Inhibitors of developmental angiogenesis in zebrafish effectively inhibit pathological ocular neovascularisation in mice

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Ocular neovascularisation is a pathological feature of human eye diseases including diabetic retinopathy, age-related macular degeneration and retinopathy of prematurity.

In an unbiased approach to discover novel anti-angiogenic drugs, we screened a chemical library in *Tg(fli1:EGFP*) transgenic fish which express EGFP in vasculature. Of the "hits" that inhibit development of the intraocular vasculature in zebrafish, two compounds (11B and 11F) had significant and reproducible anti-angiogenic activity without gross effects on ocular morphology or function. At present, it is not possible for us to disclose the chemical structure of compounds 11B and 11F as a patent application is pending.

To develop these drugs we tested their ability to inhibit Human Dermal Microvascular Endothelial Cell (HMVEC) proliferation, migration, or tubule formation over a wide range of concentrations (0.001 – 10 µM) *in vitro*. Compounds were dissolved in DMSO (0.1%) and HEPES-buffered saline solution and cells were grown in EGM®-2-MV BulletKit® (Lonza) on matrigel™ (BD Biosciences). Both compounds inhibit endothelial tubule formation *in vitro* without significant effects on cell migration or proliferation. 1 µM concentration 11B inhibited tubule formation by 40 +/- 7.8% ($p<0.05$) and 1 µM concentration 11F inhibited tubule formation by 14+/- 3.8% ($p<0.05$).

To test maximum tolerated dose, C57BL/6 adult mice (n = 3/concentration) received a 5 µl intravitreal injection, under ketamine and xylazine anaesthesia, of control, 0.05 µM, 0.5 µM or 5 µM concentration of 11B and showed no adverse effect on eye morphology (left eye was injected with compound, right eye with control of Hank's Balanced Salt Solution, HBSS) as assessed using light microscopy.

The mouse oxygen-induced retinopathy (OIR) model recapitulates features of pathological retinal neovascularisation. This experiment was repeated 3 times, using n = 3-4 pups/treatment. Briefly, mouse pups were placed in hyperoxia (75%) on postnatal day (p) 7 and returned to normal air on p12, inducing proliferative retinal vascular disease. On p13, mice were anaesthetised (ketamine and xylazine) and 1 µl of 11B (0.5 µM or 3 µM dissolved in HBSS) was injected intravitreally into the right eye with the other eye injected with 1 µl HBSS. Additionally, some mice received HBSS injection in both eyes. On p17 mice were culled and eyes fixed and retinal vasculature stained with an isolectin B4 antibody (Sigma) and with corresponding secondary antibody Alexa 568 (Invitrogen, UK). Samples were flat-mounted and photographed. The avascular area of the flatmount was quantified using NIS elements BR and expressed as a percentage of the whole flatmount. 11B significantly inhibits ocular neovascularisation increasing the avascular area by 2.4+/- 0.08 fold (p-value <0.0001) compared to control.

Statistical analysis was conducted using the Student's *t*-test and a *P* value <0.05 was considered significant. Results show mean +/- SEM.

In summary, chemical screens in zebrafish have identified novel small molecules that show potential as therapeutics for ocular neovascularisation associated with blindness.