Ibrutinib has both anti-inflammatory and anti-diabetic effects in a model of HFD induced insulin resistance and diabetic nephropathy

Gareth SD Purvis[1,2], Caroline O'Riordan[2], Haidee Aranda[1], David R Greaves[1] and Chris Thiemermann[2]

[1] Sir William Dunn School of Pathology, University of Oxford, OX1 3RE.

[2] William Harvey Research Institute, Queen Mary University of London, EC1M 6BQ.

Introduction: The link between low-grade inflammation and insulin resistance is not completely understood, however, there is compelling evidence that the activation of both NF-kB and the NLRP3 inflammasome play pivotal roles. Currently, there are no specific anti-inflammatory medicines that target the cross talk between inflammation and metabolic derangement. Here we investigate the effect of ibrutinib (an FDA approved bruton's tyrosine kinase inhibitor) as an anti-inflammatory drug in a murine model of high-fat-diet (HFD) induced insulin-resistance and microvascular disease.

Methods: Cytokine secretion was measured in BMDMs pre-incubated with ibrutinib (1-100 uM) followed by LPS (100 ng/ml) stimulation by ELISA. Ibrutinib's ability to inhibit NF-kB was measured using an NF-kB reporter cells line (InvivoGen) and inflammasome activation using Caspase Glo 1 inflammasome assay (Promega). C57BL/6 mice were fed a chow or HFD for 12 week, at week 7 HFD fed mice were randomly assigned into 2 groups and treated with ibrutinib (3 or 30 mg/kg; p.o.; 5 x per week for 6 weeks; n=9 per group). Prior to harvest mice were placed in metabolic cages to collect urine (renal function), and challenged with oral glucose (1 mg/kg) after 6 h fast (OGTT).

Results: Pre-incubation of BMDM with ibrutinib inhibited the production of pro-inflammatory cytokines and chemokines (Il-1B, TNFa, IL-6 and CXCL1 and CCL3) in a concentration dependent manner. We then tested if ibrutinib directly inhibits NF-KB activation using an NF-KB reporter cells line, ibrutinib significantly inhibited NF-kB (IC_{50} 32±2 uM) and inflammasome (IC_{50} 11±2 uM). Having shown that ibrutinib is a potent anti-inflammatory *in vitro* we wanted to test if it could be used in a model of low-grade chronic inflammation; high-fat diet induced insulin resistance. When compared to mice fed a chow diet, mice fed a HFD have an augmented OGTT, suggesting the development of severe insulin resistance. Prolonged treatment with ibrutinib to mice fed a HFD improved OGTT (3 mg/kg; 1935±49 vs. 1335±54 au; $P<0.05$). Additionally, mice fed a HFD and treated with ibrutinib had less lipid accumulation in the liver measured by Oil-Red O staining (less hepatosteatosis) and lower albumin-to-creatinine ratio (less renal dysfunction) (3 mg/kg; 69.7±6.2 vs. 46.3±4.3 au; $P<0.05$) compared to mice fed a HFD. Indeed, mice fed a HFD had increased activation of NF-kB (nuclear translocation of p65) and inflammasome (proteolytic cleavage of IL-1β) in both the kidney and liver, while treatment with ibrutinib inhibited the activation of NF-kB and NLRP3 inflammasome.

Conclusion: Ibrutinib represents a novel drug repurposing opportunity for an oncology medicine to a new indication in the treatment of type-2 diabetes.

Word 418.