Using zebrafish to model haemorrhagic stroke

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1. The zebrafish model system

- Zebrafish are vertebrates with a well conserved vascular system that is highly appropriate for studying intracerebral haemorrhage (ICH) pre-clinically.
- Highly amenable to genetic manipulation and high-throughput drug screening.
- Larval transparency + transgenic fluorescent reporter lines = powerful tool for studying cellular biology in real time, in intact animals.
- Spontaneous blood vessel rupture can be induced within the brain using chemical or genetic techniques – i.e. non-invasive.
- Using live imaging, we can make observations in zebrafish larvae that are not possible in rodent models of ICH.

3. ICH in zebrafish larvae induces a quantifiable brain injury

- Cell death was assessed using a transgenic fluorescent reporter zebrafish line that expresses a yellow/green fluorescent protein in dying cells (annexinV:ubiq:YFP).
- Significantly increased rates of cell death was observed in the brains of haemorrhaged (ICH+) larvae in comparison to non-haemorrhage siblings (ICH-).
- Cell death 'clusters' were always situated adjacent to the haemorrhage site (asterisks) from 3dpf.

5. ICH in zebrafish larvae induces an innate immune response

- Post-ICH neuroinflammation was assessed using transgenic fluorescent reporter zebrafish lines for neutrophils (*mpo:GFP*) and macrophages (*mpeg1:mCherry*).
- At 3dpf, a significant increase in the number of macrophages, but not neutrophils, recruited to the brain was observed in ICH+ larvae (A).
- Significantly increased numbers of activated (amoeboid) macrophages were also observed in ICH+ larval brains (B,C).
- Expression of the pro-inflammatory cytokine gene, *interleukin-1 β* , was significantly increased following ICH (D).

2. Non-invasive induction of spontaneous ICH

- Brain-specific bleeding induced from 2 days post-fertilisation (dpf) in zebrafish larvae
- Haemorrhages (arrows) were assessed in live animals using a double transgenic reporter zebrafish line that express a green fluorescent protein in blood vessels (*fli1:GFP*) and a red fluorescent protein in erythrocytes (*gata:DSRed*).

4. ICH in zebrafish larvae induces a quantifiable locomotor deficit

- Swimming ability was assessed using an automated tracking system (Daniovision) to quantify locomotor function following ICH induced brain injury.
- ICH+ and ICH- larval siblings were tested at 3, 4 and 5dpf.
- Significant reductions in total distance moved was recorded following ICH at all three time points.

6. Summary

- Zebrafish offer a rapid and accessible disease model system for pre-clinical ICH research.
- Unlike rodent models, spontaneous ICH can be induced in zebrafish larvae using non-invasive procedures.
- In zebrafish, bleeding in the brain causes quantifiable and reproducible brain injury, locomotor and neuroinflammatory phenotypes.
- Importantly, these phenotypes recapitulate key pathological features of the human condition, thereby validating the use of zebrafish as an alternative pre-clinical system for ICH research.
- This model can be used as a new platform for improving our understanding of basic ICH pathophysiology....
-and for future translational drug discovery studies.

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