Reviewing QACs for Biomonitoring: Metabolism, Analytical Considerations, and Effects on Cholesterol Homeostasis

Libin Xu, PhD
Assistant Professor
Department of Medicinal Chemistry
University of Washington
03/04/2020
Quaternary Ammonium Compounds

Disinfectants, pesticides, preservatives

Regularly used in a variety settings $^{1,2}$

Cleaning products, eye drops, utensils, laundry detergent, milking equipment $^{1,2}$

Dermal (eye), ingestion, inhalation exposure routes

No Public Data On QAC Exposure Levels In Humans!

Quantitation of QACs by Liquid Chromatography-Tandem Mass Spectrometry

**LC Conditions:**
- **Mobile phase:** gradient of **Solvent A** (water, 0.1% formic acid, 2 mM ammonium formate) and **Solvent B** (acetonitrile)
- **UPLC column:** Thermo Hypersil GOLD C18 (100 x 2.1 mm, 1.9 μm)
- **Run time:** < 8 min

**Internal Standards:**
- $d_7$-BACs

**MRM transition**
- 276 / 184
- 304 / 212
- 332 / 240
- 360 / 268
- 326 / 186
Exposure Levels in 100 Random Human Plasma Samples

Random human plasma obtained from BioIVT

Human QAC Exposure

% Human Subjects

Concentration (µM)

DDAC
BAC C16
BAC C14
BAC C12
BAC C10
Can Human Body Metabolize QACs?

Using benzalkonium chlorides (BACs) as examples.
Metabolism by Human Liver Microsomes

Metabolism is cofactor NADPH-dependent → cytochrome P450 involvement

Screening Recombinant CYPs Identifies Responsible CYP Isoforms

These enzymes are highly polymorphic in humans!

What Metabolites are Formed from BACs?
Primary products: $\omega$- and $(\omega-1)$-hydroxylation

Primary Products Undergo Further Metabolism

Metabolites Can Be Quantified by LC-MS/MS

Metabolites of C_{10}-BAC

QACs Detected in Kidney Tissue from Mice Exposed to QACs Via Diet

120 mg/kg/day QAC (40%BAC and 60%DDAC) for two weeks following Melin et al. Reprod. Toxicol. 2014.

QAC Metabolites are Observed in Mice Fed a QAC Diet

Metabolites of BAC-C14 and C16 are also observed!
Effect of Benzalkonium Chlorides on Cholesterol and Lipid Homeostasis: Why Are We Interested in It?
Why Do We Study Benzalkonium Chlorides (BACs)?

- 1 in 10,000 to 60,000
- Congenital malformation, mental retardation, autistic behavior
- Carrier frequency: 1 in 30

Inhibition by drugs or environmental molecules

Mutations $\rightarrow$ Smith-Lemli-Opitz syndrome (SLOS) $\rightarrow$ Neurodevelopmental Defects

Small molecule inhibitors of DHCR7:
- Pharmaceuticals: breast cancer, antipsychotics
- Disinfectants: benzalkonium chlorides

Canfrán-Duque et al. J. Lipid Res. 2013
Benzalkonium Chlorides Are Structurally Similar to Known Inhibitor of DHCR7, AY9944

“%” indicates similarity score.
Benzalkonium Chlorides Inhibit DHCR7 in a Chain Length-Dependent Manner

**Known inhibitor**

BAC: highest similarity to AY9944 (72.9%) among molecules in Tox21

**7-Dehydrocholesterol**

**Cholesterol**

**Desmosterol**

Neuro2a cells were exposed to each compound at 100 nM for 2 days

---


*, p < 0.05
**, p < 0.005
***, p < 0.0005
BACs Alter Lipid Homeostasis in Neuronal Cells

Neuro2a cells were exposed to each compound at 100 nM for 2 days

Individual BACs Alter Gene Expression Involved in Sterol and Lipid Homeostasis

Can BACs Alter Sterol and Lipid Homeostasis In Developmental Brain?
**In utero** Exposure to BACs – Experimental Design

BACs Cross the Placenta and Enter the Developing Brain

BACs Decrease Sterols in Neonatal Brains

BACs Alter Lipidome of Neonatal Brains

What Underlying Gene Expression Changes Are Associated with Alterations in Sterols and Lipids? RNA Sequencing
Global Gene Expression Changes in Exposed Neonatal Brains

Significantly up-regulated

262 46 19
BAC C12 BAC C16

Significantly down-regulated

168 31 43
BAC C12 BAC C16

Cholesterol Biosynthesis Pathway is Activated in Exposed Neonatal Brains

Upstream regulator SREBP cleavage-activating protein (SCAP) is activated in exposed neonatal brains

Regulation of Cholesterol Homeostasis by Scap and Insig

Distinct Expression Patterns of Genes Involved in Sterol-And Lipid-Homeostasis

- Cholesterol biosynthesis genes
- Insulin induced gene 1 (Insig1)
- Low density lipoprotein receptor
- ELOVL fatty acid elongase 6

Distinct Expression Patterns of Genes Involved in Sterol-And Lipid-Homeostasis

Summary

• QACs are observed in random human plasma samples.
• BACs can be metabolized by human cytochromes P450.
• BACs and metabolites can be quantified by LC-MS/MS and both should be used for biomonitoring for complete assessment of BAC exposure.
• BAC exposure leads to elevated levels of parent compounds and metabolites in dam and neonatal tissues.
• BACs disrupt cholesterol and lipid homeostasis both in vitro and in vivo.
Acknowledgements

- **Josi Herron**, Libin, Amy Li, Quynh Do, Emily Pruitt, Vanessa Lopez, Jonathan Palmer, **Ryan Seguin**, Dylan Ross, Rutan Zhang

  - Colleagues in Dept. of Med. Chem. and School of Pharmacy
  - School of Pharmacy Mass Spectrometry Center (Dale Whittington, Scott Edgar)
  - **Funding:** NIH (R01HD092659, R01AI136979), SOP Innovation Fund, UW Royalty Research Fund