UNIVERSITEIT GENT

FACULTY OF **PHARMACEUTICAL SCIENCES**

DO CYCLIC DEPSIPEPTIDE MYCOTOXINS BEAUVERICIN AND ENNIATINS CROSS THE BLOOD-BRAIN BARRIER?

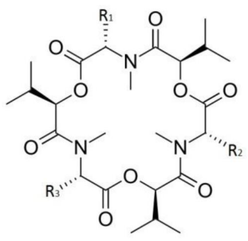
Lien Taevernier, Nathalie Bracke, Lieselotte Veryser, Evelien Wynendaele and Bart De Spiegeleer*

Drug Quality and Registration (DruQuaR) group, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium.

* Corresponding author: bart.despiegeleer@ugent.be (*O. Ref.*: 2016-065c)

INTRODUCTION

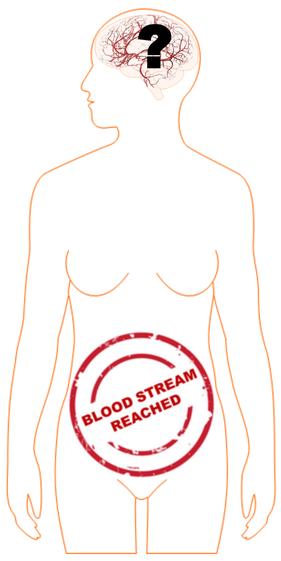
FOOD INGESTION | **DUST INHALATION** | **TOPICAL APPLICATION**



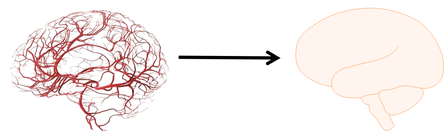
CYCLIC DEPSIPEPTIDES BEA + ENNs

HAZARDOUS:
- Cytotoxic
- Anaphylactic reactions
- Antibiotic resistance
- Genotoxic?
- …

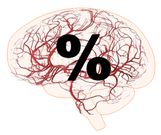
INTERESTING BIOACTIVITIES:
- ACAT inhibitors
- Antimicrobial
- Insecticidal
- Cytotoxic
- …



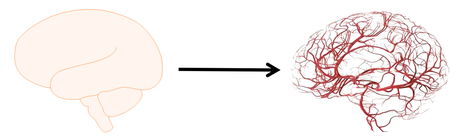
1. BLOOD-TO-BRAIN TRANSPORT?



2. CAPILLARY VS. PARENCHYMAL DISTRIBUTION?



3. BRAIN-TO-BLOOD TRANSPORT?



EXPERIMENTAL METHODS

- **Metabolic stability *in vitro*: mice serum and brain homogenate**
- **BBB transport study *in vivo* in ICR-CD-1 mice:**
  1. Blood-to-brain: multiple time regression influx (MTR) (IV)
  2. Brain distribution: capillary depletion (IV)
  3. Brain-to-blood: efflux (intracerebroventricular)

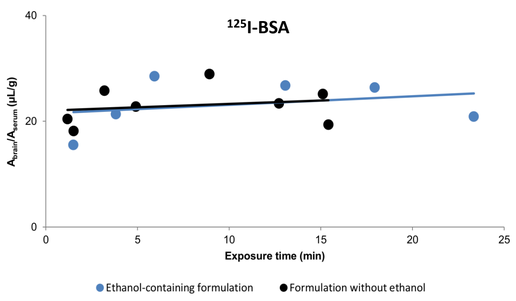
- **Bioanalytical UHPLC-MS/MS method:**
  - Sample preparation
  - LC/MS conditions
  - Validation
- **Kinetic modelling**

RESULTS

I. METABOLIC STABILITY

Stable in serum and brain during the duration of the *in vivo* study: 80-120% recoveries

II. BLOOD-TO-BRAIN TRANSPORT



→ Formulation = 0.2 mg/kg in 6:94 EtOH:Lactated Ringer's solution containing 1% BSA (V/V)

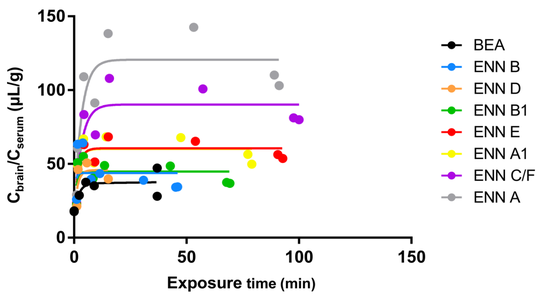
✓ Dose resembling a real-life feed contamination

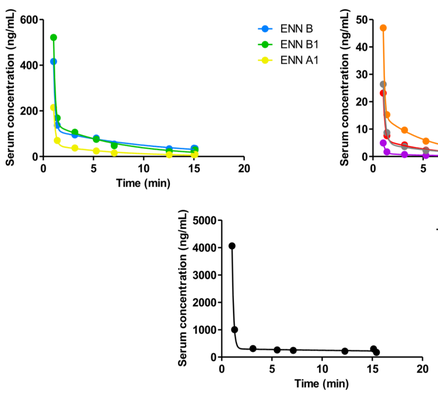
✓ No influence of the formulation (^{125}I-BSA)

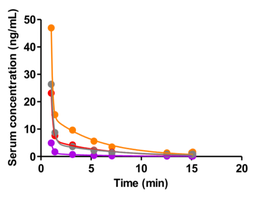
→ Gjedde-Patlak biphasic model

✓ High initial influx rate into the brain: K_1 =11 to 53 µL/(g×min)

✓ Followed by a plateau phase → negligible net brain clearance (very low K-values)







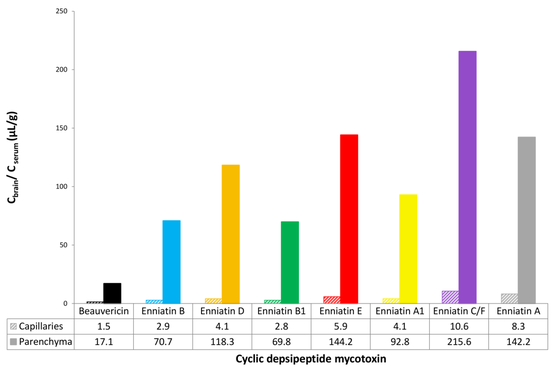
→ Serum kinetics: 2-compartment model

✓ Very fast transfer from central to peripheral compartment (distribution)

✓ Longer, slower elimination phase

Compound	Distribution half-life (min^{-1})	Elimination half-life (min^{-1})
Beauvericin	0.10	32.6
Enniatin B	0.11	7.49
Enniatin D	0.09	3.27
Enniatin B1	0.11	4.78
Enniatin E	0.12	3.58
Enniatin A1	0.13	4.50
Enniatin C/F	0.15	3.51
Enniatin A	0.16	4.27

III. CAPILLARY VS. PARENCHYMAL DISTRIBUTION

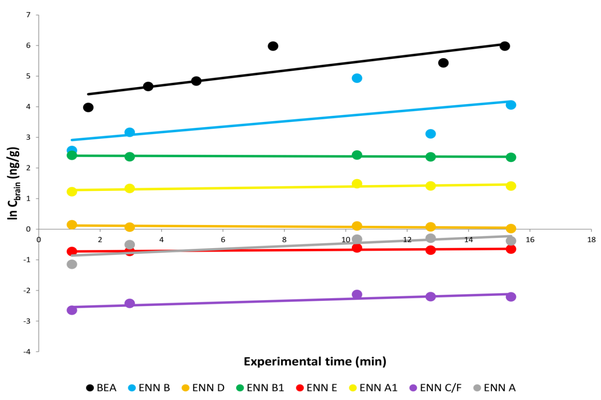


	Beauvericin	Enniatin B	Enniatin D	Enniatin B1	Enniatin E	Enniatin A1	Enniatin C/F	Enniatin A
Capillaries	1.5	2.9	4.1	2.8	5.9	4.1	10.6	8.3
Parenchyma	17.1	70.7	118.3	69.8	144.2	92.8	215.6	142.2

→ 10 min post-injection

→ 95% reached brain parenchyma after permeation through BBB lining endothelial capillaries

IV. BRAIN-TO-BLOOD TRANSPORT



→ No significant efflux (k_{out} < 0.005 min^{-1})

III. OVERVIEW

Compound	MTR blood-to-brain influx(1)			Capillary depletion		Efflux
	K (µL/(g×min))	V_g (µL/g)	K_1 (µL/(g×min))	Parenchymal fraction (%)	Capillary fraction (%)	Slope (min^{-1})
Beauvericin	0.02272 ± 0.3153	21.91 ± 9.664	11.15 ± 11.42	91.92 ± 1.41	8.08 ± 1.41	-0.1205 ± 0.04218
Enniatin B	≈ 2.071 × 10^{-16}	28.97 ± 11.07	52.95 ± 108.4	96.03 ± 0.19	3.97 ± 0.19	-0.08802 ± 0.06985
Enniatin D	0.001640 ± 0.09873	34.39 ± 6.588	21.66 ± 11.43	96.71 ± 0.15	3.29 ± 0.15	0.005063 ± 0.003091
Enniatin B1	≈ 1.444 × 10^{-16}	29.94 ± 7.594	30.03 ± 24.19	96.10 ± 0.00	3.90 ± 0.00	0.002408 ± 0.002707
Enniatin E	≈ 9.769 × 10^{-13}	45.77 ± 5.795	25.08 ± 10.86	96.20 ± 0.36	3.80 ± 0.36	-0.005929 ± 0.003019
Enniatin A1	≈ 2.185 × 10^{-16}	45.38 ± 9.303	25.38 ± 13.12	95.70 ± 0.10	4.30 ± 0.10	-0.01277 ± 0.005588
Enniatin C/F	≈ 1.845 × 10^{-16}	75.43 ± 15.39	23.53 ± 10.22	95.30(2)	4.70(2)	-0.02976 ± 0.009449
Enniatin A	≈ 1.840 × 10^{-16}	105.8 ± 21.95	32.41 ± 13.76	94.20 ± 0.71	5.80 ± 0.71	-0.04421 ± 0.02059

(1) V_0 = 14.8 µL/g of BSA
(2) n = 1 (the other sample was < limit of detection)

CONCLUSIONS

- **Very high influx rate into the brain, with a significant distribution in the brain parenchyma.**
- **No significant serum or brain metabolisation, nor significant brain efflux to the blood was observed.**

→ **Possibility that these cyclic depsipeptide mycotoxins exert local central nervous system (CNS) effects once present in the systemic circulation!**

REFERENCES

Taevernier L, Veryser L, Vandercruyssen K, D'Hondt M, Vansteelandt S, De Saeger S, De Spiegeleer B. UHPLC-MS/MS method for the determination of the cyclic depsipeptide mycotoxins beauvericin and enniatins in in vitro transdermal experiments. *Journal of Pharmaceutical and Biomedical Analysis.* 2014; **100**: 50-57, doi.org/ 10.1016/j.jpba.2014.07.021.

Taevernier L, Wynendaele E, D'Hondt M, De Spiegeleer B. Analytical quality-by-design approach for sample treatment of BSA-containing solutions. *Journal of Pharmaceutical Analysis*. 2015; **5**: 27-32, doi: 10.1016/j.jpha.2014.06.001.

Taevernier L, Bracke N, Veryser L, Wynendaele E, Gevaert B, Peremans K, De Spiegeleer B. Blood-brain barrier transport kinetics of the cyclic depsipeptide mycotoxins beauvericin and enniatins. *Submitted for publication.*