

## **siRNA Knockdown of SLC26A6 Reveals the Contribution and Some Properties of PAT-1 to Transepithelial Anion Transport, Particularly Oxalate, Across Caco-2 Monolayers**

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**Problem:** Oxalate, chloride, and bicarbonate are translocated by several possible multifunctional anion exchangers in the apical membrane of enterocytes. We employed siRNA inhibition of SLC26A6 in Caco-2 monolayers (A6KD monolayers) to establish the contribution and properties of SLC26A6 (PAT-1) to apical anion exchange (especially oxalate).

**Methods:** Caco-2 clone (bbe2) monolayers grown on 0.4  $\mu$  polycarbonate membranes were employed 6-8 days post-seeding/transfection, which were confluent and had resistances  $> 400$  ohm. Knockdown of SLC26A6 mRNA was 80 percent efficient (RT2-PCR) and PAT-1 protein at least 50 percent efficient 7 days post transfection. Scrambled siRNA (negative control) did not affect any parameter examined. Conventional Ussing chamber techniques were used to determine anion transport (mucosal to serosal [MS] and serosal to mucosal fluxes [SM]) under short-circuited conditions at 37°C. Intracellular pH was measured with BCECF on superfused monolayers grown on coverslips using standard fluorometric techniques. The relevant anions in the control buffer were (in mM): 120 Cl<sup>-</sup>, 25 HCO<sub>3</sub><sup>-</sup>, 1 SO<sub>4</sub><sup>2-</sup>, and 1.5  $\mu$ M oxalate. Bicarbonate buffers were gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> and with air when using HEPES buffers. Fluxes were measured for at least 45 min (3 x 15 min-intervals).

### **Results:**

1. In standard, symmetrical bicarbonate buffers, there was no significant net flux of oxalate or chloride in control or A6KD monolayers. PAT-1 contributes to about 60 percent of transcellular oxalate exchange in both the M-S and S-M direction.
  2. The MS and SM fluxes of oxalate were equally inhibited by mucosal DIDS with an EC<sub>50</sub> of 5.6  $\mu$ M. 100  $\mu$ M mucosal DIDS fully blocked both unidirectional oxalate fluxes in control monolayers.
  3. Knockdown of PAT-1 significantly affects the rate of change of pH<sub>i</sub> upon chloride removal and recovery of pH<sub>i</sub> following chloride readmission to the superfusate, showing PAT-1 is an important base regulator in Caco-2 cells.
  4. Symmetrical removal of Cl<sup>-</sup> increased J<sup>Ox</sup> MS 3.5-fold while HCO<sub>3</sub><sup>-</sup> removal increased J<sup>Ox</sup> MS 1.5-fold, yet no change in net oxalate transport occurred in control or A6KD monolayers. Symmetrical SO<sub>4</sub><sup>2-</sup> removal produced no discernable change in oxalate transport.
  5. Removal of mucosal chloride increased J<sup>Ox</sup> MS 9-fold and J<sup>Ox</sup> SM 0.9-fold in control monolayers producing a net oxalate flux of 160 pmole $\cdot$ cm<sup>-2</sup> $\cdot$ hr<sup>-1</sup>. Similar, albeit smaller, net fluxes (40 pmole $\cdot$ cm<sup>-2</sup> $\cdot$ hr<sup>-1</sup>) were observed in A6KD monolayers, showing that chloride is a strong competitor for mucosal-cell oxalate uptake in Caco-2 cell.
  6. The chloride-dependence of J<sup>Ox</sup> MS was examined over a range of 0 to 120 mM mucosal chloride in control and A6KD monolayers. Subtraction of DIDS-sensitive J<sup>Ox</sup> MS vs. [Cl]<sub>m</sub> of A6KD monolayers from that of control monolayers revealed a chloride affinity of PAT-1 mediated Ox-Cl exchange of about 17 mM.
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**Conclusions:** Caco-2 cells do not exhibit net oxalate transport because driver gradients for chloride or bicarbonate are insufficient. PAT-1 mediates both apical influx and efflux of the oxalate anion so the vectorial nature of this apical exchanger is most likely determined by the prevailing driver anion gradients *in vivo*. Hence, there is no compelling reason to refer to PAT-1 as only a mediator of intestinal oxalate secretion. PAT-1 is a constitutive component of the human enterocyte (Caco-2) enterocyte, yet it exhibits a chloride affinity that is significantly greater than SLC26A6 expressed in *Xenopus* oocytes.

Supported by grants from the Oxalate and Hyperoxaluria Foundation and the National Institutes of Health (DK56245, DK55944).

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