Role of P-Glycoprotein in Norbuprenorphine Transport at the Blood-Brain Barrier in Mice: Considerations for Buprenorphine Respiratory Toxicity

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Buprenorphine (BUP):

- An effective alternative to methadone in the treatment of opiate dependence
- Ceiling respiratory effects at elevated doses suggesting a high safety profile

Dahan A. Br J Anesthesiol 2005
Concern: BUP-related asphyxic deaths

Intoxications aiguës par traitement substitutif à base de buprénorphine haut dosage

29 observations cliniques - 20 cas mortels:

A. Tracqui, C. Tournoud, F. Flesch, J. Kopfierschmitt, P. Kintz, M. Deveaux, M.H. Ghysel,
P. Marquet, G. Pépin, G. Petit, A. Jaeger, B. Ludes

Kintz P. Forensic Sci Int 2001
Pirnay S. Addiction 2004

-Misuse of BUP (IV injection of crushed tablets)
-Co-ingestion of benzodiazepines (BZD)

However, the exact mechanisms of BUP-related respiratory toxicity remains unknown.
Ceiling respiratory effects
(Humans and rodents)

BUP is not a substrate for human P-gp.

Potent respiratory depression (Rats)

Tournier N. Int J Neuropsychopharmacol 2010

In vitro N-BUP is a good substrate for human P-gp.

P-gp is an efflux transporter (intestine, kidney, liver, and specifically at BBB)

60% of substances are substrates for the P-gp, among them many opioids

The P-gp-mediated efflux of opioids affects their:
1. Oral absorption
2. CNS accumulation
3. Systemic clearance
4. Antinociceptive effects
5. Adverse side effects
Our hypothesis:

Whether BUP and/or N-BUP are P-gp substrates in mice, then the pharmacological inhibition or the genetic disruption of P-gp might be responsible of BUP-related respiratory toxicity.
Objectives

1- To assess whether BUP and N-BUP are P-gp substrates \textit{in vivo}.

2- To describe the consequences of P-gp pharmacological inhibition (using PSC833) on BUP- and N-BUP-related respiratory effects.

3- To describe the consequences of P-gp gene invalidation (using KO mice) on BUP- and N-BUP-related respiratory effects.
**In situ cerebral perfusion in mouse**

**Principle:** Isolation of the brain then perfusion of radio-labeled molecules

- Study of luminal transport at the BBB

Transport parameter: \( \text{Kin} = \frac{V_d}{t} \)

\( V_d: \) volume of distribution

\( t: \) duration of perfusion

**Advantages:**

- Control of buffer parameters (composition, temperature, pH,..)
- Avoid interactions with blood cells and any possible metabolism.
- Better sensitivity *in vivo*

Ventilation study:
Plethysmography on awaken mice

Measured parameters:
- Inspiratory time ($T_I$)
- Expiratory time ($T_E$)
- Tidal volume ($V_T$)
- Respiratory rate ($f$)
- Total time ($T_{TOT}$) = ($T_I + T_E$)
- Minute volume ($V_E$) = ($V_T \times f$)

Bartlett D. Respir Physiol 1970
Experimental protocols and results
**Study 1**

*In situ* brain perfusion

Perfusion fluid + either [³H]BUP or N-BUP

Perfusion fluid + PSC833

Perfusion fluid + either [³H]BUP or N-BUP
- Result 1 -

BUP and N-BUP *in situ* brain perfusion

In contrast to BUP, N-BUP is transported by P-gp *in vivo* at the blood-brain barrier in mice.
Plethysmography
PSC833 vs. solvent pre-treatment

Study 2

Solvent or PSC833 20 mg/kg

30 min

BUP 10mg/kg or N-BUP 1mg/kg

ip

120min

Plethysmography measurements
Results 2

Inspiratory time, expiratory time, and minute ventilation in PSC833 vs. solvent pre-treatment

P-gp pharmacological inhibition significantly increased N-BUP and BUP-related respiratory effects
Study 3

Plethysmography
P-gp knock-out vs. wild-type mice

 WT
 ip

 KO

 BUP 10mg/kg
 Or
 N-BUP 1mg/kg

 120min
 Plethysmograph measurements
- Result 3 -

Inspiratory time, expiratory time, and minute ventilation in P-gp knock-out vs. wild-type mice

P-gp gene disruption significantly increased N-BUP and BUP-related respiratory effects.
BUP and N-BUP plasma concentrations following P-gp inhibition using PSC833

**Study 4**

**Solvent**
Or
PSC833 20mg/kg

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**BUP 10mg/kg**
Or
N-BUP 1mg/kg

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- Result 4 -
Plasma BUP and N-BUP concentrations following P-gp inhibition using PSC833

N-BUP, in contrast to BUP, is transported by P-gp in vivo in mice.
Discussion

**BUP respiratory safety profile**

- Ceiling respiratory effects
  
  *Cowan A. Br J Pharmacol 1977*

- BUP plays a protective role against N-BUP depressant effects due to higher affinity to Mu and delta-opioid receptors but up to certain (N-BUP/BUP) ratio of 10
  
  *Mégarbaine B. TAAP 2006*

- P-gp limits N-BUP (potent respiratory depressant) distribution into the brain
  
  (our study)
Discussion

Conditions of safety disruption

**N-BUP** is an excellent P-gp substrate in humans (*in vitro*) and mice (our study)

**Loperamide**: opioid with no central side-effects and excellent P-gp substrate

*Serious respiratory depression* in healthy volunteers pre-treated with quinidine (P-gp inhibitor)

Sadeque AJ. *Clin Pharmacol Ther* 2000

Drug poly-consumption + BUP misuse + P-gp inhibition by Drug-drug interaction or genetic polymorphism = cause of BUP-related asphyxia
Conclusion

Central respiratory toxicity

Pharmacological effect

N-BUP

Pharmacologic or Genetic

P-gp inhibition

Blood-Brain Barrier

Brain Capillary Endothelium

Brain Stem At Base of Brain

Brain

Oatp2

Oatp3

Blood

P-glycoprotein

BCRP

Mrp 1, 2, 4

Oatp2

ATP

ADP
Thank you