Evidence for “Metformin-Like” Cation Transporter-Assisted Paracellular Absorptive Transport of the Dietary Hydrophilic Organic Cation Choline Across Caco-2 Cell Monolayers

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Purpose
Choline, a component of the B-complex vitamins, is a hydrophilic cation with permanent positive charge (Figure 1A). Our previous studies demonstrated that another hydrophilic cation with permanent charge at physiologic pHs, metformin (Figure 1B), shows predominantly paracellular transport, with efficient apical (AP) uptake/efflux and inefficient basolateral (BL) efflux in Caco-2 cell monolayers, a well-established model of human intestinal epithelium. These studies led us to propose a novel intestinal absorption mechanism for metformin (Figure 2). The aim of this study was to investigate AP and BL uptake and efflux as well as transport of choline across Caco-2 cell monolayers to determine if choline, like metformin, would be absorbed orally by an AP transporter-assisted paracellular mechanism.

Methods
Caco-2 cells (passage numbers 25-45) were cultured at 37°C in Transwells™ for 21-28 days using Eagle’s minimum essential medium with 10% fetal bovine serum, 1% nonessential amino acids and 1% antibiotic-antimycotic solution. Medium was changed every 2 days and every other day thereafter. Using [14C]choline chloride at concentrations ranging from 1.8 μM to 8.3 mM (n=3 per concentration), AP and BL uptake, AP and BL efflux, and absorptive transport of choline were measured, and the rate of choline uptake and efflux, and apparent permeability coefficient (Papp) for transport across Caco-2 cells were determined. Transepithelial electrical resistance (TER) was measured to ensure monolayer integrity. Following uptake/efflux experiments, protein content from Caco-2 cells in transwells was determined by the BCA protein assay with bovine serum albumin as a standard. Data represents Mean ± S.D.; n = 3.

Results
Figure 3. Time-dependent AP and BL Uptake of Choline in Caco-2 Cell Monolayers
BL uptake of [14C] choline was 2-fold greater than AP uptake.

Figure 4. Concentration-dependent Accumulation during AP to BL Transport of Choline across Caco-2 Cell Monolayers
Concentration-dependent accumulation of [14C] choline (at 60 min) during AP to BL transport shows saturation, indicating a transporter-mediated uptake process.

Figure 5. AP to BL Transport Rate of Choline across Caco-2 Cell Monolayers
Absorptive transport rate of [14C] choline shows concentration dependence and is saturable.

Figure 6. Apparent Permeability (AP to BL) of Choline across Caco-2 Cell Monolayers Decreases with Concentration
The Papp decreased from 96.45 ± 10.41 nm s⁻¹ at 2 μM to 18.66 ± 7.24 nm s⁻¹ at 8.3 mM of [14C] choline, showing saturable transport across Caco-2 cells.

Figure 7. AP and BL Efflux of Choline from Pre-loaded Caco-2 Cell Monolayers
AP efflux of [14C] choline was 4.5-fold greater than BL efflux.

Figure 8. Absorptive and Secretory Transport of Choline across Caco-2 Cell Monolayers
Preliminary results suggest that overall absorptive (AP-BL) and secretory (BL-AP) transport of [14C] choline is similar and linear up to two hours.

Figure 9. Comparison of Uptake, Efflux, and Transport of Choline across Caco-2 Cell Monolayers
Choline is taken up efficiently across AP and BL membrane. BL efflux clearance of choline was less than 1% of absorptive transport clearance, indicating that absorptive transport is likely to be predominantly paracellular. Similarly, secretory transport also appears to be predominantly paracellular despite efficient BL uptake.

Conclusions
- The observable decrease of Papp with increasing concentration of choline provides evidence for saturable transport of choline across Caco-2 cells (AP to BL).
- In Caco-2 cells, AP and BL uptake of choline is highly efficient, whereas BL efflux appears to be rate limiting to absorptive transcellular transport.
- Efficient AP uptake of choline accompanied by poor efflux across the BL membrane would require that choline traverses Caco-2 cell monolayers via the paracellular route.
- It is anticipated that choline would share the unusual “transporter-assisted paracellular intestinal absorption mechanism” with metformin.

References

Acknowledgements
Octavio Romo-Fewell was supported by UNC Eshelman School of Pharmacy Graduate Fellowship. Tianxiang (Kevin) Han was supported by a Pre-doctoral Fellowship from Johnson and Johnson.