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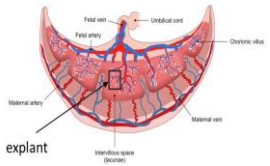

Potential of anti-CMV Immunoglobulins Cytotect® *in vitro* and *ex vivo* in first-trimester placenta model

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INTRODUCTION

We studied *in vitro* and *ex vivo* (placental model) the potential of hyperimmune globulins Cytotect CP® (Biotest, Germany) as a candidate for congenital infection prevention and curative treatment.

CONCLUSION

Suggested efficiency of Cytotect CP® in prophylaxis is sustained by our results *in vitro* and in placental villi. Additional studies will be conducted to evaluate this molecule as a curative treatment.

In vitro

Neutralizing activity of Cytotect CP®

cell-free virus stock (endotheliotropic strains TB40 and VHL) was mixed with Cytotect CP®.MOI of 0.1 and Cytotect CP® concentrations of 0.005 U/mL, 0.015 U/mL, 0.05 U/mL, 0.15 U/mL, 1.5 U/mL). After 1 hour, mix was incubated on a cell monolayer (Fibroblasts cells (FEH): MRC-5 (bioMérieux) and Retinal epithelial cells: ARPE (ATCC)) in 48-well plate for 3h at 37°C, before renewing the medium. After 5 days of incubation at 37°C cells were fixed and stained by immunocytochemistry. Foci were counted to determine the 50% and 90% neutralizing doses (DN50 and DN90)

Viral strain	HEF		ARPE	
	ND_{50} +/- SD (U/mL)	ND_{90} +/- SD (U/mL)	ND_{50} +/- SD (U/mL)	ND_{90} +/- SD (U/mL)
VHL/E	0.014 ± 0.01	0.069 ± 0.02	0.011 ± 0.01	0.067 ± 0.02
TB40/E	0.033 ± 0.01	0.10 ± 0.01	0.032 ± 0.01	0.11 ± 0.02

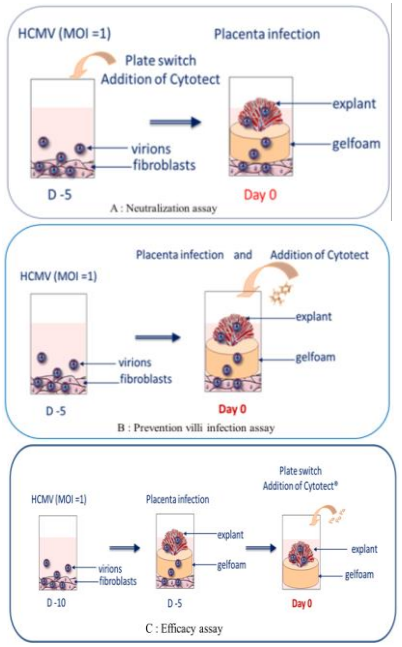
50% and 90% neutralizing doses (ND50 and ND90) were determined graphically for each strain

Cells viability

Measures the percent of cell death (CC) in 96-well plates (Promega France). The percentages of cell death due to the molecule were null even for the highest concentration tested (20 units/mL). The CC50 and CC90 were therefore not reached.

Ex vivo

Three different protocols to evaluate the efficacy of Cytotect CP®. Cytotect CP® was used at 0.015 U/mL, 0.15 U/mL, 1.5 U/mL and 5 U/ml with a viral concentration at a MOI of 1. Trials were carried out in triplicate and on 3 different placentae.



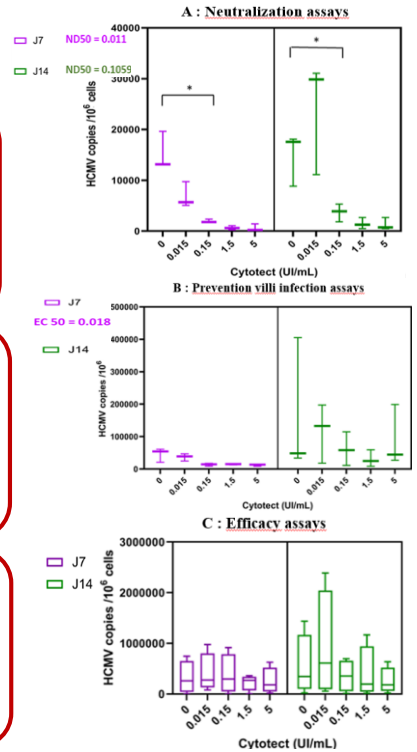
Explants viability
- Harvesting: Days 7, 14
- DNA extraction
- qPCR: ratio copies CMV / 10^6 cells
- β-HCG quantification in supernatants (Cobas, Roche)

Antiviral efficacy
- Harvesting: Days 7, 14
- Viral strain: endotheliotropic strainTB40 (supernatant produced in ARPE)
- DNA extraction
- qPCR: ratio copies CMV / 10^6 cells

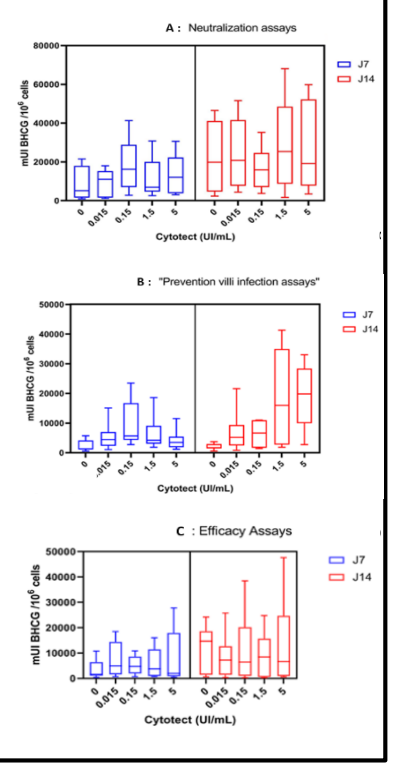
Placenta collection

Placentae were collected from voluntary pregnancy termination (8-14 weeks of gestation) from HCMV seronegative women, after consent, in collaboration with *Collection Biologique HME, Limoges* (CRBioLim).

Cytotect® impact on viral replication in 1st trimester villi



Placenta viability



➟ Cells and villi viability were not impacted by Cytotect CP®. *In vitro* and *ex vivo* neutralization tests have shown Cytotect® CP ability to inhibit the development of infection by endotheliotropic strains. For prevention of villi infection, EC50 was 0.018 U/ml at day 7. Cytotect-CP® did not inhibit viral growth in infected villi.

Bibliography: Morere et al. Ex vivo model of congenital cytomegalovirus infection and new combination therapies. Placenta, 36 (2015), pp. 41-47

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