

Monomethyl fumarate inhibits angiogenesis and fibrosis in neovascular age-related macular degeneration models

Swathi Priya MN¹, Fergus C. McLellan¹, Peter R Wich², Tushar Kumeria³, Mark Willcox¹, Daisy Y. Shu¹

¹School of Optometry & Vision Science, University of New South Wales, Sydney, NSW 2052, Australia

²School of Chemical Engineering, University of New South Wales, Sydney, NSW 2052, Australia

³School of Materials Science & Engineering, University of New South Wales, Sydney, NSW 2052, Australia

swathi_priya.mn@unsw.edu.au

Abstract

INTRODUCTION: Neovascular age-related macular degeneration (nAMD) is a sight-threatening disease, characterized by abnormal blood vessel growth (choroidal neovascularization) and fibrosis. These pathological changes occur through a process involving endothelial cell migration and endothelial-mesenchymal transition (EndMT). We previously showed that dimethyl fumarate (DMFu), an FDA-approved drug for multiple sclerosis, has anti-inflammatory and anti-angiogenic activity in the retina. We explored whether monomethyl fumarate (MMF), the active metabolite of DMFu, could suppress angiogenesis and fibrosis in primary human retinal endothelial cells (HREC) and mouse choroidal explants, offering a novel therapeutic approach for nAMD. **METHODS:** HREC were treated with 100 μ M MMF or DMSO (vehicle control) in the presence of transforming growth factor- β 2 (TGF- β 2, 10 ng/mL; pro-fibrotic) or vascular endothelial growth factor (VEGF, 10 ng/mL; pro-angiogenic) to model nAMD pathology. Angiogenic and fibrotic responses were assessed using scratch wound migration (n=6), tube formation (n=4), and cytotoxicity assays (n=6). Expression of EndMT markers, alpha-smooth muscle actin (α -SMA) and vimentin, was evaluated by immunofluorescence (n=3). In an *ex vivo* mouse choroidal sprouting model (n=9), choroidal explants isolated from C57BL/6 mice (4-5 weeks old) were cultured, treated with VEGF and/or MMF. Sprouting area was quantified to assess anti-angiogenic capacity. **RESULTS:** MMF was tested from concentrations 50 μ M to 200 μ M, at 100 μ M showed minimal cytotoxicity ($\leq 15\%$). MMF significantly reduced TGF- β 2-induced cell migration, blocking wound closure up to 14 hours (TGF- β 2 vs. TGF- β 2 + MMF: $p < 0.0001$). MMF also suppressed VEGF-induced tube formation ($p < 0.05$). Furthermore, MMF reduced TGF- β 2-induced upregulation of EndMT markers α -SMA and vimentin. The anti-angiogenic potential was further demonstrated using the mouse choroidal sprouting assay, VEGF increased sprouting response compared to the control. MMF significantly suppressed VEGF-induced sprouting compared to vehicle control (< 0.05). **CONCLUSION:** MMF demonstrates anti-angiogenic and anti-fibrotic efficacy positioning it as a promising therapeutic agent for nAMD. However, MMF undergoes rapid clearance and poor bioavailability. Future studies will investigate *in vivo* efficacy and explore nanoparticle-based drug delivery systems to enhance bioavailability and achieve sustained release.