Investigating murine sex and strain differences in kidney microsomal and cytosolic scaling factors

Michael J. Doerksen[1], Robert S. Jones[2], Michael W.H. Coughtrie[1], and Abby C. Collier[1]

[1] Faculty of Pharmaceutical Sciences, The University of British Columbia, 2405 Wesbrook Mall, Vancouver, BC V6T 1Z3, Canada

[2] Genentech Inc., 1 DNA Way, South San Francisco, California 94080, USA

Introduction

- The scaling factors microsomal protein per gram of kidney (MPPGK) and cytosolic protein per gram of kidney (CPPGK) are used to extrapolate *in vitro* renal metabolism data to help predict *in vivo* kinetics[1].
- MPPGK/CPPGK scalars have been defined for some species (e.g., human[2,3] and dog[3]), but are missing for other species.
- Additionally, strain and sex differences haven't been investigated.
- Objective: Parameterize mice MPPGK and CPPGK scaling factors and examine any potential sex and strain differences.

Methods

- Mice kidneys (n = 5 per strain and sex) were evenly dissected and homogenized in either a sucrose-HEPES buffer condition or Tris-HCl buffer condition.
- Microsomal and cytosolic fractions were generated following differential centrifugation (10 000x *g*, then 100 000x *g*)[4] and protein content of the fraction was quantified using the bicinchoninic acid (BCA) assay[5].
- Scalars were parameterized by accounting for fraction protein content, fraction volume, and original tissue mass.
- Microsomal intactness, a measure of membrane integrity and quality, was quantified using the mannose-6-phosphatase activity assay[6].

Discussion

- Parameterization of MPPGK and CPPGK scaling factors could reduce the number of animals used in science – microsomes and cytosol from a cohort of 5 animals could be used to characterize the *in vitro* kinetics of multiple compounds instead of using a cohort to characterize kinetics *in vivo* for one compound.
- Scalars may also improve first-in-human trials and reduce attrition rate of drug candidates by improving *in silico* models that use metabolism data generated *in vitro*.

References

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Results

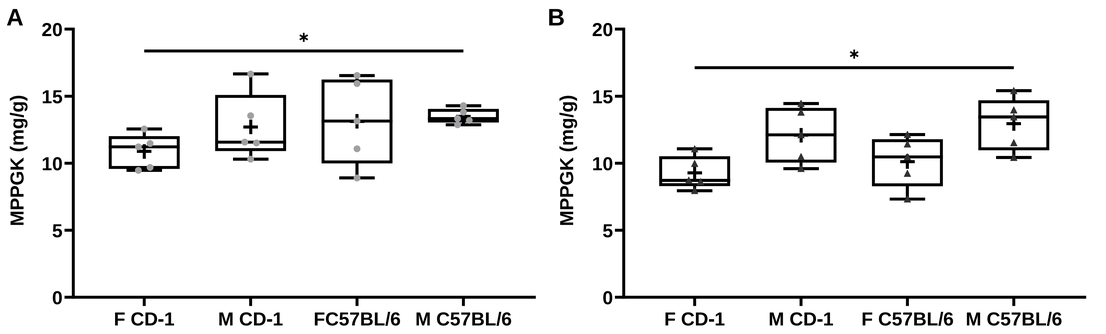

Figure 1. Microsomal protein per gram of kidney (MPPGK) scalars for C57BL/6 and CD-1 mice parameterized in two different buffer conditions. Sucrose-HEPES buffer conditions (A) and Tris-HCl buffer conditions (B) are denoted by grey circles and black triangles, respectively. + denotes mean. F, female; M, male; * $p < 0.05$.

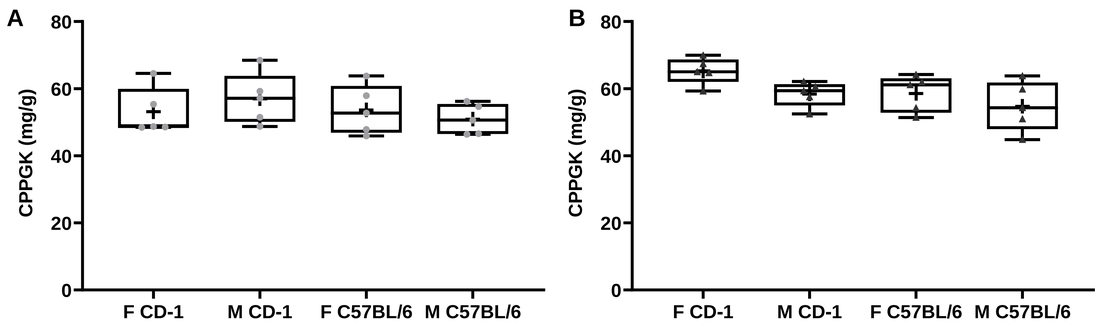

Figure 2. Cytosolic protein per gram of kidney (CPPGK) scalars for C57BL/6 and CD-1 mice parameterized in two different buffer conditions. Sucrose-HEPES buffer conditions (A) and Tris-HCl buffer conditions (B) are denoted by grey circles and black triangles, respectively. + denotes mean. F, female; M, male.

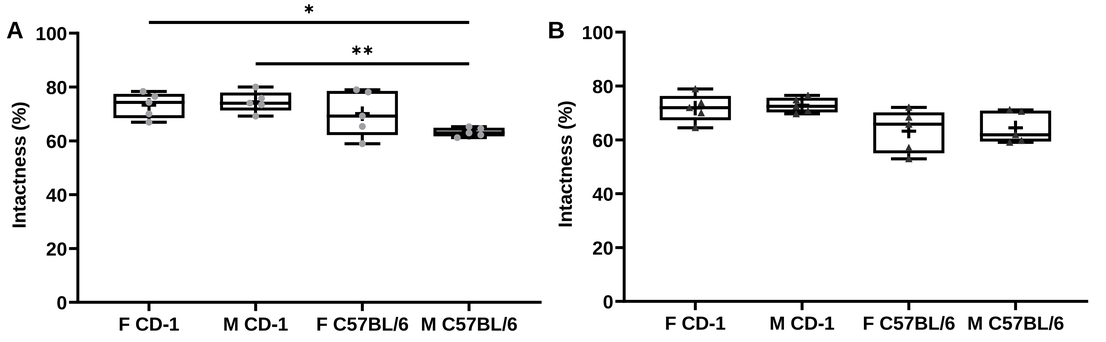

Figure 3. Microsomal intactness, determined through mannose-6-phosphatase activity, for C57BL/6 and CD-1 microsomes generated in two different buffer conditions. Sucrose-HEPES buffer conditions (A) and Tris-HCl buffer conditions (B) are denoted by grey circles and black triangles, respectively. + denotes mean. F, female; M, male; *, $p < 0.05$; **, $p < 0.01$.

Future Directions

- Parameterize kidney scalars in other mice strains (e.g, Swiss Webster) and compare to CD-1 & C57BL/6 scalars.
- Parameterize liver and kidney scalars in rats (Sprague Dawley, Wistar, Long Evans).
- Analyze microsomal and cytosolic fraction purity through semi-quantitation of markers (western blotting) – Pi-class glutathione S-transferase (GST-P) & Cytochrome P450 reductase (POR).

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