Cellular and Molecular Gastroenterology and Hepatology Editorial

Hidden dangers of antibiotic use: increased gut permeability mediated by increased pancreatic proteases reaching the colon

Professor Robin Spiller

Nottingham Digestive Diseases Biomedical Research Unit

Nottingham Digestive Diseases Centre

University of Nottingham

Queens Medical Centre

E Floor West Block

Nottingham

NG7 2UH

United Kingdom

As we learn more about the importance of our microbiome in inflammatory, metabolic and functional disorders, we are coming to appreciate the risks of disturbing this with broad-spectrum antibiotics. We have known for many years that as antibiotics have dramatically reduced the risk of infectious diseases, the incidence of other diseases like inflammatory bowel disease, obesity and type 2 diabetes mellitus have increased. While the causes are undoubtedly multifactorial, meta-analysis shows that for Crohn's disease (CD) antibiotic exposure nearly doubles the risk [1]. The current paper provides one possible mechanism where by this might occur. The authors studied 32 patients who provided stool samples before and after taking a range of antibiotics. They found that 8 patients showed a marked increase in faecal protease activity, mostly due to increased pancreatic proteases. Supernatants from the stools with increased protease activity increased permeability when applied to a polarised monolayer of cultured colonic epithelial cells. This was particularly evident in those given antibiotics such as levofloxacin and metronidazole which are known to markedly reduce the faecal microbiota, while much less obvious after antibiotics like rifaximin, which has less impact on the microbiota. The pancreas secretes around 500mg of tryptase daily into the gut yet only a about 1mg is excreted and early animal experiments showed that this degradation was largely prevented by broad spectrum antibiotics[2].

This paper takes these ideas forward by examining the impact of antibiotic induced increased fecal proteases on gut barrier function using cell line and animal models. They showed that in mice, broad-spectrum antibiotics (vancomycin and metronidazole) increased fecal proteolytic activity, mostly due to rises in pancreatic serine proteases like trypsin and chymotrypsion. This lead to a rise in permeability, which in normal wild type mice lasted around after 14 days but did not lead to an inflammatory response. However in genetically susceptible IL10 knock out mice, repeated antibiotic courses lead to the development of a chronic colitis which could be blocked by a specific protease inhibitor. While Crohn's disease is important, it is also thankfully rare and the majority of antibiotic courses do not lead to the development of Crohn's disease. However, Irritable Bowel

Syndrome (IBS) is around 100 times more common, affecting around 1 in 10 of the population. IBS is also associated with antibiotic use, with a 3 fold increase in risk of developing functional GI symptoms in the 4 months after antibiotic consumption[3]. IBS with diarrhoea (IBS-D), which is characterised by rapid colonic transit and hypersensitivity of the gut to distension, is associated with increased fecal proteases. Furthermore in animal studies IBS-D fecal supernatants have been shown to act via protease activated receptors type 2 (PAR2) to sensitise murine colons to distension, suggesting fecal proteases may account for the visceral hypersensivity in IBS-D[4], whether the same is true for CD remains unexplored. Rapid transit, which is a feature of both IBS-D and Crohn's disease and reduces the time available for protease degradation is another mechanism whereby fecal proteases could be increased. Observational studies show fast transit in IBS-D is correlated with elevated fecal protease activity, which has been shown to be of pancreatic origin[5]. Furthermore purging the bowel using an osmotic laxative depletes the faecal bacteria and increases faecal protease substantially. These studies in IBS-D patients may be relevant for understanding CD whose features overlap with IBD including increased gut permeability, low grade immune activation and increased mast cell activation in some, though not all, IBS patients.

Manuscript Click here to access/download;Manuscript;Commentary

Spiller.docx

The effect demonstrated in this paper is short-lived so how could it cause chronic diseases? The authors argue that while the increased permeability does not induce inflammatory response in normal animals in genetically susceptible individuals it could initiate a self-perpetuating circle of increased permeability allowing access of microbial antigens leading to immune activation which in turn increases permeability. Several studies have found significant alterations in gut microbiota in both CD and IBS-D which have been linked to mucosal expression of inflammatory genes [6]. The effect of antibiotic shown in the current study supports the idea that an altered gut microbiota might be an important part of the pathogenesis of both conditions. However not all bacteria degrade proteases so specifically examining whether the altered microbiota in these diseases have impaired protease degradation would be a logical next step. Gastroenterologists are perhaps more aware than most doctors through their experience in treating *Clostridium difficile* of the risks of depleting the microbiota with broad spectrum antibiotics and new better targeted agents are being developed to treat this condition[7;8]. However most antibiotics are given for non-GI infections and until recently, their impact on gut microbiota has been largely accepted as inevitable. The current study provides an important reason by we should be developing more specifically targeted antibiotics and thus achieve the benefits of antibiotics without doing harm to long-term health.

Reference List

(1) Ungaro R, Bernstein CN, Gearry R, Hviid A, Kolho KL, Kronman MP et al. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a meta-analysis. *Am J Gastroenterol* 2014; 109(11):1728-1738.

(2) Genell S, Gustafsson BE. Impaired enteric degradation of pancreatic endopeptidases in antibiotic-treated rats. *Scand J Gastroenterol* 1977; 12(7):801-809.

(3) Maxwell PR, Rink E, Kumar D, Mendall MA. Antibiotics increase functional abdominal symptoms. *Am J Gastroenterol* 2002; 97(1):104-108.

(4) Gecse K, Roka R, Ferrier L, Leveque M, Eutamene H, Cartier C et al. Increased faecal serine protease activity in diarrhoeic IBS patients: a colonic lumenal factor impairing colonic permeability and sensitivity. *Gut* 2008; 57(5):591-599.

(5) Tooth D, Garsed K, Singh G, Marciani L, Lam C, Fordham I et al. Characterisation of faecal protease activity in irritable bowel syndrome with diarrhoea: origin and effect of gut transit. *Gut* 2014; 63(5):753-760.

(6) Jalanka-Tuovinen J, Salojarvi J, Salonen A, Immonen O, Garsed K, Kelly FM et al. Faecal microbiota composition and host-microbe cross-talk following gastroenteritis and in postinfectious irritable bowel syndrome. *Gut* 2014; 63(11):1737-1745.

(7) Louie T, Nord CE, Talbot GH, Wilcox M, Gerding DN, Buitrago M et al. Multicenter,

Double-Blind, Randomized, Phase 2 Study Evaluating the Novel Antibiotic Cadazolid in Patients with Clostridium difficile Infection. *Antimicrob Agents Chemother* 2015; 59(10):6266-6273.
(8) Vickers RJ, Tillotson GS, Nathan R, Hazan S, Pullman J, Lucasti C et al. Efficacy and safety of ridinilazole compared with vancomycin for the treatment of Clostridium difficile infection: a phase 2, randomised, double-blind, active-controlled, noninferiority study. *Lancet Infect Dis* 2017; 17(7):735-744.