentagrophytes have been developed by MedPharm.

Model considers barrier properties of the nail (and the skin).

Provides data for formulation optimisation, selection and application.

Methods
- Placebo and active Control 1 (100 µL) and Amorolfine NF 7% and 10% w/v (100, 10, or 3X10 µL) tested against T. rubrum across 5 µm and full thickness nail samples
- Medium: Sabouraud dextrose agar
- Following formulation application flux measured by adapted ZOI

Encouraging results for 5 µm nail sections
CONCLUSION:

Application of techniques to support formulation development and optimization

Efficient formulation development for onychomycosis treatments requires understanding of:
- Nail anatomy
- Disease state
- API physicochemistry and how this can influence interactions with the nail and associated tissues

Consistent and robust evaluation and selection processes also maximizes potential for success:
- Clear understanding of the product needs
- Conduct extensive preformulation studies
- Develop multiple prototypes with contrasting compositional elements
- Ensure appropriate stability
- Characterize formulation appropriately
- Utilize various performance tests
  - Permeation through and deposition into the nail plate, layer by layer
  - Permeation-MIC relationships for each API
  - Local tolerance
- Understand capabilities of each test to enable appropriate interpretation

Select most appropriate formulation with the knowledge that there is no substitute for clinical data

Formulation selection for Non-clinical and Clinical studies
Acknowledgements

- Galderma team, Sophia Antipolis
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  - Transderma Systems

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