Pathologic neovascularisation and increased ocular permeability are hallmarks of proliferative Diabetic Retinopathy and advanced Age-related Macular Degeneration which are the leading cause of blindness in the working and aged population, respectively.

Current pharmacologic interventions targeting VEGF are effective in only a subset of patients and require multiple intraocular injections associated with iatrogenic infection. Therefore, there is a clinical need to develop novel VEGF independent therapies with sustained ocular release, that are anti-angiogenic and reduce retinal vascular permeability (RVP).

From 1780 compounds screened from ChemBridge DiverSET library, one lead compound was selected and a bespoke small molecule drug series was developed and patented (US 13/979,447; WO2014012889 A1). These molecules are anti-angiogenic and anti-inflammatory. 

The parent compound quininib (QB) was formulated into quininib-hyaluronan (QB-HA) microneedles; sustained release of this formulation was assessed in vitro, and the safety and efficacy evaluated in vivo.

METHODS

- Quininib-HA Microparticle Fabrication
- Hyaloid Vessel Assay
- Ts(fli1:EGFP) Zebrafish
- HPLC
- Evans’s Blue Assay

RESULTS

- Figure 1. Characterisation and Early Release of Quininib from Quininib-HA Microneedles
- Scanning Electron Microscopy analysis of empty-HA microparticles revealed needle shaped microparticles (a), this microneedle conformation was also apparent in the QB-HA microparticles (as indicated by a red arrow) (b). Electrophoretic Light Scattering of the microparticles indicated a charge of -35.5 mV (c). The early release of QB (d) over 72 hours indicated an initial burst of compound in the first 3 hours as determined by High Performance Liquid Chromatography.

Figure 2. Short Term Evaluation of Safety and Anti-Angiogenic Activity of QB-HA Microneedles in Vivo

Empty- and QB-HA microparticles were incubated with zebrafish larvae for 72 hours from post fertilisation day 2-5. Empty-HA microparticles (0.3 mg/ml) and QB-HA microparticles (0.1 and 0.3 mg/ml) significantly reduced the number of hyaloid vessels (HV) compared to vehicle control (**p<0.01, ***p<0.001), n=3, N=30 (arrows). Treatment was well tolerated with no truncated growth in tail to head length and no pericardial oedema observed (arrows), representative images from 3 independent experiments, with 10 zebrafish per treatment group (n=3, N=30) (c).

Figure 3. Long Term In Vitro QB Release From HA Microparticles and Anti-Angiogenic Activity in Vivo

Approximately 800 µM of QB was released from QB-HA microparticles over 16 weeks corresponding to 20% of QB contained in the microparticles (a). Hyaluronidase induced an increase in QB release, approximately 2,400 µM correlating to 60% of QB contained in the microparticles was released (triangles) (b). QB (10 µM) released into 10% DMSO in PBS, or increasing concentrations of hyaluronidase in 10% DMSO in PBS from QB-HA microparticles were incubated with zebrafish larvae from post fertilisation day 2-5, and significantly inhibited the formation of primary HV (white arrows) compared to vehicle control (c), this was comparable to the inhibition observed with neat QB (10 µM) indicated in red (d); (**p<0.01), n=3, N=30. Treatment was well tolerated with no truncated growth in tail to head length and no pericardial oedema as indicated by arrows (e).

CONCLUSIONS

- We have developed a formulation of novel QB-HA microneedles which allow for a sustained release of active QB for at least 16 weeks in vitro and 4 weeks in vivo.
- We have established for the first time a cysteiny leukotriene induced model of RVP in Brown Norway rats.
- We have successfully inhibited RVP in this model with novel QB-HA microneedles.
- QB-HA microneedles provide a potential adjunctive or alternative treatment option for proliferative Diabetic Retinopathy and advanced Age-related Macular Degeneration by promting a sustained drug delivery mechanism of a novel compound which has a different mechanism of action to current therapies.

REFERENCES


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