Lopinavir (an HIV Protease Inhibitor) Impairs Uterine Remodeling During Pregnancy

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background

Combination antiretroviral therapy (cART) is now recommended for all pregnant women living with HIV, for maternal health and to prevent vertical HIV transmission.

Early initiation of Protease Inhibitor (PI)-based cART is associated with adverse pregnancy outcomes including preterm delivery and small for gestational age babies.

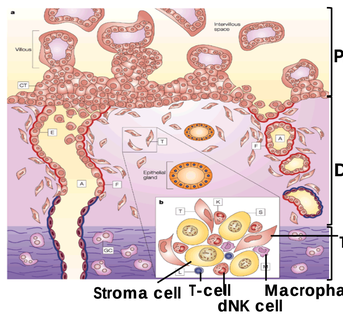
PIs contribute to these adverse events by lowering progesterone (P4) levels and by altering placental angiogenesis.

A critical P4-dependent process in early pregnancy is formation of the Decidua - the maternal component of the placenta.

In this process of decidualization, endometrial stroma cells undergo proliferation and differentiation to form decidual stroma cells.

Decidual spiral arteries undergo remodelling into highly dilated vessels to adequately supply blood to the placenta and fetus.

Decidual natural killer (dNK) cells are the most prominent immune cells in the placental bed during early pregnancy, and are key regulators of spiral artery remodelling.



Decidual Spiral Artery Remodelling at the Maternal-Fetal Interface

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hypothesis

As the decidua is highly dependent on P4 to remodel or 'decidualize' so the decidualization process would be affected by exposure to PI based cART.

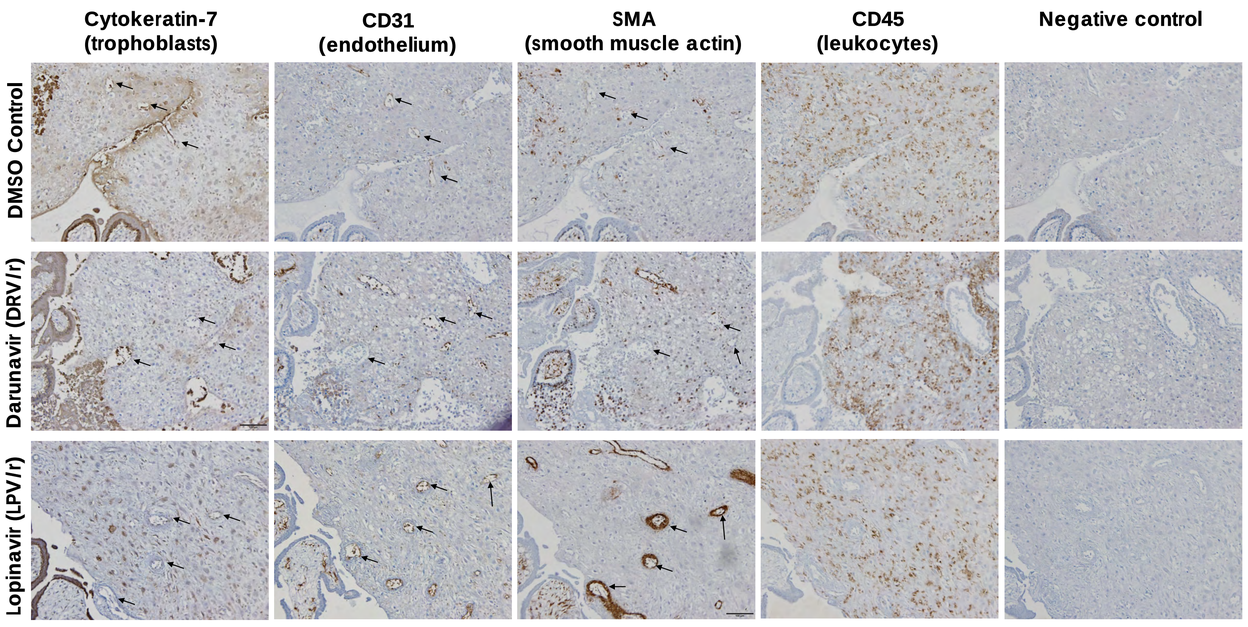
Objective

To investigate the effect of PI based cART on decidualization and spiral artery remodelling in first trimester (human) placenta/decidua samples.

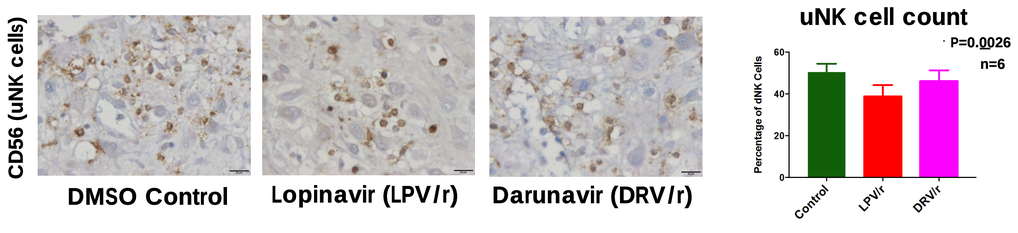
methods

- Human HIV-negative decidua and placenta samples were collected from elective first trimester terminations.
- The placental-decidual co-culture model was used to examine the effects of PIs on spiral artery remodelling by immunohistochemistry.
- The placental villous explant culture was used for studying extravillous trophoblast (EVT) invasion across matrigel.
- A primary decidual cell culture system was used to assess PI-induced changes in the expression of the biomarkers of decidualization and decidual angiogenesis, using ELISA and multiplex approaches.
- The effects of PIs on the viability of various decidual cell types was examined by flow cytometry.
- **Antiretrovirals used: Protease Inhibitors**- (Ritonavir boosted) Lopinavir (LPV/r), Atazanavir (ATV/r) and Darunavir (DRV/r). **NRTI**- Combivir (Zidovudine+Lamivudine).

result 1- LPV Treatment Impairs Spiral Artery Remodelling



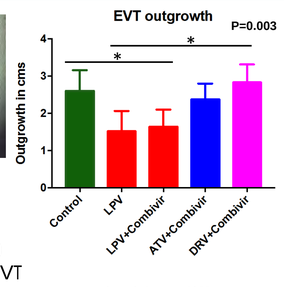
Immunohistochemical identification of remodelling vessels in serial cross-sections of placental-decidual co-cultures, treated with ritonavir (r)-boosted protease inhibitors or control as indicated. Remodelled arteries and invading trophoblasts are indicated by arrows. LPV treatment results in un-remodelled vessels, as opposed to completely remodelled vessels in control or DRV treatment.



CD56 staining of uNK cells reveals unhealthy, apoptotic cells in LPV treatment and a lower uNK cell count as measured by stereology. Statistical significance (P<0.05) is indicated by * for the treatments grouped by a bar.

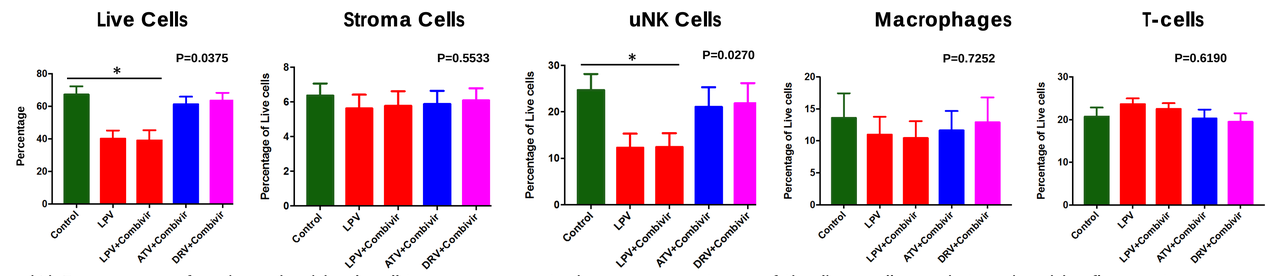
result 2- LPV Treatment Inhibits Trophoblast Outgrowth





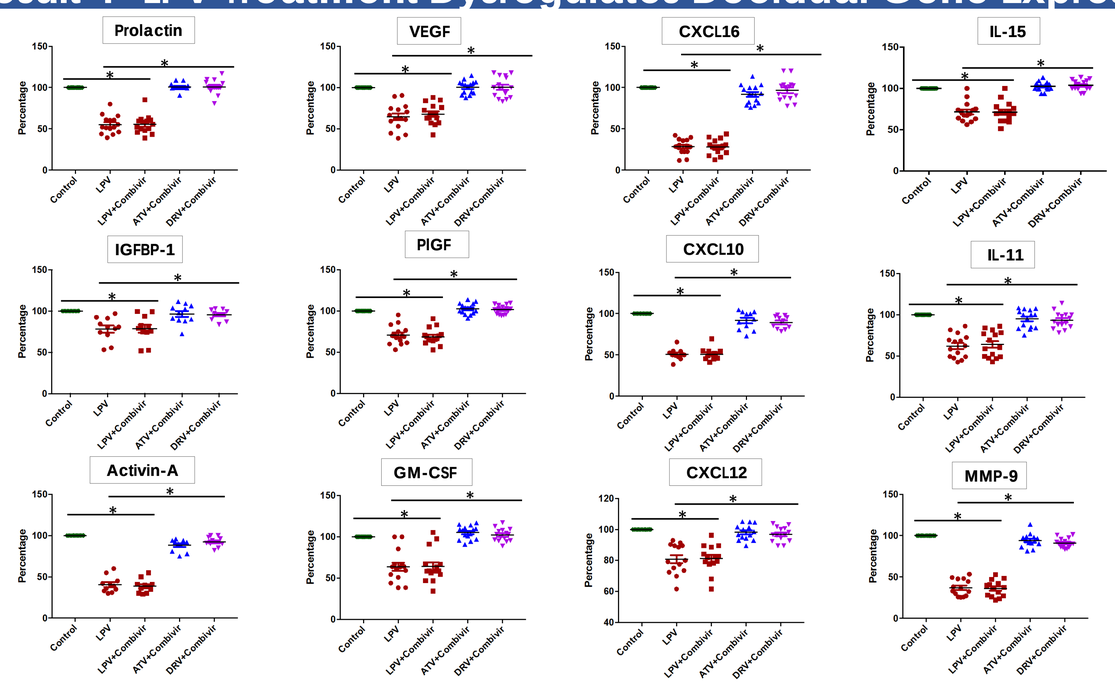
Primary placental explants containing EVT columns were incubated with drugs as indicated, in the presence of decidua conditioned media (DCM) obtained from primary decidual cell culture (also) treated with the same drugs. The explants photographed 48 hours after treatment show reduced EVT outgrowth upon LPV treatment, both with and without the Combivir backbone. The experiment was repeated with 7 different placenta samples and distance migrated from the villous tips is presented as mean ± SEM from 7 independent observations. Statistical significance (P<0.05) is indicated by * for the treatments grouped by a bar.

result 3- LPV Treatment Selectively Depletes uNK Cells



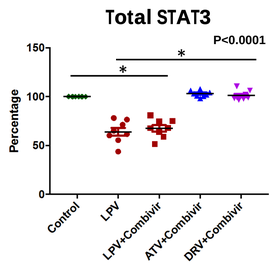
(A) Frequency of various decidual cell types represented as a percentage of the live cells, as determined by flow-cytometry analysis. Decidual cell cultures were treated for 48 hours with PI-cART or DMSO control as indicated. Data are presented as mean ± SEM of n=9 individual experiments using 9 different decidua. Statistical significance (P<0.05) was calculated using ANOVA with Tukey's posthoc analysis. * indicates P<0.05 in the post-test analyses for the treatments grouped by a bar.

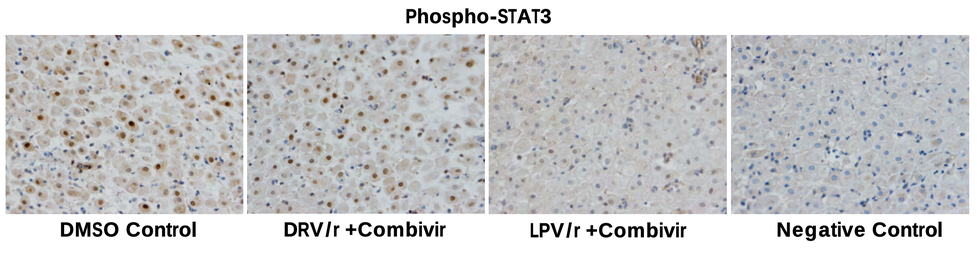
result 4- LPV Treatment Dysregulates Decidual Gene Expression



Multiplex protein analysis of various factors after 48 hour treatment of the decidual cell culture with PI-cART or DMSO control as indicated. A total of 11-15 decidua samples were used to isolate cells which were cultured in the presence of cAMP, progesterone and estradiol. Data presented as mean ± SEM were normalized to the control, which is at 100%. Statistical significance (P<0.05) was calculated using ANOVA with Tukey's posthoc analysis and is indicated by * (P< 0.0001)for the treatments grouped by a bar.

result 5- LPV Treatment Inhibits The Transcription Factor STAT3





ELISA analysis of Total STAT3 using cell lysates of decidual cell cultures (n=8) treated with PI-cART or DMSO as indicated. Statistical significance (P<0.05) is indicated by * for the treatments grouped by a bar.
Immunohistochemical identification of phospho STAT3 in sections of decidual tissue treated with PI-cART or DMSO as indicated.

Conclusions

- LPV treatment impaired trophoblast outgrowth and spiral artery remodelling in an *in-vitro* placenta-decidua co-culture model.
- A dysregulation of decidualization was observed, marked by reduced stromal expression of Prolactin, IGFBP-1 and Activin-A.
- The viability of uNK cells was affected, concomitant with changes in the secretion profile of uNK and stroma cell specific growth-factors and cytokines/chemokines such as VEGF, PlGF, IL-15, CXCL16, MMP-9 etc.
- The effects of LPV treatment could be attributed to a decrease in the expression of transcription factor STAT3, known to regulate decidualization.
- Overall, our data reveal that LPV based cART causes dysregulation of decidualization and impairment of spiral artery remodeling, thereby possibly contributing to inadequate placentation and hence poor birth outcomes in pregnant women living with HIV, upon early initiation of LPV based cART.

acknowledgements

CIHR IRSC
CANADIAN FOUNDATION FOR AIDS RESEARCH CANFAR
CROI Conference on Retroviruses and Opportunistic Infections
UHN