Targeting the intestinal lymphatic system using an oral triglyceride mimetic prodrug enhances immunosuppressant activity *in vivo*

R. Kochappan,[1,2] S. Han,[1,2] L. Hu,[1,2] T. Quach,[2,3] D. Senyschyn,[1,2] E. Cao,[1] G. Lee,[1,2] F. Lim,[2,3] J.S. Simpson,[2] J. Mintern,[4] N.L. Trevaskis[1] and C.J.H. Porter,[1,2] [1]Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, 399 Royal Parade, Parkville, Melbourne, VIC 3052, Australia, [2]ARC CoE in Convergent Bio Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, 399 Royal Parade, Parkville, Melbourne, VIC 3052, Australia, [3]Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, 399 Royal Parade, Parkville, Melbourne, VIC 3052, Australia and [4] Bio21 Molecular Science & Biotechnology Institute, The University of Melbourne, Unit 400/30 Flemington Rd, Parkville, Melbourne, VIC 3052, Australia.

Purpose: Intestinal lymph is important for immune surveillance in the gastrointestinal tract, with approximately 50 - 70% of the immune cells present within the gut associated lymphoid tissue (GALT) and the mesenteric lymph nodes (MLN). It also serves as a channel for the transport of dietary lipids from the gut to the systemic circulation (Trevaskis *et al.*, 2015). The aim of this study was to harness endogenous lymphatic transport processes for dietary lipids and to use them to promote the delivery of an immunosuppressant to the immune cells present in the gut. To facilitate this we synthesised a triglyceride mimetic prodrug of mycophenolic acid (MPA-2-TG) (Han *et al.*, 2014) and investigated the pharmacodynamic benefit of targeting the intestinal lymph using the prodrug version of mycophenolic acid (MPA).

Methods: The intestinal lymphatic transport of MPA and MPA-2-TG, was assessed after intraduodenal infusion by cannulating the mesenteric lymph duct in anesthetized mice (100 mg/kg ketamine and 10 mg/kg xylazine, ip). The lymph samples were assayed by HPLC-MS/MS. Immunosuppression was studied using an oral ovalbumin challenge model in mice. Ovalbumin specific $CD4^+$ T cells were purified, labelled with proliferation tracking dye and ~2×10^6 of these cells adoptively transferred into syngeneic mice. Feeding ovalbumin to recipient mice resulted in the stimulation and proliferation of T cells. The mice were treated orally, twice daily for 3 days, with 50 mg/kg MPA or the equimolar dose of MPA-2-TG. At the end of the treatment, mice were killed and T cell proliferation in lymph nodes was studied using flow cytometry. A similar protocol was followed using ovalbumin specific $CD8^+$ T cells to evaluate the effect on $CD8^+$ T cell proliferation.

Results: The lymphatic uptake of MPA-2-TG (17.3 % dose) was higher than MPA (0.14 %). In the immunosuppression studies, MPA-2-TG treatment significantly reduced the proliferation of $CD4^+$ T cells with most cells (~80%) found in generation 2 or lower in MLN. In contrast, MPA had no significant effect when compared to the ovalbumin group. Similar observations were seen in case of $CD8^+$T cells where MPA-2-TG treatment significantly reduced cellular proliferation in the MLN, with most dye labelled $CD8^+$ cells (~80%) being found in generation 4 or lower after OVA stimulation. MPA again had no significant effect on the proliferation of $CD8^+$ T cells.

Conclusions: Targeting MPA to lymphocytes in the intestinal lymph and MLN significantly enhances immunosuppressive activity in an oral ovalbumin challenge model. This approach has potential benefit in disease states where an aberrant immune response is initiated in the gut.

Han S, Quach T, Hu L, Wahab A, Charman WN, Stella VJ, Trevaskis NL, Simpson JS, Porter CJH. (2014) *J Control Release*, **177:** 1-10.

Trevaskis NL, Kaminskas LM, Porter CJH. (2015) *Nat Rev Drug Discov*, **14:** 781-803.