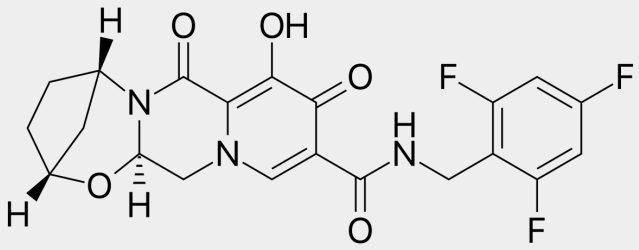
Potential Interaction Between Dolutegravir and Folate Transporters/Receptor in Human Placenta



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Conflict of Interest Disclosure

I have no conflicts of interest.

Background

- Dolutegravir (DTG) is recommended by the World Health Organization as part of first/second-line antiretroviral therapy for adults living with HIV
- Tsepamo study from Botswana reported an increased risk for neural tube defects (NTDs) in infants from mothers taking DTG during conception.

Treatment / Year (% with NTD)	DTG from conception	Non-DTG from conception
2018	0.94% (n=426)	0.12% (n=11173)
2019	0.30% (n=1683)	0.10% (n=14792)

- Folates are critical for fetal development and folate deficiency is associated with an increased risk of NTDs.
- Placental folate transport is primarily mediated by folate receptor alpha (FRα), reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT).
- Recent study demonstrated that DTG acts as a partial antagonist of FRα and causes developmental toxicity in zebrafish.
- It is unclear whether DTG can interact with folate receptor/transporters in placenta cells and impair folate delivery to the human fetus, potentially leading to NTDs.

Hypothesis and Methods

Hypothesis: INSTIs, in particular, DTG, inhibit the functional expression of folate transporters and/or receptor in human and rodent placenta, and could impair adequate delivery of folates to the fetus resulting in a higher risk of fetal NTDs.

Methods

- Immunohistochemical staining was performed to localize RFC and PCFT in human first-trimester placenta tissues.
- First-trimester human trophoblast cell lines, HTR-8/SVneo and JAR, were treated with DTG (500-4000ng/ml) for 1-24 hours. qPCR and western blot analyses were performed to assess mRNA and protein expression of FRα, RFC and PCFT, respectively.
- Transport assays, using 50nM [^{3}H]-methotrexate (MTX) and 50nM [^{3}H]-folic acid (FA), were conducted to assess the functional activity of RFC and PCFT
- Human first-trimester and term placenta tissues were examined for the mRNA and protein expression of FRα, RFC and PCFT by qPCR and western blot.

Results

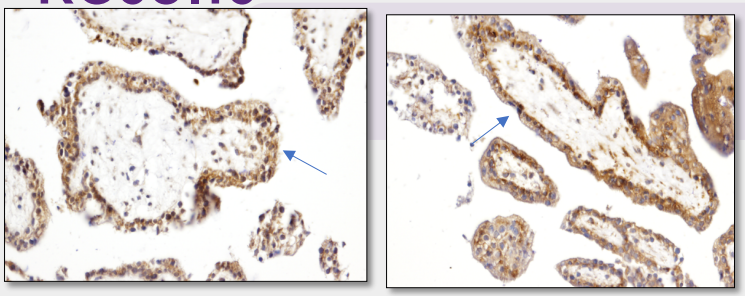

Figure 1. Immunohistochemical localization of RFC (left) and PCFT (right) in human first-trimester placenta tissues (4 weeks). Blue arrowheads show syncytiotrophoblast. Both RFC and PCFT are highly

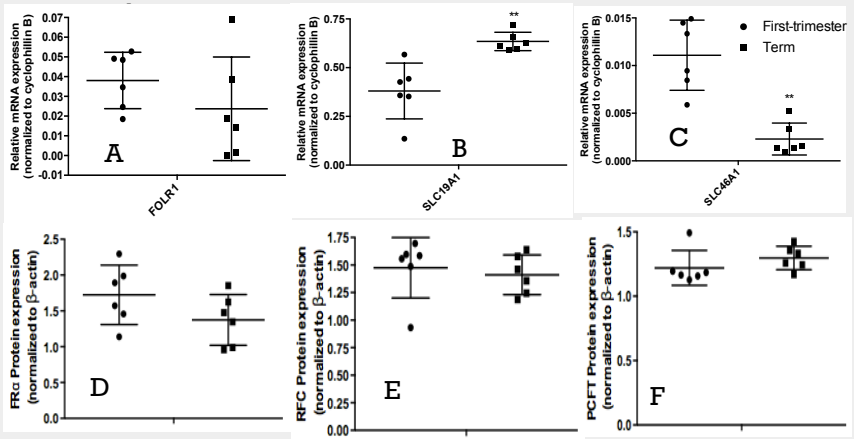

Figure 2. Relative mRNA expression of FRα (A), RFC (B), and PCFT (C) genes was determined in human placental tissues from non-infected donors, first-trimester (5-7 weeks) and term (38-40 weeks). Results are presented as mean relative mRNA expression ± SEM normalized to the housekeeping human Cyclophilin B gene (n=6). Relative levels of FRα (D), RFC (E), and PCFT (F) protein in human first-trimester and term placenta tissues, expression were determined by densitometric analyses. n=6

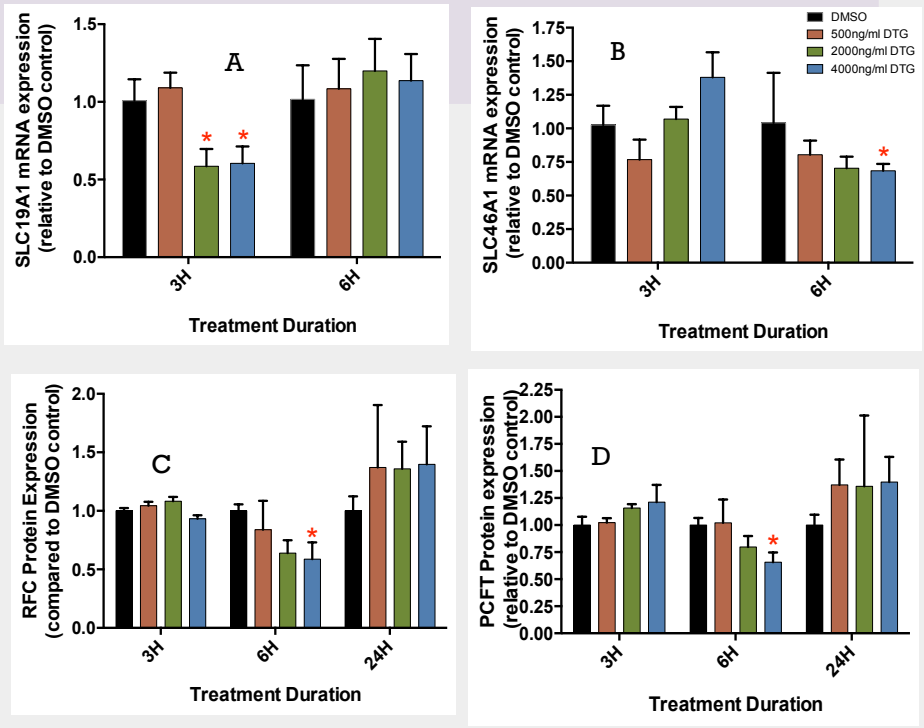

Figure 3. Effect of DTG treatment on RFC (A), and PCFT (B) mRNA expression and RFC (C) and PCFT (D) protein expression in HTR-8/SVneo cells. Results are expressed as percentage change normalized to vehicle (DMSO) control and reported as mean ± S.E.M. for $n = 3$ independent experiments. *, $p < 0.05$.

Results and Conclusion

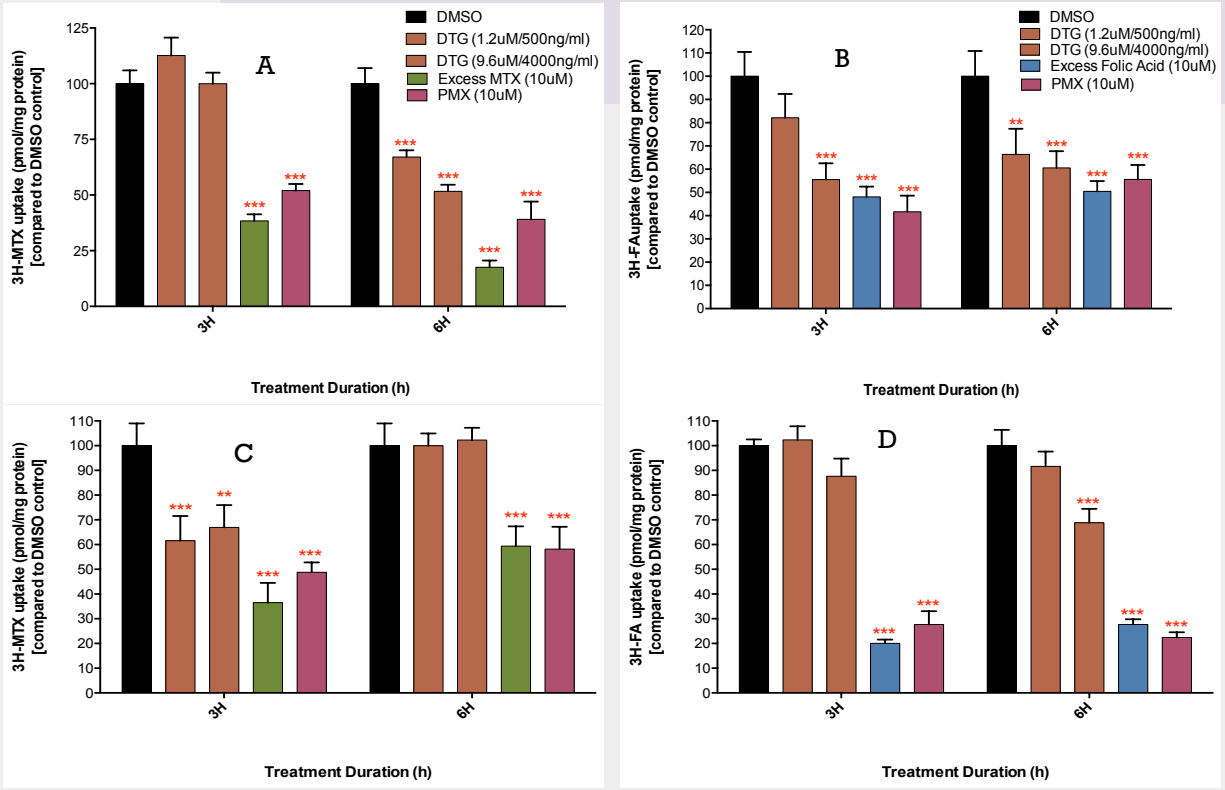

Figure 4. Effect of DTG treatment on uptake of [^{3}H]-MTX (A) and [^{3}H]-FA (B) by HTR-8/SVneo and uptake of [^{3}H]-MTX (C) and [^{3}H]-FA (D) by JAR cells. Cellular uptake of [^{3}H]-MTX (50 nM) and [^{3}H]-FA (50 nM) were measured over 1 min at pH 7.4 or pH 5.5 at 37°C. Results are presented as mean ± S.E.M. for $n = 3$ independent experiments. **, $p < 0.01$; ***, $p < 0.001$.

Conclusion: RFC and PCFT functional expression is decreased by DTG treatment in first-trimester human placental cell lines, suggesting that DTG could potentially impair effective placental folate delivery to the fetus.

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