Citation: Clinical and Translational Gastroenterology (2016) 7, e195; doi:10.1038/ctg.2016.52
 2155-384X/16

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EDITORIAL

Rifaximin, Microbiota Biology, and Hepatic Encephalopathy

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Rifaximin is beneficial in the treatment of minimal hepatic encephalopathy (MHE). Kang *et al.* (*Clin Transl Gastroenterol* 7: e187; doi:10.1038/ctg.2016.44) investigated the effects of rifaximin in a mouse model of MHE-associated microbiota without concomitant liver disease. In addition to some impact on the composition of microbiota, rifaximin altered bacterial functions, ameliorated local and systemic inflammation, and reduced enterocyte glutaminase activity. We discuss these effects as well as the interpretation of the permeability studies, given the potential interaction of dysbiosis with dysfunctional intestinal barrier, leading to systemic inflammation and increased uptake of bacterial metabolites that contribute to MHE in the presence of hepatic dysfunction.
Clinical and Translational Gastroenterology (2016) **7,** e195; doi:10.1038/ctg.2016.52; published online 6 October 2016

There is an altered profile of the gut microbiome in patients with cirrhosis and its complications;[1,2] modulation of the microbiome with rifaximin treatment is associated with improvement in hepatic encephalopathy.[3] In this issue of the journal, Kang *et al.*[1] assessed the actions of rifaximin on intestinal barrier, inflammatory milieu, and ammonia generation through studies conducted in four groups of germ-free (GF) mice: GF, GF+rifaximin, GF humanized with stools from a patient with minimal hepatic encephalopathy (MHE), and humanized plus rifaximin.

The beneficial effects on encephalopathy include: first, reduction in bacterial production and absorption of ammonia in the intestine, particularly, the colon; second, a change in other intraluminal metabolites such as amino acids or bacterial products that might lead to hepatic encephalopathy; or third, a decrease in intestinal permeability that would reduce absorption of such factors. The authors concluded that rifaximin has more mechanisms of action in addition to its bactericidal and bacteriostatic effects. Importantly, the investigators used a formulation of rifaximin that was soluble in water and did not require bile acids to enhance rifaximin's solubility. This precaution is important; whereas, the standard formulation of rifaximin is soluble in the small intestine where the concentration of bile acids is high enough; it is typically insoluble in the colon after the active reabsorption of bile acids in the distal ileum.[4]

Through the experiments conducted, humanized microbiota increased endotoxemia, systemic inflammation (demonstrated by serum inflammatory cytokine profile), and local inflammation (demonstrated by the increased messenger RNA (mRNA) expression of cytokines such as interleukin (IL)-1β and IL-6). Treatment with rifaximin significantly ameliorated the systemic inflammation and local inflammation. Perhaps more directly related to the etiology of MHE, rifaximin decreased intestinal ammonia generation in GF mice (lower serum ammonia, decreased small intestinal glutaminase, and higher cecal glutamine content), apparently without changing measurements of intestinal barrier. In the humanized mice, rifaximin resulted in similar effects, except that the numerical reduction in serum ammonia did not reach statistical significance.

Rifaximin had predictable effects on microbiota function such as reduced endotoxin, decreased deconjugation, and formation of secondary bile acids, but the nonabsorbed antibiotic did not significantly alter microbial composition in humanized mice. The modest influence of rifaximin on fecal microbiota composition is consistent with earlier findings.[5] The humanized mice had increased serum endotoxin, which was reduced by rifaximin and may reflect a change in the microbiome. Rifaximin has been shown to alter metabolism of colonic bacteria[6] and reduce the virulence factors of pathogenic bacteria[7] that ultimately impact the gut microenvironment, enhancing cytoprotection and resistance to bacterial colonization.[8] Thus, *in vitro* experiments have established several effects of rifaximin that are well beyond its antibacterial effects.

Could these effects observed with rifaximin be explained by alternative mechanisms? For example, would humanization increase mucosal permeability and allow for greater absorption of bacterial toxins that are present in the gastrointestinal lumen? As discussed below, the studies of Kang *et al.*[1] provide indicators for increased mucosal permeability with humanization, which were partly reversed with rifaximin.

Several details in the experimental methods and concepts are worthy of further analysis and comparison with the previously reported effects of rifaximin on some of these biological mechanisms in patients with non-constipation irritable bowel syndrome (IBS-non-C).[9]

First, the microbiota composition from the one patient with MHE was similar to microbiota from a group of 78 cirrhotic patients without hepatic decompensation and significantly different from the microbiota of healthy controls. Therefore, the results pertain to the potential effects of microbiota in mild hepatic encephalopathy and cannot be extrapolated to

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Received 15 August 2016; accepted 22 August 2016

potential effects of microbiota from patients with more severe hepatic encephalopathy. In fact, Bajaj *et al.*[2] had previously shown correlation between the ratio of beneficial to potentially harmful bacteria (such as *Enterobacteriaceae* and *Enterococceae*), and increased model for end-stage liver disease score and episodes of hepatic encephalopathy.

Second, the dose of rifaximin used was 100 mg/kg, which is ~6 times higher per kg body weight than the dose typically administered to patients with hepatic encephalopathy (550 mg b.i.d. or 16 mg/kg in a 70 kg person).

Third, rifaximin predictably decreased deconjugation and formation of secondary bile acids in the humanized mice; these effects were not previously observed in patients with IBS-non-C treated with rifaximin 550 mg t.i.d. for 2 weeks.[9] It is conceivable that the sixfold difference in the dose of antibiotic or the availability of the soluble formulation for these animal studies (and lack of availability for the human study reported[9]) may explain the differences in observed effects on bile acids in the mice and humans.

Fourth, *in vivo* measurement of intestinal permeability was based on blood sampling 3 h after oral administration of fluorescein isothiocyanate (FITC)-dextran. Table 1 in the paper by Kang *et al.*[1] shows that mRNA expressions of zonulin-1 and e-cadherin were both reduced in small intestine and colon samples in the humanized mice, consistent with reduced tight junction barrier. In addition, the improved inflammatory mediators, and expressions of zonulin-1 and e-cadherin, in humanized mice treated with rifaximin contrast with the lack of effect on mucosal permeability measured using the FITC-dextran method. However, it is relevant to note that the molecular weight of the permeability probe may be 1,386 daltons (or more likely 4,386 daltons, if the standard dextran was used in these studies). This molecular size is considerably larger than the molecular weights of typical probe molecules used to study permeability *in vivo*, such as monosaccharides, disaccharides, or ethylenediaminetetraacetic acid, all of which have molecular weights <600 daltons.[10] Although FITC-dextran is a good probe molecule for measurement of mucosal permeability *in vitro*, this is a very different experimental situation from the *in vivo* state, in which other factors such as surface mucus and submucosal neuronal input may alter the barrier function. Thus, it is conceivable that the lack of effect of rifaximin on the FITC-dextran measurements of intestinal mucosal permeability in these studies may reflect the lack of sensitivity of the probe molecule used for *in vivo* studies. Nevertheless, it is worth noting that the prior study in IBS-non-C also showed no significant effects of rifaximin on small intestinal or colonic permeability, using mannitol and lactulose as probe molecules.[9] Overall, the expression data using small bowel biopsies show humanization changes to the expression of tight junction proteins, and that there is an effect of rifaximin on intestinal permeability that may impact local and systemic inflammation.

Fifth, the authors justify the absence of a separate GF group that was "humanized" with the stool of a healthy control by their observation that the microbiota of the patient was similar to that of other MHE patients and the prior studies that have not demonstrated intestinal inflammation in mice simply by humanization. Nevertheless, an additional control group of GF humanized with stool from a healthy volunteer would have been desirable, for example, to ensure that the serum endotoxemia, and local and systemic inflammation after humanization of the microbiota in the GF animals were specific to MHE-associated microbiota. Although serum endotoxin (lipopolysaccharide, molecular weight 1 million daltons) has been used to appraise mucosal permeability in humans,[11] it appears more likely that this observation reflects an inflammatory reaction to the human microbiota. These *in vivo* observations of reduced endotoxemia and inflammation with rifaximin also correspond with properties of rifaximin *in vitro*. Thus, rifaximin (dissolved in dimethyl sulfoxide) enhanced epithelial repair in a Caco-2 cell line[12] (via activation of the pregnane X receptor) and reduced local intestinal inflammatory responses through inhibition of nuclear factor kappa B.[13]

Sixth, although the humanized GF mice (with normal hepatic function) did not have hyperammonemia (and paradoxically GF mice did), the mice treated with rifaximin had decreased intestinal glutaminase without significant impact on serum ammonia levels. The role of glutaminase in the production of ammonia is more relevant in the absence of a microbiome (that is the GF group). Indeed, the studies by Kang *et al.*[1] show reduction in both ammonia levels and intestinal glutaminase with rifaximin treatment (GF+rifaximin group). These data are consistent with the concept that glutaminase is a relevant and alternative source of ammonia in the absence of a microbiome; the novel observation in the current report[1] is that rifaximin directly affects enterocyte glutaminase.

Overall, the lack of effect on high ammonia levels in the presence of MHE microbiome does not provide a clear model to study the potential effects of rifaximin on hepatic encephalopathy. On the other hand, humanized mice showed lower gamma-amino butyric acid (GABA) compared with GF and an increase in cecal GABA after treatment with rifaximin. This would be consistent with rifaximin reducing colonic mucosal permeability and preventing absorption of GABA, which is not cleared in the presence of hepatic dysfunction and contributes to hepatic encephalopathy by increasing "GABA-ergic tone".[14]

Thus, Kang *et al.*[1] demonstrate an impact of rifaximin on bacterial function without significantly affecting intestinal bacterial composition, and effects on local and systemic inflammation, enterocyte glutaminase, as well as intestinal barrier function. Changes in gut bacterial and intestinal barrier function, and pathogenic potency of MHE-associated microbiota, influence complications associated with hepatic dysfunction such as spontaneous bacterial peritonitis and encephalopathy.

The authors are congratulated for performing very significant studies on a variety of mechanisms potentially relevant to rifaximin's beneficial effect on hepatic encephalopathy. Further research will build upon these in-depth studies to shed further light on the beneficial effects of rifaximin, including the effect on MHE-associated microbiome in the presence of concomitant liver disease. The availability of soluble formulations of rifaximin will facilitate evaluation of these effects better than the standard formulation of rifaximin that is relatively insoluble, especially in the presence of low bile acid concentrations. We believe the new soluble formulation(s) may also potentially enhance the clinical benefit of rifaximin by greater antibacterial and other biological actions in the colon.

CONFLICT OF INTEREST

Guarantor of the article: Michael Camilleri, MD.
Specific author contributions: Both authors performed the literature review, and drafting and revision of the manuscript.
Financial support: M.C. is funded by grant R01-DK92179 from National Institutes of Health.
Potential competing interests: M.C. has carried out research on rifaximin funded by Salix. P.C. declares no conflict of interest.

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