QUALITY-BY-DESIGN RISK ASSESSMENT OF TOPICAL FORMULATION VARIABILITY

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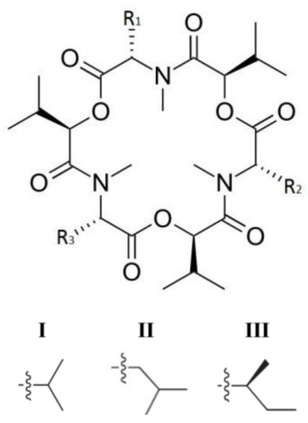
INTRODUCTION and OBJECTIVES

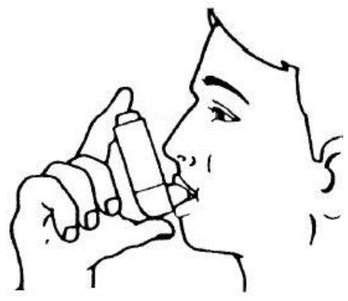
FUSAFUNGINE

- Mixture of cyclic hexadepsipeptide enniatins
- Produced by fungi, *i.a. Alternaria* and *Fusarium*
- Marketed as oral/nasal sprays, patented in 1953
- Topical treatment of upper respiratory tract infections
- Claimed anti-inflammatory and bacteriostatic effects
- SmPC indicates no systemic absorption

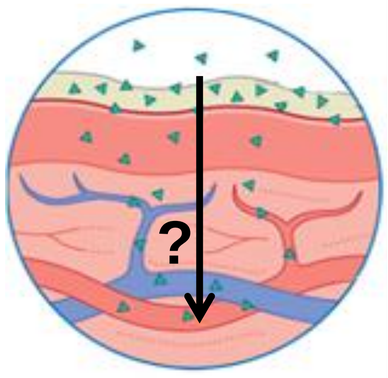


Enniatin	R1	R2	R3
Enniatin A	III	III	III
Enniatin A1	III	I	III
Enniatin B	I	I	I
Enniatin B1	I	III	I
Enniatin C	II	II	II
Enniatin D	I	I	II
Enniatin E1	I	II	III
Enniatin E2	I	III	II
Enniatin F	II	III	III

- Enniatins have been shown to permeate human skin
- Generally mucosal permeation > skin permeation
- Formulated in ethanol (EtOH) and isopropyl myristate (IPM)



MUCOSA



1. **Do enniatins permeate mucosa and reach blood circulation?**
2. **Influence of excipient variability on mucosal permeation?**

→ **Quantify transmucosal kinetics**

EXPERIMENTAL

1. GC-FID

- Five different batches of a fusafungine market preparation
- Determination of EtOH and IPM concentration

2. Franz Diffusion Cell (FDC)

- Buccal pig mucosa
- Dose solutions: 1 mg/mL enniatins mix in different EtOH:IPM mixtures

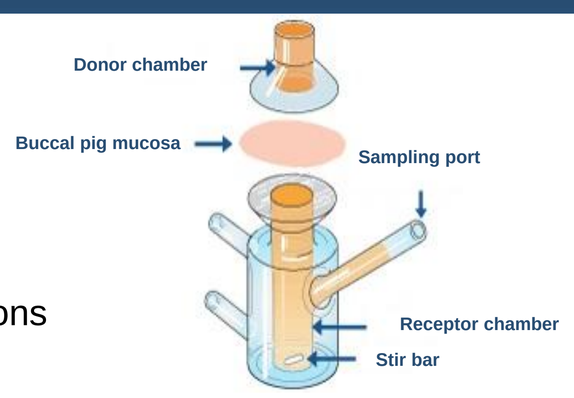
→ 1:99, 3:97, 5:95 and 10:90 EtOH:IPM (V/V)

3. UHPLC-MS/MS (MRM)

Analysis of the FDC samples

4. Calculations

- Transmucosal kinetics
- Steady-state plasma concentrations



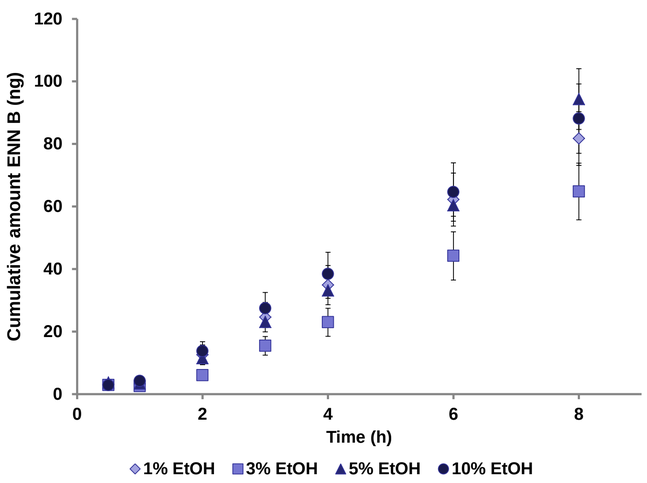
RESULTS and DISCUSSION

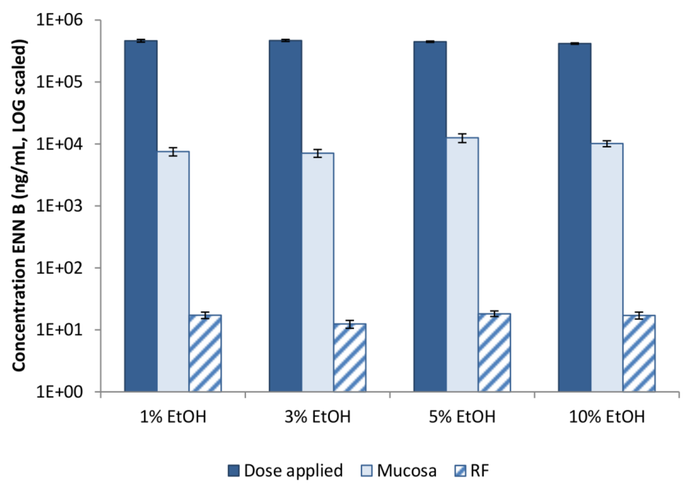
1. Determination of EtOH and IPM content

- EtOH: 1.67 ± 0.03% (mean ± SEM, n =5)
- IPM: 91.60 ± 2.02% (mean ± SEM, n =5)
- No statistical significant difference between batches ($p > 0.10$)

2. Transmucosal kinetics

- Enniatins able to permeate buccal mucosa!
- Example enniatin B (most abundant):





- No statistical significant difference between dose solutions ($p > 0.05$)
- Inverse relationship between log P versus $k_{p,v}$, t_{lag} and Q_{8h}
- Local mucosa concentrations up to 33 µM (total enniatins)
  → marketed preparations x 10 dosage = 330 µM

EtOH:IPM (V/V)	1:99	3:97	5:95	10:90	1:99	3:97	5:95	10:90
2° parameters	J_{ss} (ng/(cm²×h))				Q_{8h} (%)			
ENN B	20.11 ± 2.00	15.16 ± 1.98	21.24 ± 1.76	19.43 ± 2.13	0.048 ± 0.006	0.035 ± 0.005	0.053 ± 0.005	0.053 ± 0.007
ENN B1	5.54 ± 0.79	3.45 ± 0.62	5.96 ± 0.73	5.36 ± 0.93	0.018 ± 0.003	0.014 ± 0.002	0.020 ± 0.003	0.021 ± 0.004
ENN A1	0.68 ± 0.12	0.56 ± 0.16	0.90 ± 0.19	0.81 ± 0.12	0.006 ± 0.001	0.006 ± 0.002	0.008 ± 0.002	0.008 ± 0.001
ENN D	1.13 ± 0.14	6.79 ± 0.12	1.18 ± 0.10	1.16 ± 0.16	0.034 ± 0.005	0.024 ± 0.004	0.033 ± 0.004	0.038 ± 0.006
ENN E	0.22 ± 0.04	0.14 ± 0.02	0.24 ± 0.04	0.22 ± 0.04	0.014 ± 0.003	0.011± 0.002	0.015 ± 0.003	0.014 ± 0.002
1° parameters	$k_{p,v}$ (× 10^{-5} cm/h)				Lag time (h)			
ENN B	4.36 ± 0.43	3.25 ± 0.43	4.75 ± 0.40	4.68 ± 0.51	1.24 ± 0.22	1.56 ± 0.16	0.93 ± 0.20	0.94 ± 0.19
ENN B1	1.62 ± 0.23	1.06 ± 0.19	1.79 ± 0.22	1.87 ± 0.32	1.16 ± 0.24	0.73 ± 0.21	1.13 ± 0.05	0.93 ± 0.08
ENN A1	0.50 ± 0.08	0.43 ± 0.12	0.67 ± 0.14	0.70 ± 0.11	0.67 ± 0.38	n.d.	0.53 ± 0.20	0.68 ± 0.21
ENN D	3.04 ± 0.39	2.14 ± 0.33	2.95 ± 0.25	3.47 ± 0.48	1.20 ± 0.14	1.31 ± 0.09	0.94 ± 0.18	1.16 ± 0.13
ENN E	1.20 ± 0.22	0.80 ± 0.14	1.36 ± 0.21	1.45 ± 0.30	0.86 ± 0.12	0.50 ± 0.11	0.92 ± 0.05	0.86 ± 0.12

3. Clinical interpretation

- Neglecting *in-vivo* saliva flow, GI absorption, metabolisation
- Steady-state plasma concentrations

$$C_{pl,ss,buccal} = (A \times k_{p,v} \times C_v)/Cl$$

Cl = plasma clearance
$k_{p,v}$ = transmucosal permeability coefficient
C_v = enniatin concentration in vehicle
A = exposed mucosal area

- Ranging from 0.026 mg/L for ENN E to 1.339 mg/L for ENN B
  → x 10 dosage = up to 13.4 mg/L for ENN B alone

CONCLUSIONS

- Enniatins in topical medicines are capable of permeating the mucosa barrier!
- QbD approach → no risk of a significantly different systemic enniatin availability in terms of composition variability.
- Worst-case scenario → question use of enniatins in topical treatment of innocent upper respiratory tract infections → long-term chronic effects?

REFERENCES

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