Longitudinal investigation of microbiota dynamics in a model of mild chronic DSS-induced colitis in wild-type (WT) C57BL/6 mice receiving diets with different iron contents

Mahalhal A, Campbell B.J., Pritchard D.M., Probert C.S.

Introduction: Iron deficiency anaemia is common in inflammatory bowel disease (IBD). Iron supplementation may induce or exacerbate colitis in rats (*APT* 2001; 15:1989-99). Dysbiosis is common in IBD and iron contributes to this as it is a growth factor for some bacteria. We investigated the long-effect of dietary iron supplementation and iron reduction on the intestinal microbiome in a chronic murine model of colitis. We report results of changes at the phylum level.

Methods: Studies were performed on 6 groups of 8 WT mice. Chronic colitis was induced with 1.25% dextran sodium sulphate (DSS) for 5 days, followed by 16 further days on water [for three consecutive cycles]. DSS-treated mice were fed one of three diets (start from day-1 of experiments): low iron [LI] (100ppm), normal iron [NI] (200ppm) and high iron [HI] (400ppm) supplemented chow. Also, three non-DSS-treated groups were studied and fed similarly. Half of the mice in each control group were treated with one cycle of acute 2% DSS for 5 days at day 53, followed by 5 further days on water. All mice were sacrificed at day 63. Clinical and pathological data were compared at day-1, 21, 42 and 63 (chronic) and day-1 and 10 (acute); bacterial gDNA was extracted from faeces and microbiota composition determined from the sequence of V4 region of *16S* rDNA on the Illumina MiSeq platform. Statistical inferences were made using Kruskal-Wallis H-test with post-hoc analysis (*Bioinformatics* 2010; 26:715-21).

The results: DSS-induced colitis in all treated mice. Chronic DSS colitis was not associated with significant weight loss, while weight loss in acute DSS mice was greatest in the LI diet group ($p<0.001$ LI vs. HI; $p<0.05$ for LI vs. NI). Histologically, the colitis features were worse in LI ($p<0.001$) than HI and NI ($p<0.01$ each) and more prominent in acute DSS-treated mice ingesting low and high iron diets, with median colitis scores 6 & 5.5 respectively. However, faecal phyla changes were seen in both LI and HI DSS-treated groups and controls fed a HI diet for chronic experiments only: *Proteobacteria* were increased significantly at day-63 ($p<0.01$) in LI, HI DSS groups and HI controls. *Actinobacteria* were also increased in the latter group whereas, a reduction was observed for *Bacteroidetes* ($p<0.028$) in the HI DSS group.

Conclusions: Changes in nutritional luminal iron exacerbate colitis. Oral administration of DSS causes a reproducible acute colitis, followed by a slow recovery phase with a concomitant chronic inflammation. Chronic colitis was worse in mice fed low or high iron diets. Dysbiosis was found in mice with chronic colitis and altered iron intake as well as controls receiving high iron diets. Iron therefore appears to contribute to the dysbiosis associated with IBD.