The blood-brain barrier (BBB) permeability properties of plant $N$-alkylamides

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**N-alkylamides**

- Plant secondary metabolites
- Occurrence > 25 plant families
- Wide structural diversity

- Various functionalities *i.e.* antimicrobial, insecticidal, sensory, anti-inflammatory, immune-modulating, *central nervous system effects (CNS):*
  * analgesic, anticonvulsant, antidepressant, anti-oxidant, anti-inflammatory activity
  * protection against neurodegeneration
  * cognitive enhancing effects

- 2 model *N*-alkylamides (NAAs): spilanthol (logP 3.39) and pellitorine (logP 3.65)

**Spilanthol**
deca-2\(E\),6\(Z\),8\(E\)-trienoic acid isobutylamide
(present in *Spilanthes acmella*)

**Pellitorine**
deca-2\(E\),4\(E\)-dienoic acid isobutylamide
(present in *Anacyclus pyrethrum*)
**N-alkylamides (spilanathol and pellitorine) enter the systemic blood circulation after different routes of administration:**

- **Oral:**
  *In vitro* Caco-2 cell monolayer experiment\(^1\)
  *In vivo* oral gavage experiment with rats\(^1\)
  → penetrate the intestinal barrier

- **Topical:**
  *In vitro* transdermal Franz diffusion cell experiment using human skin and pig mucosa\(^2-4\)
  → penetrate the stratum corneum

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**NAAs in blood → brain ??**

To investigate the permeability of spilanthol and pellitorine through blood-brain barrier (BBB)

1. Influx: from blood-to-brain
   - a) Multiple time regression (MTR) experiment
   - b) Capillary depletion experiment (brain distribution)

2. Efflux: from brain-to-blood

Investigation using the *gold-standard in vivo* method
In vivo blood-brain barrier (BBB) experiment with mice

1. Influx (blood-to-brain transport)
   - Dose = spilanthol: 2.77 mg/kg mouse, pellitorine: 1.23 mg/kg mouse
   - IV injection

   a) Multiple time regression (MTR) experiment
      - Collection of serum and isolation of brain at specified time points

   b) Capillary depletion (brain distribution) experiment
      - Collection of serum and isolation of brain after 10 min.

2. Efflux (brain-to-blood transport)
   - Dose = spilanthol: 0.14 mg/kg mouse, pellitorine: 0.06 mg/kg mouse
   - Intraventricular injection
   - Collection of serum and isolation of brain at specified time points

Samples analysed using a bio-analytical UPLC-MS method
1. Influx: blood-to-brain
   a) MTR results

➢ Serum profiles

**Spilanthol**

one-compartmental model

\[ C = C_0 \times e^{-k_e t} \]

**Pellitorine**

two-compartmental model

\[ C_p = C_1 \times e^{-\alpha t} + C_2 \times e^{-\beta t} \]

**Kinetic parameters of spilanthol**

- \( t_{1/2,\text{elimination}} = 3.16 \text{ min} \)
- \( k_{\text{elimination}} = 0.22 \text{ min}^{-1} \)
- \( C_0 = 3.05 \text{ µg/ml} \)

**Kinetic parameters of pellitorine**

- \( t_{1/2,\text{elimination}} (\beta) = 4.48 \text{ min} \)
- \( \alpha = 1.56 \text{ min}^{-1} \)
- \( \beta = 0.15 \text{ min}^{-1} \)
- \( C_1 = 7.02 \text{ µg/ml} \)
- \( C_2 = 0.44 \text{ µg/ml} \)
Brain/serum concentration profiles: biphasic model

**Spilanthol**

- \( K_1 = 796 \, \mu l/g \cdot min \)
- \( V_g = 652 \, \mu l/g \)

**Pellitorine**

- \( K_1 = 153 \, \mu l/g \cdot min \)
- \( V_g = 792 \, \mu l/g \)

(If \( V_0 \) and \( K > 0 \): \( V_g = 344 \, \mu l/g; K_1 = 217 \, \mu l/g \cdot min \))

(If \( V_0 \) and \( K > 0 \): \( V_g = 807 \, \mu l/g; K_1 = 159 \, \mu l/g \cdot min \))

\[
\frac{A_m(t)}{C_p(t)} = K\Theta + V_g \left( 1 - e^{-\left( K_1 - K \right) \frac{t}{V_g}} \right) + V_0 
\]

\[
A_m(t) = \text{the concentration of NAA in the brain at time } t \text{ (ng/g)}
\]

\[
C_p(t) = \text{the concentration of NAA in serum at time } t \text{ (ng/µl)}
\]

\[
K_2 = \text{unidirectional clearance (µl/(g·min))}
\]

\[
K = \text{the net clearance (µl/(g·min)): } 10^{-16} \sim 0
\]

\[
V_g = \text{tissue brain distribution volume (µl/g)}
\]

\[
V_0 = \text{vascular brain distribution volume (µl/g): 14.8 (BSA)}
\]

\[
\Theta = \text{exposure time: x-axis}
\]
**b) Capillary depletion: brain distribution**

Spilanthol

![Bar chart for Spilanthol](chart1.png)

- Concentration in tissue vs. serum for Spilanthol:
  - 10.63 min: 95.28%
  - 12.12 min: 98.89%

- Concentration in parenchyma vs. serum for Spilanthol:
  - Concentration in capillaries vs. serum for Spilanthol:
  - Concentration in parenchyma vs. serum for Spilanthol:

Pellitorine

![Bar chart for Pellitorine](chart2.png)

- Concentration in tissue vs. serum for Pellitorine:
  - 11.33 min: 96.27%
  - 11.23 min: 93.78%

- Concentration in parenchyma vs. serum for Pellitorine:
  - Concentration in capillaries vs. serum for Pellitorine:
  - Concentration in parenchyma vs. serum for Pellitorine:
2. **Efflux: brain-to-blood**

**Spilanthol**

Kinetic parameters of spilanthol

\[ k_{\text{out}} = 0.11 \text{ min}^{-1} \]
\[ t_{1/2,\text{brain}} = 6.38 \text{ min} \]

**Pellitorine**

Kinetic parameters of pellitorine

\[ k_{\text{out}} = 0.05 \text{ min}^{-1} \]
\[ t_{1/2,\text{brain}} = 13.78 \text{ min} \]
Conclusions

- Spilanthol and pellitorine are able to pass the BBB

- Both NAAs show a rapid and high influx rate, with pellitorine somewhat higher BBB influx permeation compared to spilanthol

- Similar \( K_{in} \) values were obtained compared to CNS small molecules

- > 95% of NAAs was found in parenchyma of the brains, < 5% in the capillaries → possibility to exert CNS effects

- There is also efflux from the brain into the blood
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