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Effects of dolutegravir in cardiovascular system development using zebrafish embryos as a model

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Introduction

- Dolutegravir (DTG), is one of the most prescribed antiretroviral drugs around the world for treating people that live with HIV infection, due to its efficacy, safety and low resistance but it was recently associated with a higher risk of hypertension and cardiovascular events (1). Notwithstanding, data on associations between INSTIs and specific cardiovascular outcomes are convoluted, and this question remains unsolved.
- The aim of our study was to characterize the effect of DTG on heart development and angiogenesis, a very important process during the embryogenesis, by using zebrafish embryos, a vertebrate animal model widely accepted for in vivo assessment of chemical and drug toxicity and for developmental studies.

Methods

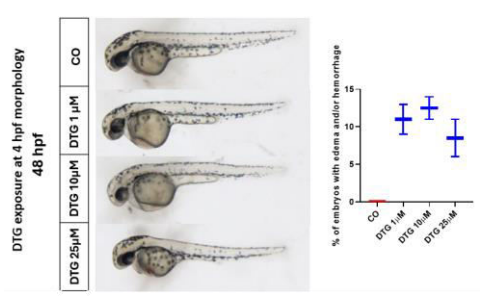
- Wild-type zebrafish embryos were exposed to DTG doses in the range 1-25 µM from gastrula stage (4 hours post fertilization, hpf) up to 144 hpf. We established the dose-curve response in order to find optimal concentration for the next experiments, which was 1 µM (subtherapeutic dose).
- Angiogenesis and heart morphology were analyzed by Whole Mount In situ hybridization (WISH) with specific probes for genes that are crucial in vascular development, by a phosphatase assay and by using transgenic zebrafish lines.

Results

EARLY EMBRYONIC EXPOSURE TO DOLUTEGRAVIR INDUCES PERICARDIAL EDEMA AND/OR HEMORRHAGE

- Wild-type zebrafish embryos were treated at 4 hours post fertilization (hpf) with a subtherapeutic DTG dose (1 µM) by the classic static immersion method and we observed morphological malformations in the developing heart. Notably, we observed pericardial edema and/or hemorrhage at 48 hpf.

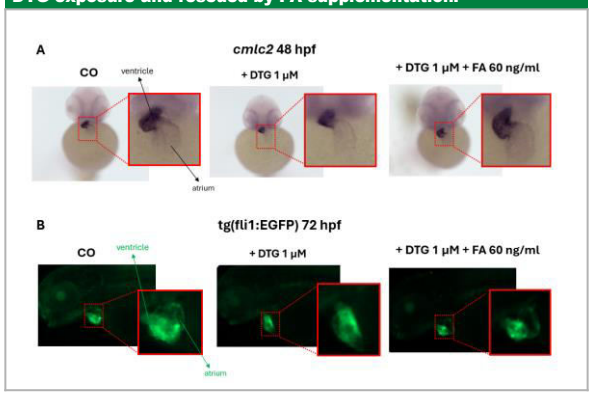
Figure 1 **Morphology assessment at 48 hpf of zebrafish embryos after exposure to dolutegravir (DTG) at 4 hpf (blastula stage) and percentage of pericardial edema and/or hemorrhage.**



DTG EXPOSURE INDUCES DEFECTS IN HEART DEVELOPMENT THAT ARE RESCUED BY POSTEXPOSURE FOLATE SUPPLEMENTATION

- By WISH using the specific probe *cmlc2*, a pan-cardiac marker, we observe that DTG induced an incomplete D-loop formation with a slight bradycardia.
- Using the transgenic zebrafish line tg(*fli1*:EGFP), at 72 hpf we observed morphological malformations in the developing heart.
- In both cases, the supplementation of folic acid (FA) at the concentration of 60 ng/ml rescued alterations.

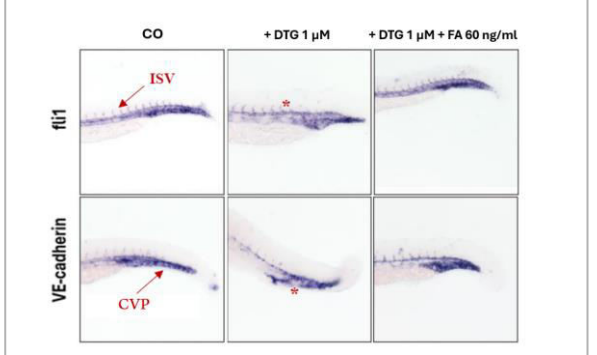
Figure 2 **Morphological malformations of developing heart of zebrafish embryos at 48 hpf (A) and at 72 hpf (B) induced by DTG exposure and rescued by FA supplementation.**



ANGIOGENESIS ALTERATIONS INDUCED BY EARLY EMBRIONIC EXPOSURE OF ZEBRAFISH EMBRYOS TO DOLUTEGRAVIR ARE RESCUED BY POST-EXPOSURE TO FOLIC ACID

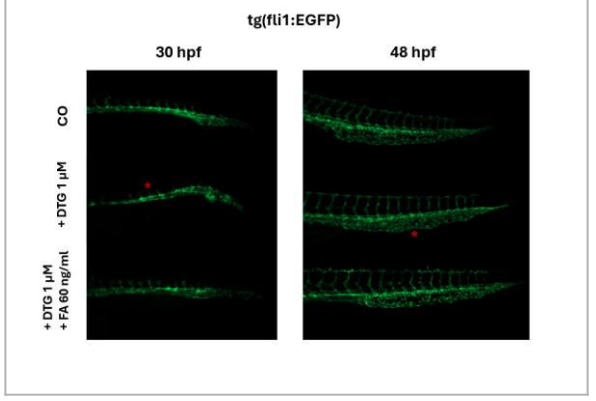
- At 30 hpf, the expression of key-genes in angiogenesis (*fli1* and *Ve-Cadherin*) was reduced in embryos exposed to DTG, while the post-exposure of FA restored the normal expression of the genes.

Figure 3 **DTG exposure decreases the expression of fli1 and Ve-Cadherin, while FA supplementation partially rescues the deficit.**
ISV: Intersegmental Vessels
CVP: Caudal Venous Plexus



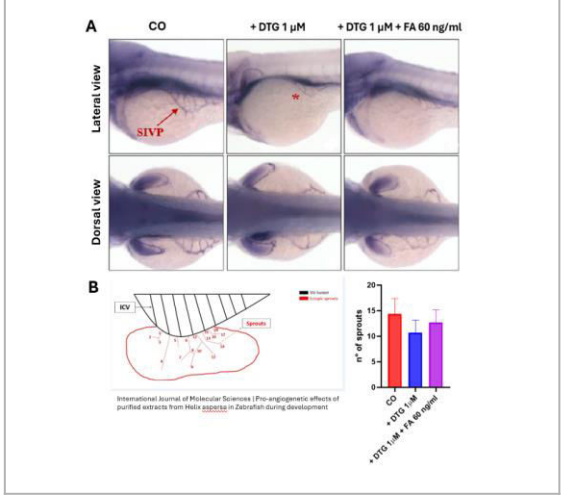
- Moreover, using the transgenic line tg(*fli1*:EGFP), we observed an impaired formation of the intersegmental vessels (ISVs) and the caudal venous plexus (CVP), also in this case FA supplementation restored correct angiogenesis.

Figure 4 ***fli1*-dependent EGFP fluorescence in zebrafish embryos exposed to DTG at the blastula stage, with or without FA supplementation.**



- By phospatase assay, we analysed the generation of the sub-intestinal venous plexus (SIVP) at 72 hpf. DTG exposure decrease the number of sprouts of SIVP, while the FA supplementation restored the physiological number of sprouts.

Figure 5 **DTG exposure decreases the number of sprouts in SIVPS, while FA supplementation rescued this loss. (A) Lateral and dorsal view of zebrafish embryos. (B) Number of sprouts of SIVP.**
SIVP: Sub-Intestinal Venous Plexus



Conclusion

- Our results suggest that DTG may interfere with vessels and heart development, while the folic acid may rescued these alterations. Further studies are however needed to understand the molecular mechanisms underlying these findings.

References

1. Sempere, A., Assoumou, L., González-Cordón, A., Waters, L., Rusconi, S., Domingo, P., Gompels, M., de Wit, S., Raffi, F., Stephan, C., Masiá, M., Rockstroh, J., Katlama, C., Behrens, G. M. N., Moyle, G., Johnson, M., Fox, J., Stellbrink, H. J., Guaraldi, G., Florence, E., … NEAT 022 Study Group (2023). Incidence of Hypertension and Blood Pressure Changes in Persons With Human Immunodeficiency Virus at High Risk for Cardiovascular Disease Switching From Boosted Protease Inhibitors to Dolutegravir: A Post-hoc Analysis of the 96-week Randomised NEAT-022 Trial. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 77(7), 991–1009. https://doi.org/10.1093/cid/ciad297