Influence of iron on gut microbial composition in acute DSS-induced colitis in C57BL/6 mice

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 acute effect of dietary iron supplementation and/or reduction on the intestinal microbiome in a murine model of colitis. We report results of changes at the phylum level.

Methods: Studies were performed on six groups of wild-type mice (6/group). Acute colitis was induced with 2% dextran sodium sulphate (DSS) for 5 days, followed by 5 further days on water. DSS-treated mice were fed one of three diets (from day-1 of DSS treatment): Low iron [LI] (100ppm), normal iron [NI] (200ppm) and high iron [HI] supplemented (400ppm) chow. Also, three non-DSS-treated groups were studied and fed similarly. Clinical and pathological data were compared. At day-1 vs. day-10, 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 Welch's t-test with post-hoc analysis (*Bioinformatics* 2010; 26:715-21).

The results: DSS-induced colitis was exacerbated in LI and HI groups, more so in LI mice (as determined by mean total body weight loss [9.2%, at day-8] and significantly worse histology; median score 2.5, at day-10). However, faecal phyla changes were only seen following DSS treatment in the HI group at day-10 vs. day-1 with increased *Proteobacteria* (*P* <1.38e-3), *Actinobacteria* (*P* <5.13e-3) and *Fusobacteria* (*P* <3.25e-3), and decreased *Firmicutes* (*P* <6.96e-3) and *Bacteroidetes* (*P* <5.98e-3).

Conclusion: DSS-induced colitis occurred in each group, but the severity was greatest in mice on low or high iron diets. Dysbiosis was only evident in mice receiving the high iron diet. These data suggest that 1) luminal iron may influence the severity of colitis, but 2) dysbiosis occurred when DSS was administered with the high iron diet, but not with low and normal iron diets. These findings suggest a role for iron in the dysbiosis seen during relapse of IBD and likely GI symptoms following iron supplementation.

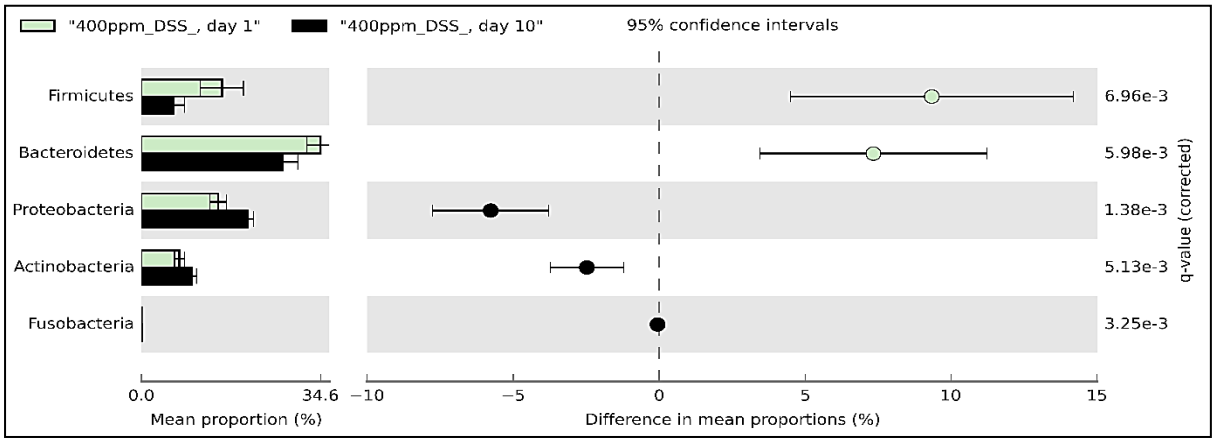

Figure 1: Extended error bar plot for the five phyla (*Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* and *Fusobacteria*) that have a difference between the proportions of day-1 and day-10 for 400pm iron DSS-treated mice. This post-hoc plot for each phylum indicating ***1)*** the mean proportion of sequences at day-1 and-10, ***2)*** the difference in mean proportions for each phylum comparing pre and post-DSS treatment, and ***3)*** a p-value indicating if the mean proportion is equal for each time point. Statistical differences were assessed by Welch's t-test followed by Storey's FDR multiple test correction.