In Vitro Characterization of Oral Absorption of Non-Pharmaceutical Chemicals

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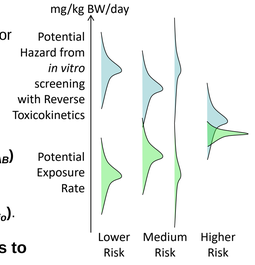
Background

- Data from high throughput *in vitro* screening assays describe potential **hazard**.
- **Toxicokinetics** may be used to determine corresponding oral equivalent doses for comparison to potential **exposure** rate (Wetmore *et al.* 2015).
- The extrapolation of *in vitro* hazard to *in vivo* oral equivalent doses may be improved by accurate definition of the **fraction absorbed (F_{abs})** through the intestine.
- F_{abs} are not frequently available for non-pharmaceuticals.
- The **Caco-2** assay allows for measurement of an apparent **permeability rate (P_{AB})** that is highly correlated with F_{abs} (Artursson *et al.* 2001).
- F_{abs} , combined with first pass hepatic clearance (F_{FP}) determined from intrinsic hepatic clearance, may be used to estimate the **fraction oral bioavailability (F_{bio})**.



In this work, the Caco-2 assay was run for over 400 ToxCast chemicals to measure apparent permeability and estimate fraction absorbed.

Potential improvements in toxicokinetics using more accurate estimates of F_{abs} from Caco-2 data may enable the use of *in vitro* toxicity data to inform regulatory decisions.

Methods

Experimental

- **Caco-2 cells**, developed from human colon carcinoma cells, form a polarized monolayer that behaves similarly to the human intestinal epithelium.
- Caco-2 cells were seeded on 96-well format plates and cultured for 20 days.
- Transepithelial electrical resistance (TEER) measurement was used to verify the formation of tight junctions between the cells and lucifer yellow, a membrane integrity marker, was co-incubated with the test compound at the start of the experiment.
- Chemical stock solutions were administered in duplicate to the apical or basolateral side to measure apparent permeability from apical to basolateral (P_{AB}) or basolateral to apical (P_{BA}).
- Media were collected from the receiver and donor wells 120 minutes after the addition of chemical.
- Control chemicals of warfarin (high AB permeability control), talinolol (p-gp efflux control), and ranitidine (low AB permeability control), were measured for each monolayer batch.

Data analysis

- apparent permeability (P_{AB} or P_{BA}) determined by amount of compound transported per time divided by filter area and initial concentration
- F_{abs} calculated as a function of P_{AB}
- F_{FP} determined using previously measured values for intrinsic clearance and fraction unbound (Pearce *et al.* 2017)

Equations

$$P_{AB} = \frac{1}{area * C_{Apical}} \frac{dA_{Basolateral}}{dt}$$

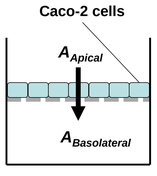
$$F_{abs} = A_{abs} / A_{gut} \approx \text{function}(P_{AB})$$

$$F_{FP} \approx \frac{Q_{liv}}{Q_{liv} + f_{up} Cl / R_{b2p}}$$

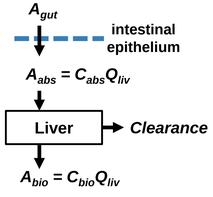
$$F_{bio} = F_{abs} F_{FP}$$

(assume $F_{abs,rat} = F_{abs,human}$)

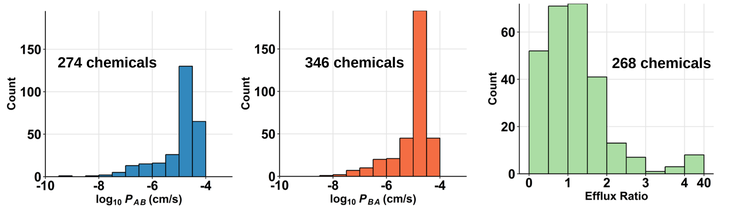
Schematic of Caco-2 assay



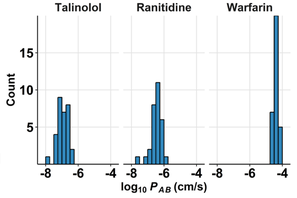
Schematic of absorption and first pass metabolism *in vivo*



Caco-2 Assay Results



- Majority of chemicals (71%) have high apparent P_{AB} (>1x10^{-5} cm/s), similar to that of the high permeability control (warfarin)
- Generally, P_{BA} slightly larger than P_{AB}
- Most chemicals (88%) had efflux ratio (P_{BA} /P_{AB}) < 2
  - efflux ratio > 2 indicates active transport
- Issues encountered:
  - P_{AB}: 120 low recovery, 7 high recovery, 16 with other issues
  - P_{BA}: 53 low recovery, 2 high recovery, 16 with other issues
- Reproducibility was reasonable for the high permeability control (warfarin), but worse for the controls with low permeability (talinolol and ranitidine)

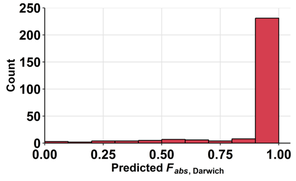


Estimates for F_{abs} based on P_{AB}

Darwich *et al.* 2010

Empirical model fitted to pharmaceutical data

$$\log_{10}(P_{eff}) = 0.6532 * \log_{10}(P_{AB} * 10^6) - 0.3036$$
$$F_{abs} = 1 - (1 + 0.54 * P_{eff})^{-7}$$



Model reported by Darwich *et al.* estimates:

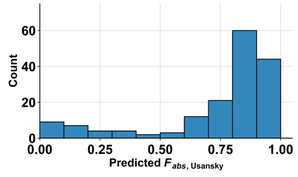
- **87% of chemicals have F_{abs} ≥ 0.80**
- remaining chemicals have median F_{abs} = 0.49

Usansky and Sinko 2005

From a compartmental model

$$F_{abs} = \frac{P_{AB} S / V_d}{k_i + P_{AB} S / V_d}$$

absorptive surface area: $S = 200\ m^2$
intestinal transit rate: $k_i = 5 * 10^{-3}\ min^{-1}$
volume of distribution (V_d) determined using *httk* (limited to chemicals with f_{up} measured *in vitro*)



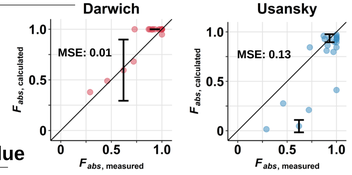
Model reported by Usansky *et al.* estimates:

- **63% of chemicals have F_{abs} ≥ 0.80**
- remaining chemicals have median F_{abs} = 0.63

Predicted vs measured values of F_{abs} and F_{bio}

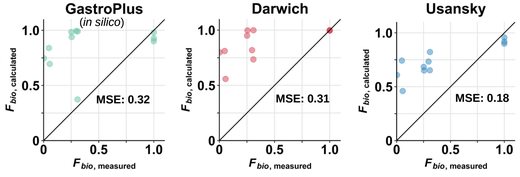
F_{abs} human calculated vs literature measured value

- Literature values for measured F_{abs} mostly for pharmaceuticals (from *httk* R package, multiple sources)
- Darwich provides better estimates of F_{abs} for this set of chemicals
- Median values of $F_{abs,\ calculated}$ with error bars (± 2 std. dev.) for controls of ranitidine and warfarin

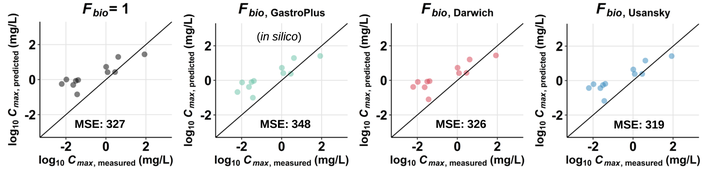


F_{bio} rat calculated vs literature measured value

- F_{bio} measured *in vivo* rat data from Wambaugh *et al.* 2018
- GastroPlus F_{bio} determined *in silico*, otherwise $F_{bio} = F_{abs} F_{FP}$ based on *in vitro* results
- Usansky gives the best result
- F_{FP} :
  - Restrictive clearance assumed
  - Nonrestrictive clearance would result in lower F_{bio}



Influence of F_{bio} on prediction of toxicokinetics



- C_{max} predicted using a 1 compartment model (Wambaugh *et al.* 2018)
- Minimal difference when using estimated F_{bio} in prediction of toxicokinetics observed for this limited set of chemicals

Summary

- **P_{AB} was measured for 274 ToxCast chemicals**
- **While 87% of chemicals are estimated to be highly absorbed (F_{abs} ≥ 0.80), the remainder of chemicals have significantly lower F_{abs} (median of 0.49)**
- F_{abs} predicted using measured P_{AB} was highly correlated with literature values
- F_{bio} was poorly correlated with measured values reported in literature; different assumptions in hepatic clearance (restrictive vs. nonrestrictive) may influence estimates for F_{bio}

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