Development of a zebrafish model to study barrier breakdown in retinal and brain capillaries under pathological conditions

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Background

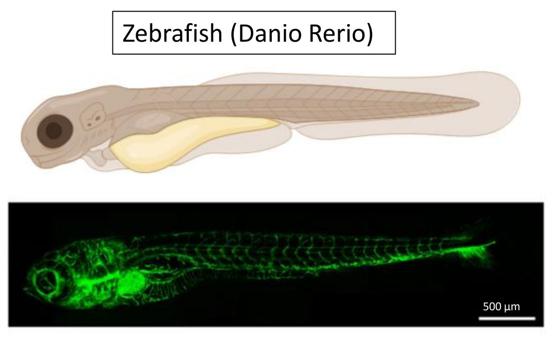
The **blood-retinal barrier** (BRB) and **blood-brain barrier** (BBB) are crucial in protecting the nervous tissue in the retina and brain, respectively. Loss of the proper function of the neuroprotective BBB and BRB is a critical and chronic event in numerous neurological disorders. Further research is required to elucidate how the barrier is affected and how this can be reversed. Zebrafish can be a suitable early *in vivo* model as zebrafish already form these barriers at the larval stage.

The **zebrafish** is the smallest vertebrate model with a functional BBB that is **similar to the human BBB** and offers several advantages, such as high fecundity, fast development, small size and availability of transgenic lines with fluorescent blood vessels or proteins of interest.

The aim of our study is to develop a **zebrafish model** that can be employed to **study pathological breakdown of the BBB**, as a model for both the BBB and **BRB**.

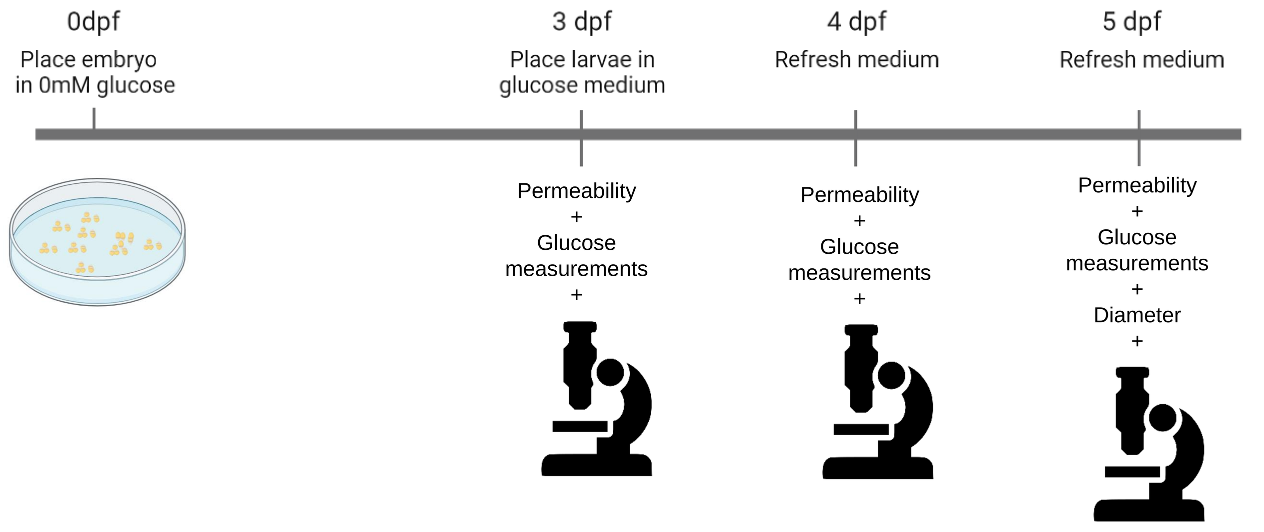
Experimental set-up

Zebrafish (Danio Rerio)



The reporter zebrafish line **Tg(*fli1*:eGFP)** expresses EGFP in the vascular endothelium and is used to visualize blood vessels *in vivo*.

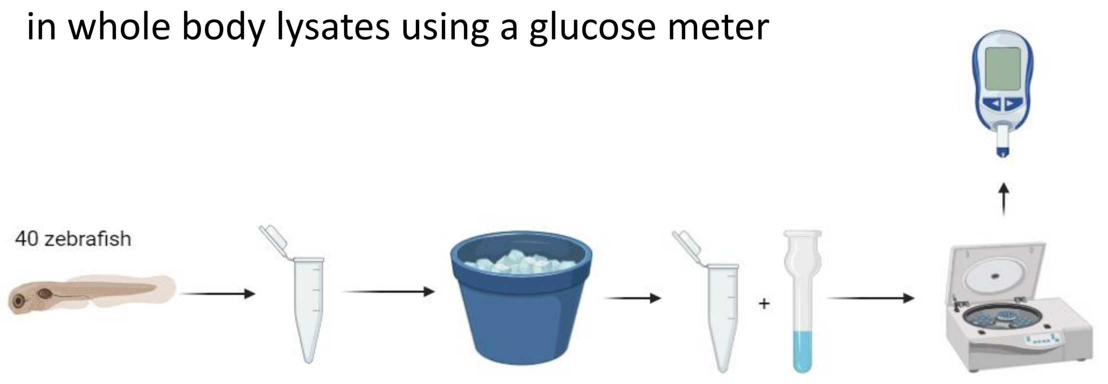
The reporter zebrafish line **Tg(*cldn5a*:eGFP)** expresses EGFP at tight junctions (between endothelial cells)

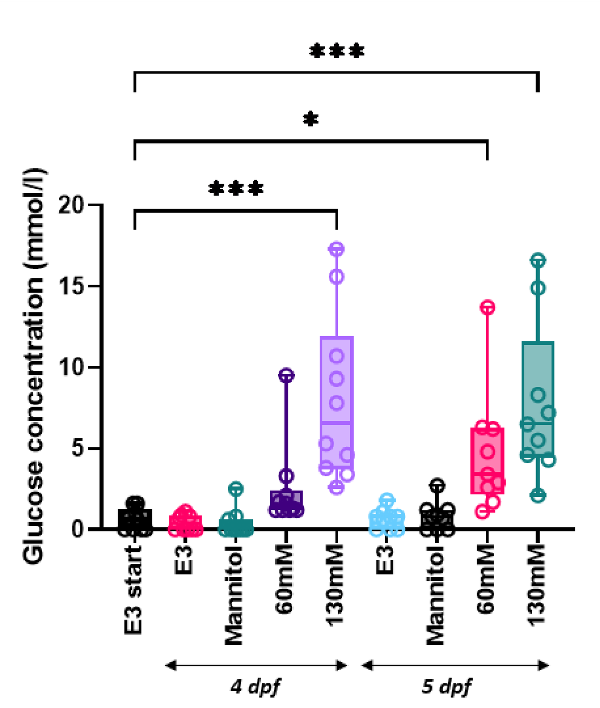


Four conditions:
1. E3 medium (control)
2. 130 mM mannitol (osmotic control)
3. 60 mM glucose
4. 130 mM glucose

1. Glucose uptake

Internal glucose levels at 3, 4 and 5 dpf were measured in whole body lysates using a glucose meter



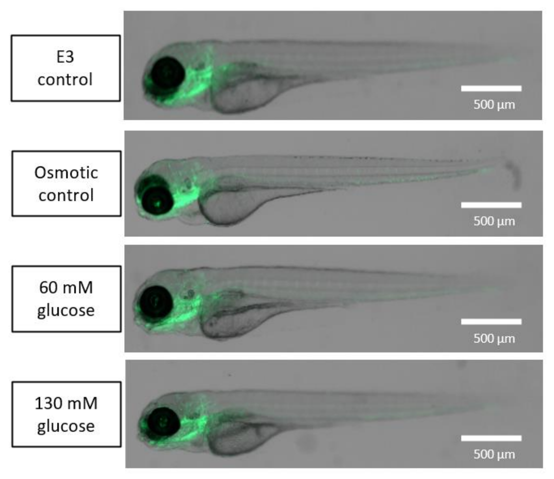


Increased internal glucose levels were found after one day and two days of adding glucose to E3 medium, in a dose-dependent relationship

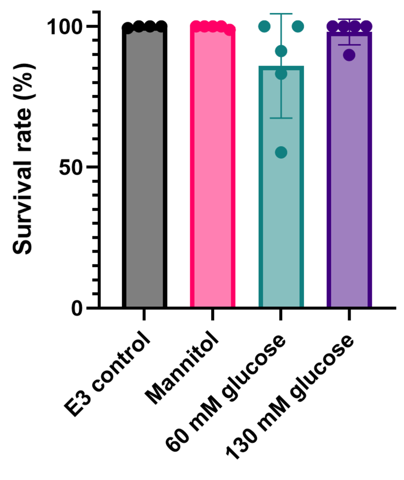
* p < 0.05
** p < 0.01
*** p < 0.001

2. Survival and morphology

Morphology of Tg(fli1:EGFP) zebrafish at 5 dpf after hyperglycemia



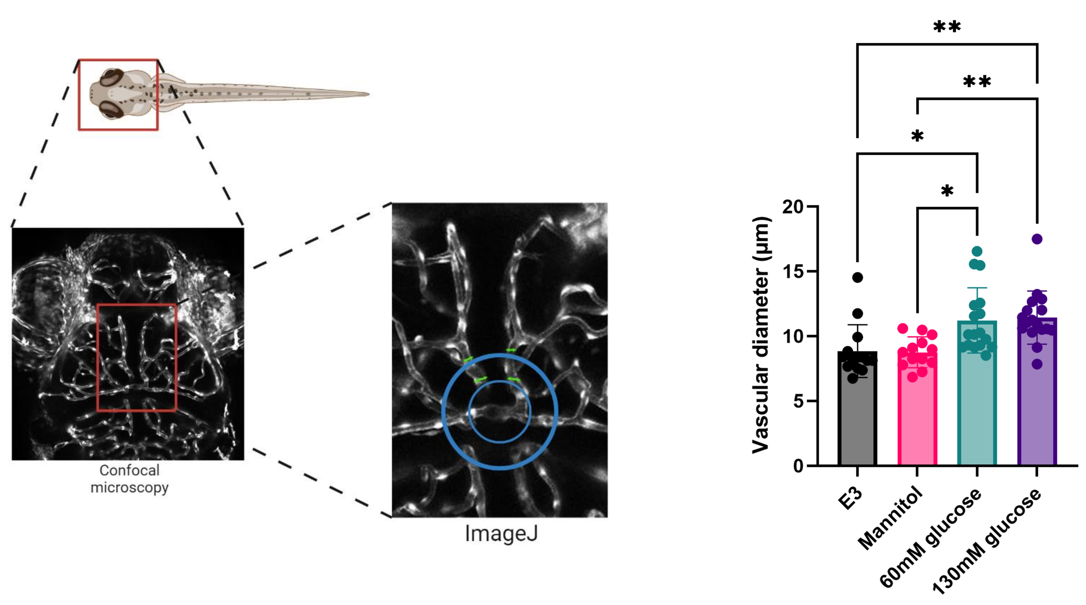
Survival of zebrafish at 5 dpf after hyperglycemia



No effect on morphology and survival rate was found after hyperglycemia

3. Blood-brain barrier characterization

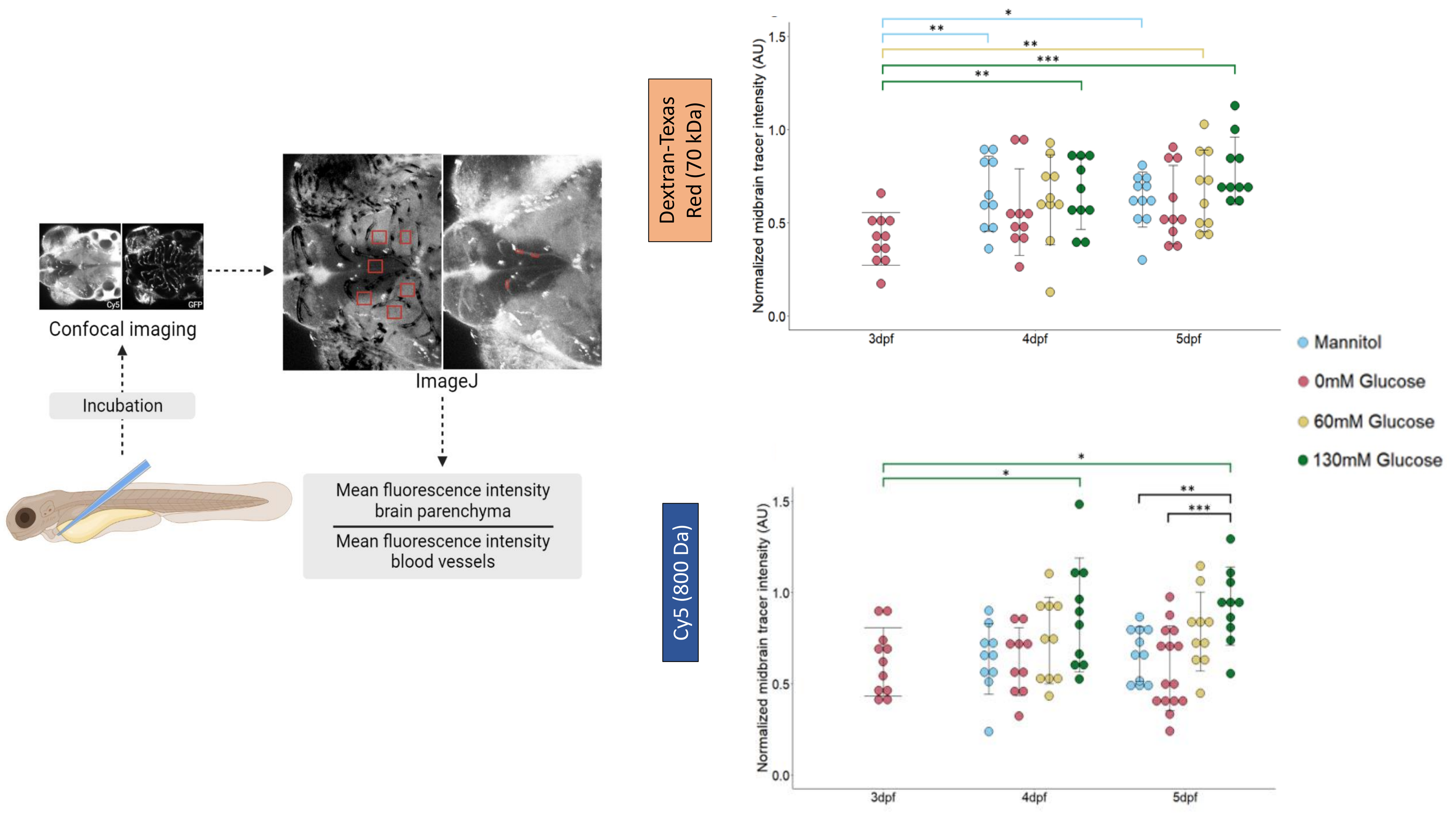
Cerebral blood vessel diameter after hyperglycemia



The diameter of the mesencephalic vein was increased after hyperglycemia compared to control conditions

Cerebral vascular permeability after hyperglycemia

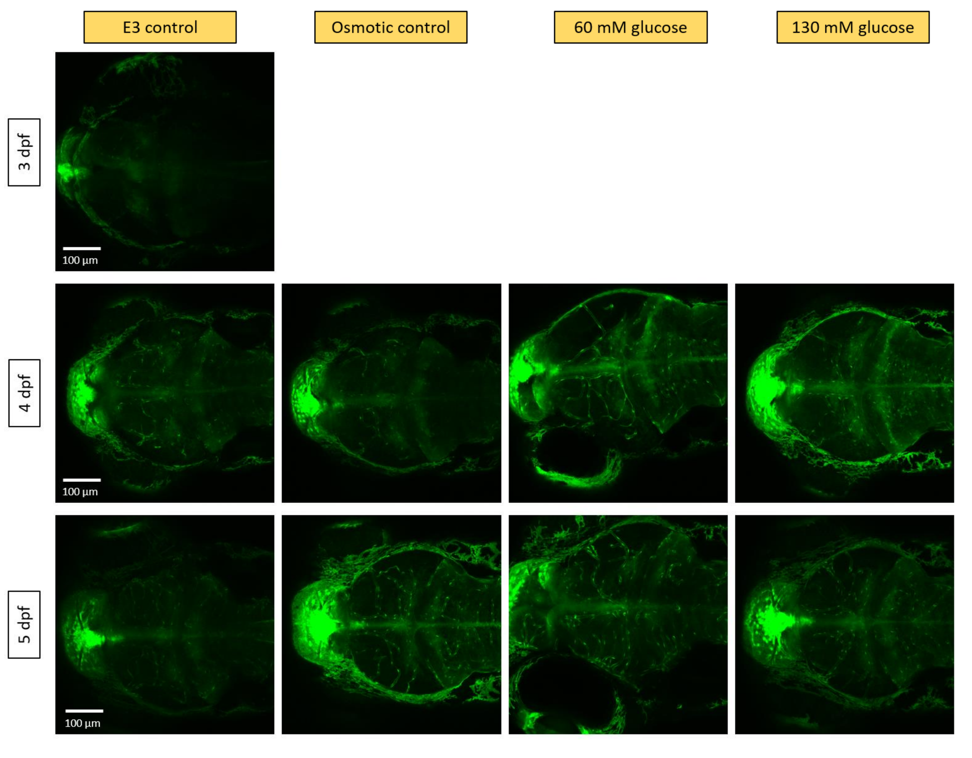
Fluorescent tracers of small and large molecular weight were injected in the zebrafish heart sac. Tracer leakage into the brain parenchyma was studied using confocal microscopy



Tracer leakage was increased after two days of hyperglycemia, and a significant increase in permeability after hyperglycemia was found for the small tracer

Paracellular pathway under hyperglycemia - Claudin-5 expression

Tight junction claudin-5 reporter zebrafish Tg(*cldn5a*:eGFP)



No clear difference in CLDN5 expression was found after hyperglycemia

Conclusion and future directions

Zebrafish can be used as a model to study pathological breakdown in the BBB. As an example, hyperglycemia was used here to induce pathology in the zebrafish brain. Hyperglycemia in zebrafish embryos did not affect morphology and survival, but increased vascular permeability was observed compared to control zebrafish. The mechanism underlying the increase in vascular permeability after hyperglycemia needs to be explored further.

Funding

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