Retention of illicit drugs in dental plaque – a model study regarding plaque as alternative matrix in forensic toxicology

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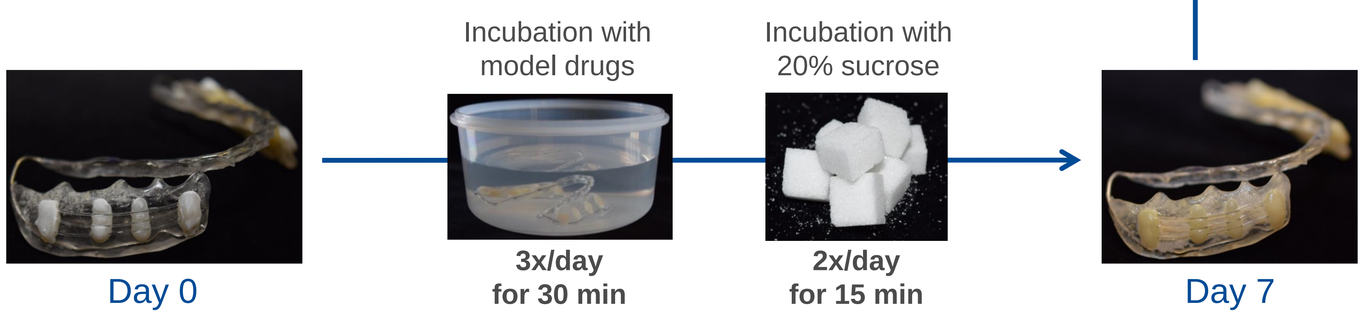
Aims

Analysis of non-mineralised dental biofilm (plaque) might extend the spectrum of alternative matrices commonly used in forensic toxicology, but only little is known about the retention of extraneous substances in this material. The present pilot study aimed to explore whether the investigated drugs of abuse are retained in plaque and at which concentrations. For this purpose, a model was developed simulating plaque growth and regular drug uptake as authentic as possible.

Methods

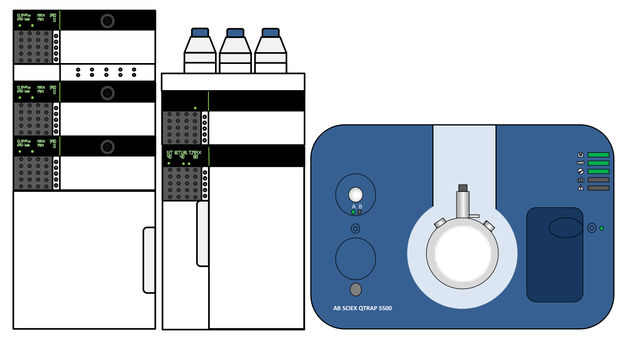
Study Design

Plastic braces were customised for 5 volunteers facilitating plaque growth on their surface. The braces were worn for at least 21 h/day for 7 days. In order to mimic regular drug uptake, the braces were repeatedly placed in a 10 µg/mL solution of the model drugs. Additionally, the braces were treated regularly with a sucrose solution to nourish the biofilm-associated microorganisms.



Sample Preparation

- Grown plaque was scraped off with a dental scaler and air-dried
- Extraction: 10 min ultrasonication with 500 µL ACN
- Evaporation of the supernatant, reconstitution in solvent A/B (95/5)
- Quantitative LC-MS/MS analysis (fully validated method)



Model drugs:

- Amphetamines: amphetamine, methamphetamine, MDA, MDEA, MDMA
- Opiates: morphine, codeine, 6-acetylmorphine
- Cocaine and benzoylecgonine (BE)

LC parameters:

- Solvent A: 0.1% HCOOH (v/v) + 1 mM $NH_4^+HCOO^-$ in H_2O
- Solvent B: 0.1% HCOOH (v/v) in MeOH
- Instruments: Shimadzu UHPLC + Sciex QTrap 5500
- Column: Phenomenex Luna PFP (150 x 2 mm, 5 µm)

Results & Discussion

The weight of the dried biofilm samples varied from 1.2 – 9.5 mg, showing a wide inter-individual range in plaque growth which corresponds with literature describing ‘heavy and light plaque-formers’ [1].
All model drugs were retained in plaque and could be detected 16 h after the last drug exposure.

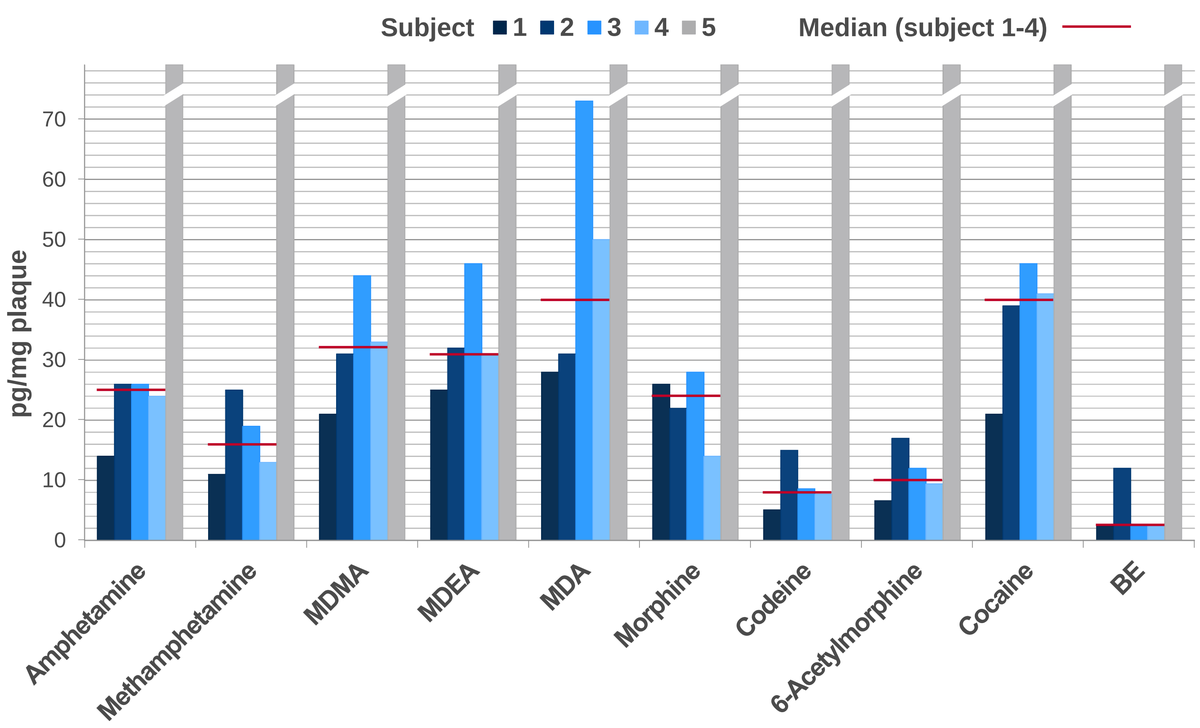
Concentration range in 4 of 5 subjects:

- Amphetamines: ~ 11 – 73 pg/mg
- Opiates: ~ 5.1 – 28 pg/mg
- Cocaine / BE: ~ 21 – 46 / 2.4 – 12 pg/mg

Concentrations 10 to 100 times higher were detected in the plaque sample of the 5th subject.

Incorporation levels:

- Amphetamines ≥ Opiates
- Cocaine ~ Amphetamines
- BE << Cocaine



Conclusions

This study represents a first model feasible to investigate the retention of drugs in plaque. The results show that all model substances are retained for at least 16 h and can be detected with the here-presented method.
The incorporation levels observed in this first study could be dependent on the physico-chemical properties of the substances (e.g. molecular size). But the high inter-individual variances of the drug concentrations found in the plaque samples demonstrate that not only substance properties but also individual factors seem to have an important impact on the incorporation.
The here-presented model can be easily modified for further investigations on incorporation rates but also on the window of detection of the model drugs or other substances of interest.

References

[1] J.M. ten Cate, Biofilms, a new approach to the microbiology of dental plaque, Odontology 94(1) (2006) 1-9.

Acknowledgement

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