AP&T Alimentary Pharmacology and Therapeutics

Randomised clinical trial: vancomycin or metronidazole in patients with primary sclerosing cholangitis - a pilot study

J. H. Tabibian*,[1], E. Weeding*,[1], R. A. Jorgensen*, J. L. Petz*, J. C. Keach*, J. A. Talwalkar* & K. D. Lindor†

*Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA.
†Health Solutions, Arizona State University, Tempe, AZ, USA.

Correspondence to:
Dr K. D. Lindor, Health Solutions, Arizona State University, 500 North 3rd Street, Phoenix, AZ 85004, USA.
E-mail: keith.lindor@asu.edu

[1]Co-first authors.

Publication data
Submitted 17 November 2012
First decision 24 December 2012
Resubmitted 10 January 2013
Accepted 14 January 2013
EV Pub Online 5 February 2013

SUMMARY

Background
Emerging data suggest that oral antibiotics may have therapeutic effects in primary sclerosing cholangitis (PSC), but published studies are limited.

Aims
To investigate the safety and efficacy of oral vancomycin and metronidazole in patients with PSC.

Methods
Thirty-five patients with PSC were randomised in a double-blind manner into four groups: vancomycin 125 mg or 250 mg four times/day, or metronidazole 250 mg or 500 mg three times/day for 12 weeks. The primary endpoint was decrease in alkaline phosphatase (ALK) at 12 weeks. Secondary end points included serum bilirubin and Mayo PSC risk score; pruritus; and adverse effects (AEs). Nonparametric tests were used for analysis.

Results
The primary endpoint was reached in the low-dose (−43% change in ALK, $P = 0.03$) and high-dose (−40%, $P = 0.02$) vancomycin groups, with two patients in the former experiencing ALK normalisation. Bilirubin decreased significantly in the low-dose metronidazole group (−20%, $P = 0.03$) and trended towards significance in the low-dose vancomycin group (−33%, $P = 0.06$). Mayo PSC risk score decreased significantly in the low-dose vancomycin (−0.55, $P = 0.02$) and low-dose metronidazole group (−0.16, $P = 0.03$). Pruritus decreased significantly in the high-dose metronidazole group (−3.4, $P = 0.03$). AEs led to medication discontinuation in six patients, four of whom were receiving metronidazole.

Conclusions
Both vancomycin and metronidazole demonstrated efficacy; however, only patients in the vancomycin groups reached the primary endpoint, and with less adverse effects. Larger, longer-term studies are needed to further examine the safety and efficacy of antibiotics as a potential treatment for patients with primary sclerosing cholangitis (clinicaltrials.gov NCT01085760).

Aliment Pharmacol Ther 2013; **37:** 604–612

doi:10.1111/apt.12232

INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic idiopathic liver disease characterised by inflammation and concentric fibrosis of the bile ducts for which there is no known effective pharmacotherapy.[1, 2] Liver transplantation (LT) is the only existing treatment shown to prolong survival; however, PSC recurs in up to one-third of deceased donor LT and up to two-thirds of living-related donor LT patients.[3, 4] The most widely studied pharmacological agent in the treatment of cholestatic liver diseases, ursodeoxycholic acid (UDCA), improves serum liver tests in PSC, but unlike in primary biliary cirrhosis, does not appear to delay disease progression.[5–7] Furthermore, a recent large randomised trial has found high-dose UDCA to be associated with serious adverse effects (AEs) in patients with PSC.[5, 8] Numerous other drugs, including but not limited to azathioprine, budesonide, methotrexate and pentoxifylline, have also been evaluated in the treatment of PSC but without evidence of benefit.[2, 4, 9–15] The negative results of these clinical trials have highlighted the importance of considering treatment approaches that target alternative pathophysiological pathways.

Primary sclerosing cholangitis is now generally considered to be an idiopathic, likely immune-mediated disease with various aetiopathogenic components. One hypothesis is that enterohepatic circulation of (as of yet undetermined) bacterially derived molecules plays a critical role in eliciting pro-inflammatory, pro-fibrotic hepatobiliary responses that lead to the development of PSC (hereinafter 'PSC microbiota hypothesis').[16–18] The entry of such molecules into the enterohepatic circulation may, in some patients, be related to the enteric dysbiosis and increased intestinal permeability associated with inflammatory bowel disease (IBD), a condition diagnosed in 75% of those with PSC.[19–23] Further supporting the PSC microbiota hypothesis is the observation, for example, that patients with PSC often have a leucocyte differential exhibiting increased neutrophils, even in the absence of signs or symptoms of acute cholangitis, suggesting circulation of endotoxins or other immunoactive molecules.[24] Collectively, these and animal model[25, 26] findings point toward a role for bacteria and bacterially derived molecules in the aetiopathogenesis of PSC.[2]

To date, there have been a few studies of antibiotics in patients with PSC (Table 1), some of which have yielded favourable results based primarily on reduction in serum aminotransferases or alkaline phosphatase (ALK).[27–30] Aminotransferase values are independently associated with prognosis and are a component of the

Table 1 | Previously reported results of antibacterial treatment in primary sclerosing cholangitis

					% change from baseline post-therapy			
Drug	Year	*n*	Antibiotic dose	Months of therapy	ALK	AST	ALT	GGT
Tetracycline[32]†	1959	5	500 mg/day	1–10	−45	−60	−45	–
Tetracycline[36]‡	1965	5	500 mg/day	48 (mean)	+21	–	–	–
Sulfasalazine (+UDCA)[34]§	1998	2*	–	30	−79	−38	−70	−26
				45	−35	−87	−95	−94
Vancomycin[28]	1998	3*	375–1000 mg/day	9 (mean)	–	–	−89	−93
Sulfasalazine (+UDCA)[35]	2002	1	50 mg/kg/day	37	–		−92	−83
Metronidazole (+UDCA)[38]	2004	39	600–800 mg/day	36	−52.4	−41.0	−67.9	–
Sulfasalazine[29]	2006	1	2–4.5 g/day	24	−74	–	−84	–
Azithromycin (+UDCA)[33]	2007	1	500 mg/day, 3 days/week	5	−72	−31	−33	−54
Vancomycin[27]	2008	14*	50 mg/kg/day	54 ± 43	–	–	−78	−89
Minocycline[39]	2009	16	200 mg/day	12	−19.7	−2.8	–	–

ALK, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; q.d.s., four times a day; UDCA, ursodeoxycholic acid.

Months of treatment and follow-up are absolute unless otherwise indicated.

Table adapted from Elfaki and Lindor.[37]

* Paediatric patients.

† Includes one patient who also received prednisone but was not separable from the other four patients.

‡ Does not include two patients who received prednisone.

§ Does not include a third patient who also received prednisolone and mizoribine.

Mayo PSC risk score, and ALK values have been recently associated with prognosis,[31] thus making these two readily measured liver biochemistries important end points in studies of PSC. However, the number of patients in the aforementioned antibiotic studies has been small (and often case report-based), the end points assessed relatively few and the selection of antibiotics often arbitrary or for another condition (e.g. reactive airways disease, after which incidental improvement of PSC was noted).[28, 29, 32–36]

Given the evidence supporting the PSC microbiota hypothesis yet the limited studies investigating the potential therapeutic effects of antibiotics, we conducted a randomised, double-blind pilot study of oral vancomycin or metronidazole as a treatment for patients with PSC. We chose these agents based on prior experience and evidence of seemingly greater therapeutic response. We assessed safety and efficacy of both antibiotics, each at two different doses, in improving liver biochemistries and liver-related symptoms in patients with PSC.

MATERIALS AND METHODS

Patients

Thirty-five adult patients with PSC were enrolled in this study between February 2010 and November 2011. Diagnosis of PSC was established by the following criteria: ALK greater than 1.5 times the upper limit of normal for at least 6 months and cholangiography demonstrating intrahepatic and/or extrahepatic biliary strictures, beading or irregularity consistent with PSC. Exclusion criteria were (i) treatment with any investigational agents, such as UDCA or other antibiotics, within three months of the study, (ii) prior history of allergic reactions to vancomycin and/or metronidazole, (iii) evidence of decompensated liver disease such as recurrent variceal bleeding, refractory ascites, or spontaneous hepatic encephalopathy, (iv) anticipated need for liver transplant within one year as determined by Mayo PSC risk score, (v) findings highly suggestive of liver disease of an alternative or concomitant aetiology, such as chronic alcoholic liver disease, chronic hepatitis B or C infection, haemochromatosis, Wilson's disease, α1-antitrypsin deficiency, non-alcoholic steatohepatitis, primary biliary cirrhosis, or secondary sclerosing cholangitis, (vi) pregnancy or lactation, (vii) active illicit drug or alcohol abuse, and (viii) extremes of age (younger than 18 or older than 75 years of age).

Informed consent was obtained from each patient, and in addition, written material containing information about the study was provided, including the potential side effects of antibiotic therapy. A complete history and physical examination was performed for each patient at study entry if one had not been done within the previous year at Mayo Clinic. The study was approved by the Mayo Foundation Institutional Review Board and was carried out in accordance with the ethical principles of the current revision of the Declaration of Helsinki. (clinicaltrials.gov registration number: NCT00483938).

Study medication and monitoring

Patients were randomised into four treatment groups: vancomycin 125 mg orally four times a day ($n = 8$), vancomycin 250 mg orally four times a day ($n = 9$), metronidazole 250 mg orally three times a day ($n = 9$) or metronidazole 500 mg orally three times a day ($n = 9$) for 12 weeks. These groups are hereafter referred to as low-dose vancomycin, high-dose vancomycin, low-dose metronidazole and high-dose metronidazole respectively. Randomisation was stratified according to initial ALK level (≤ 320 U/L or >320 U/L); this cut-off was the mean value among the patients in a previous high-dose UDCA trial.[8] Patients were advised to abstain from alcohol due to the potential for serious interactions with metronidazole. Drugs were packaged in identical gelatin capsules, and patients and investigators were blinded to the type and dose of the drug.

Serum liver biochemistries were measured at 3 weeks and at 12 weeks. Phone calls were made monthly to monitor for AEs related to the study medications.

Variables and definitions

Serum ALK, aspartate aminotransferase (AST), total bilirubin, C-reactive protein (CRP), and albumin were measured at baseline (i.e. study entry), 3 weeks and 12 weeks (i.e. study end). For CRP results below the 3 mg/L sensitivity limit of the conventional CRP assay used, a value of 1.0 mg/L was assigned for purposes of statistical analysis.

The Mayo PSC risk score was calculated for each patient at baseline and at 12 weeks, where Risk = 0.03 [age (years)] + 0.54 Ln [total bilirubin (mg/dL)] + 0.54 Ln [AST (IU/L)] + 1.24 (variceal bleeding) − 0.84 [albumin (g/dL)].[37] As no patients had a history of variceal bleeding, the Mayo PSC risk score in the present study was a reflection of only patient age and serum biochemical tests.

Severity of two symptoms, fatigue and pruritus, was assessed at baseline and at 12 weeks. Fatigue was assessed using the Fisk Fatigue Impact Scale (FFIS), a 40-item instrument with a response range from 0 to 4

for each item. Pruritus was graded using a 10-cm visual analogue scale (VAS).

Study end points

The primary end point was decrease in ALK at 12 weeks of treatment compared with the baseline value. Secondary end points were: (i) decrease in AST, total bilirubin, Mayo PSC risk score and CRP at 12 weeks compared with the baseline value, (ii) decrease in fatigue severity and pruritus VAS score at 12 weeks compared with baseline and (iii) AEs anytime during the 12 weeks of treatment.

Sample size

In patients with PSC, there is often spontaneous variation in the ALK level within ±5% of the baseline level based on our unpublished experience. Therefore, it seemed reasonable to consider a response to therapy in PSC patients as an improvement in ALK levels from 5% to approximately 55% from baseline. A study with nine patients would have a power of 0.8 to detect a response rate of 50% with a two-sided alpha level of 0.05. Therefore, we planned to enrol 10 patients in each arm, which would have a power of 0.8 to detect a positive response rate of 50% (55% minus 5% spontaneous variation of baseline) at a two-sided alpha level of 0.05 while allowing for one patient drop-out per arm. However, we were only able to enrol 35 patients, and thus the study was conducted with three arms of nine patients and one arm of eight patients.

Statistical analysis

Considering the small number of patients per treatment group as well as the heterogeneous nature of PSC, statistical analyses were conducted using conservative (e.g. nonparametric) tests. Baseline data from the four treatment groups were compared across groups using the Wilcoxon rank-sum test and Fischer's exact test. Changes in liver biochemistry values, Mayo PSC risk score, FFIS scores and VAS pruritus scores from study entry to study end were compared within groups using the Wilcoxon signed-rank test. All tests were two tailed, and an α level of 0.05 was used for significance.

RESULTS

The median patient age in the sample was 40 years (range: 20–70), 60% of patients were men, 71% had IBD and median baseline ALK value was 383 U/mL. All patients had a stably elevated ALK (at least two separate measurements) for $\geq$6 months (median 19 months) prior to enrolment. Five patients were previously treated with UDCA but had been off of this drug for at least 3 months prior to enrolment and throughout the study.

There were no statistically significant differences across treatment groups at baseline except for fatigue and gender (both $P < 0.05$), with FFIS scores being highest (worst) in patients in the high-dose metronidazole group and the proportion of male patients being greatest in the high-dose vancomycin group. These and other baseline characteristics of each treatment group are shown in Table 2.

Serum biochemical tests

The changes in biochemical end points from baseline to study end are summarised in Table 3. As shown in Figure 1, a significant decrease in ALK, the primary end point, was only observed in the high-dose vancomycin group (−40%, $P = 0.02$). The low-dose vancomycin group, however, had a larger decrease in ALK, and after excluding an outlier (corresponding to a patient who did not take the study medication for 1 month), the change in ALK became statistically significant (−43%, $P = 0.03$). Furthermore, two patients in the low-dose vancomycin group experienced normalisation of ALK as compared with none in the other three groups.

With respect to secondary end points, total bilirubin decreased significantly in the low-dose metronidazole group (−20%, $P = 0.03$), and there was a trend towards a significant decrease in the low-dose vancomycin group (−33%, $P = 0.06$) (Figure S1). Mayo PSC risk score decreased significantly in the low-dose vancomycin group (−0.55, $P = 0.02$) and low-dose metronidazole group (−0.16, $P = 0.03$) (Figure S2). CRP decreased significantly in the low-dose metronidazole group (−49%, $P = 0.03$), and there was a trend towards a decrease in CRP in the low-dose vancomycin group (−69%, $P = 0.06$). AST did not change significantly (data not shown).

Fatigue

Among the patients reporting fatigue at baseline (i.e. eight in the low-dose vancomycin group, seven in the high-dose vancomycin group, eight in the low-dose metronidazole group and six in the high-dose metronidazole group), the median change in FFIS score in each group was −1, 0, −15 and −16 units respectively. None of these changes were statistically significant (data not shown).

Pruritus

The number of patients reporting pruritus at baseline is shown in Table 2. The median baseline VAS pruritus

Table 2 | Characteristics of the four treatment groups at study entry

	Vancomycin groups		Metronidazole groups	
	Low-dose (n = 8)	High-dose (n = 9)	Low-dose (n = 9)	High-dose (n = 9)
Age, year	35 (23–57)	42 (27–70)	35 (21–64)	40 (20–60)
Male gender, *n* (%)*	4 (50%)	9 (100%)	4 (44%)	4 (44%)
IBD, *n* (%)	6 (75%)	5 (56%)	8 (89%)	6 (67%)
ALK, U/L	406 (210–545)	345 (187–788)	354 (137–1007)	424 (186–721)
AST, IU/L	102 (48–154)	107 (73–234)	65 (34–333)	115 (36–245)
Total bilirubin, mg/dL	0.8 (0.3–1.8)	1.0 (0.3–5.4)	1.0 (0.3–1.7)	0.8 (0.4–2.4)
Direct bilirubin, mg/dL	0.3 (0.1–1.1)	0.4 (0.1–3.4)	0.5 (0.1–1.2)	0.3 (0.2–0.7)
CRP, mg/L	3.2 (1.0–48.3)	4.3 (1.0–48.0)	9.7 (1.0–29.6)	1 (1.0–14.2)
Albumin, g/dL	4.3 (3.9–4.5)	3.9 (2.8–4.5)	4.2 (4.0–4.9)	4.1 (3.8–4.5)
Mayo PSC risk score	−0.10 (−1.09–0.71)	0.30 (−0.43–2.76)	−0.49 (−1.66–0.89)	0.34 (−0.73–0.90)
Fatigue score (FFIS)*	7 (2–35)	6 (0–38)	37 (0–107)	53 (0–102)
Pruritus, *n* (%)	5 (63%)	5 (56%)	8 (89%)	7 (78%)

ALK, alkaline phosphatase; AST, aspartate aminotransferase; CRP, C-reactive protein; FFIS, Fisk Fatigue Impact Scale; IBD, inflammatory bowel disease.

Values reported as median (range).

* Statistically significant differences between groups at study entry.

Table 3 | Serum biochemical tests before and after 12 weeks of vancomycin or metronidazole treatment

	Study entry	Study end	Change	*P*-value
Alkaline phosphatase, U/L				
Vancomycin low-dose	406 (210–545)	218 (163–710)	−46%	**0.03***
Vancomycin high-dose	345 (187–788)	209 (169–766)	−40%	**0.02**
Metronidazole low-dose	354 (137–1007)	400 (53–669)	+13%	0.47
Metronidazole high-dose	424 (186–721)	286 (84–763)	−33%	0.22
Total bilirubin, mg/dL				
Vancomycin low-dose	0.8 (0.3–1.8)	0.5 (0.2–1.0)	−33%	0.06
Vancomycin high-dose	1.0 (0.3–5.4)	1.0 (0.2–15.1)	0%	0.48
Metronidazole low-dose	1.0 (0.3–1.7)	0.8 (0.2–1.4)	−20%	**0.03**
Metronidazole high-dose	0.8 (0.4–2.4)	0.9 (0.3–2.8)	+6%	0.78
Mayo PSC risk score				
Vancomycin low-dose	−0.10 (−1.09–0.71)	−0.65 (−1.13–0.21)	−0.55	**0.02**
Vancomycin high-dose	0.30 (−0.43–2.76)	0.27 (−0.41–3.91)	−0.03	0.98
Metronidazole low-dose	−0.49 (−1.66–0.89)	−0.64 (−1.22–0.48)	−0.16	**0.03**
Metronidazole high-dose	0.34 (−0.73–0.90)	0.06 (−0.82–0.68)	−0.28	0.16
C-reactive protein				
Vancomycin low-dose	3.2 (1.0–48.3)	1.0 (1.0–1.0)	−69%	0.06
Vancomycin high-dose	4.3 (1.0–48.0)	5.4 (1.0–76.6)	26%	0.78
Metronidazole low-dose	9.7 (1.0–29.6)	4.9 (1.0–23.9)	−49%	**0.03**
Metronidazole high-dose	1.0 (1.0–14.2)	3.5 (1.0–14.6)	250%	1.00

Bold values indicate statistical significance.

Values reported as median (range).

* As described in the results section, when an outlier value was omitted from this group for this variable, the difference became statistically significant (−43%, P = 0.03) as shown.

score among these patients was 1 in the low-dose vancomycin group, 0.25 in the high-dose vancomycin group, 2 in the low-dose metronidazole group and 4 in the high-dose metronidazole group. Individual changes in VAS score are shown in Figure S3. Overall, the median change in VAS scores from baseline to study end was −0.8 points in the low-dose vancomycin group, −0.1 point in the high-dose vancomycin group, −1.0 point in the low-dose

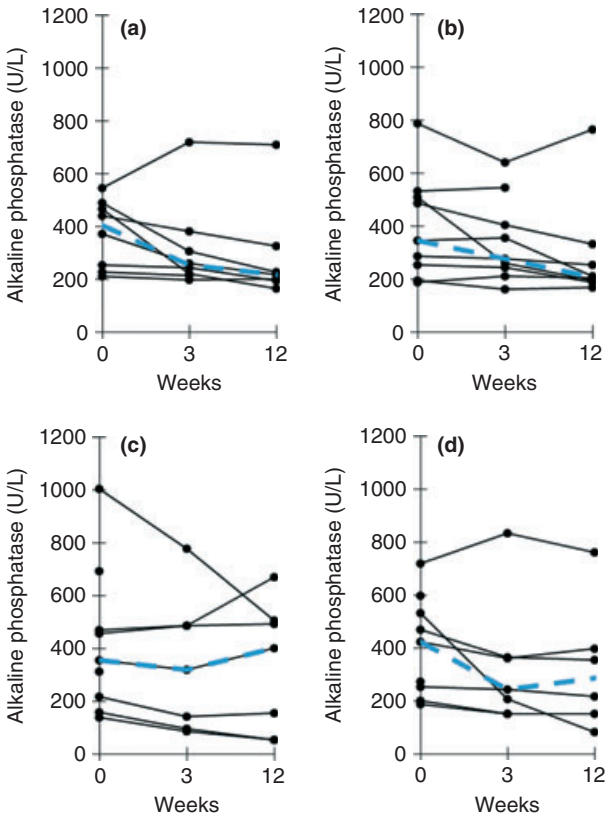

Figure 1 | Change in alkaline phosphatase in low- and high-dose vancomycin and metronidazole groups: (a) low-dose vancomycin, (b) high-dose vancomycin, (c) low-dose metronidazole, (d) high-dose metronidazole. Decrease in alkaline phosphatase was significant in the low- and high-dose vancomycin groups ($P = 0.03$ and $P = 0.02$ respectively). Note: Bold, dashed lines represent the group medians. Outlier present (top curve) in low-dose vancomycin group.

metronidazole group and −3.4 points in the high-dose metronidazole group. Change in VAS score was only significant in the high-dose metronidazole group ($P = 0.03$). No patients developed pruritus de-novo.

Adverse effects

A list of AEs experienced in this study is provided in Table S1. Importantly, several patients stopped treatment indefinitely due to AEs or were severed by the investigators: in the low-dose vancomycin group, one patient stopped treatment indefinitely due to migraine headaches and increased diarrhoea. In the high-dose vancomycin group, one patient stopped treatment indefinitely due to diarrhoea and increased fatigue. In the low-dose metronidazole group, one patient stopped treatment indefinitely due to persistent dyspepsia and one patient was severed because of noncompliance. In the high-dose metronidazole group, three patients stopped treatment indefinitely; one due to nausea and developed flu, the second due to dyspepsia and burning in the eyes and the third due to dyspepsia, diarrhoea and anorexia. The remainder of the patients completed the study.

DISCUSSION

Despite numerous prior studies evaluating an array of pharmacological treatments for PSC, including immunosuppressants and anti-inflammatories, there remains to be an effective medical treatment for this disease.[2] Several recent studies, mostly small case series, have suggested a therapeutic effect with antibacterial agents while having only mild side-effect profiles. Here, we performed the first randomised, double-blinded trial of the two most promising antibacterial agents based on prior experience, vancomycin and metronidazole, and tested their efficacy and safety at two different doses for each drug. Although individual responses were variable, overall, both drugs appeared to have some favourable effects at both doses; however, only the vancomycin groups reached the primary end point—significant decrease in ALK at 12 weeks. Furthermore, two patients in the low-dose vancomycin group experienced normalisation of ALK; although spontaneous normalisation of ALK is seen in a subset of patients with PSC,[31] it would be unlikely after a median of 19 months of persistently elevated ALK and should occur with equal probability (i.e. not only in the low-dose vancomycin group) given the randomised nature of this study.

In addition to reaching the primary end point, both vancomycin groups also reached some of the secondary end points. The low-dose vancomycin group experienced a significant decrease in Mayo PSC risk score and a trend towards a significant decrease in total bilirubin and CRP. The high-dose vancomycin group experienced significant improvement in pruritus. Importantly, AEs in both vancomycin groups were generally mild and relatively infrequent, and unlike the majority of pharmacotherapies previously tested in patients with PSC, there are few known long-term safety concerns with oral vancomycin (although societal effects of chronic use of this and other antibiotics should be considered, e.g. vancomycin-resistant enterococci). Although not reaching the primary end point, the metronidazole groups also experienced some therapeutic effects, namely a decrease in total bilirubin, Mayo PSC risk score and CRP in the low-dose metronidazole group and a decrease in pruritus in the high-dose metronidazole group. The decrease in pruritus in the high-dose metronidazole group may,

however, have been related to the high baseline prevalence and greater severity of pruritus in this group as compared with the other groups, thus resulting in a relatively larger opportunity to demonstrate improvement (potentially as a result of regression toward the mean). Regardless, this improvement came at the expense of more AEs, as all but one patient in the high-dose metronidazole group experienced AEs, and some patients experienced multiple. It is possible, although, that some of the AEs (e.g. increased fatigue, burning sensation in the eyes) in the vancomycin and/or metronidazole groups may not have been related to the study medication and instead may have been coincidental.

As shown in Table 1, there have been several reports of antibiotic use in treating PSC. The earliest of these dates back to 1959, when Rankin *et al.* used tetracycline in five patients with ulcerative colitis and "chronic pericholangitis" (later recognised as PSC) for 1–10 months; a dramatic improvement was noted in liver tests in all five patients and in clinical symptoms in three patients.[32] More recently, Farkkila *et al.* randomised 39 patients to UDCA combined with metronidazole and 41 patients to UDCA alone and noted a significant improvement in serum liver tests and a trend towards less cholangiographic progression in the combination group.[38] Boner *et al.* found a decrease in cholestasis-related signs and symptoms over 5 months of azithromycin treatment in a patient previously diagnosed with PSC; the azithromycin was originally prescribed to treat severe reactive airways disease, and the patient continued to take it after experiencing improvement in several clinical symptoms.[33] Cox *et al.* reported three children with PSC and IBD who experienced normalisation of liver tests and resolution of symptoms with oral vancomycin treatment.[28] A recent pilot study from the same group evaluated 14 children diagnosed with PSC and IBD who were treated with oral vancomycin for 54 $\pm$ 43 months[27]; the authors noted normalisation or significant improvement in liver tests, erythrocyte sedimentation rate and clinical symptoms in nearly all patients. In addition, when vancomycin treatment was discontinued, there was recurrence of clinical symptoms and an increase in liver enzymes in several patients and re-treatment again resulted in normalisation of liver enzymes.[27] Most recently, in a pilot study by Silveira *et al.*, 16 patients with PSC were treated with minocycline for one year; although a substantial proportion of patients withdrew from the study due to AEs, those who continued treatment were found to experience a significant reduction in ALK.[39]

Although the use of antibiotics in PSC seems logical and promising based on the clinical experience described above, there is very little data on humans supporting their role in the pathophysiology of this disease. One line of evidence that supports the PSC microbiota hypothesis is that bacterially derived molecules such as lipopolysaccharide (LPS), lipoteichoic acid and bacterial DNA fragments have been detected in bile, cholangiocytes and/or portal tracts of patients with chronic cholestatic liver disease.[40–43] It is believed that these molecules enter the enterohepatic circulation via a leaky gut, although this has been questioned based on a study that failed to show increased intestinal permeability in adults with PSC[44]; it should be noted though, that none of the patients in the study had active IBD, thus this remains a subject of uncertainty. With respect to animal models, it has been shown that rodents with bacterial and/or chemically induced enterocolitis develop hepatobiliary inflammation and histological injury (e.g. fibrosis).[43, 45–48] Interestingly, these findings can be mitigated by antibiotic administration and by selective degradation of enteric bacterial components.[25, 26] We believe that antibiotics such as metronidazole (effective against anaerobic bacteria) and vancomycin (effective against Gram-positive bacteria) may decrease the biosynthesis of bacterially derived immunoactive molecules and thus mitigate the aberrant (pro-inflammatory, pro-fibrotic) hepatobiliary immune responses and signalling cascades that they may trigger in immunogenetically susceptible individuals. To that effect, it has been speculated, for example, that the convergence of both agents' antibacterial activity on clostridial organisms, the bacteria primarily responsible for bile acid metabolism, may point towards a mode of action for the therapeutic effects seen with these agents in PSC. This is an area that merits further investigation.

This study has several limitations. As commonly seen with pilot studies, group sizes were small, and there was no control arm. The study was not powered to detect small changes or perform subgroup analyses, and at least one patient stopped treatment indefinitely in each group, thus further limiting overall power. We cannot fully exclude the effect of regression towards the mean, although it is mitigated by several features of our study, including: (i) random allocation of patients to treatment groups, (ii) similar baseline values across treatment groups, (iii) selection of patients based on multiple abnormal ALK values over a minimum of 6 months (median 19 months) and (iv) the observation that treatment groups and/or individuals with the highest baseline values did not necessarily demonstrate the largest decreases (e.g. in ALK) while on therapy. Other limitations of this study include the relatively short-treatment duration and follow-up. Also, all patients in the

high-dose vancomycin group were men, thus generalisation to female patients should be avoided until more data are available. Lastly, the FFIS was used to measure fatigue but has not been validated in patients with PSC.[49] Considering the limitations herein, we believe a larger-scale randomised trial is warranted before the results of this study can be applied broadly in the clinic. This shows that, there is growing interest in conducting such a trial in the near future, ideally as a multi-centre collaboration.

Based on this randomised, double-blind pilot study, vancomycin appears to be the better-tolerated drug and may have greater promise as a potential emerging therapy for patients with PSC. Although both low and high-dose vancomycin groups achieved the primary outcome of significant decrease in ALK after 12 weeks of treatment, only patients in the low-dose group experienced normalisation of ALK. In addition, patients in the low-dose group also experienced other biochemical improvements, including a decrease in Mayo PSC risk score, total bilirubin and CRP. Collectively, these findings encourage larger, randomised controlled trials of antibiotics in PSC (particularly low-dose vancomycin). These trials should have longer treatment and follow-up periods to demonstrate improvements in more definitive end points, such as liver histology and/or cholangiographic findings, and identify subgroups of PSC patients who may benefit most from antibiotic therapy. In addition, alternative regimens, such as other non-absorbable antibiotics (e.g. rifaximin) and/or intermittent (e.g. 2 weeks on, 1 week off) antibiotic dosing, also merit investigation, as these could be more efficacious, better tolerated, less costly and associated with less bacterial resistance. In conclusion, by focusing on the enteric microbiota as a novel therapeutic target in PSC, the experience thus far appears promising, and we may be one step closer to finding a safe and effective pharmacotherapy for patients with this illness.

AUTHORSHIP

Guarantor of the article: J. H. Tabibian and E. Weeding.
Author contributions: JHT drafted the manuscript and contributed to research design, acquisition and interpretation of data. EW co-drafted the manuscript and analysed data. RAJ revised the manuscript and contributed to study design. JLP contributed to study design and data acquisition. JCK contributed to study design and data acquisition. JAT revised the manuscript and contributed to data interpretation. KDL revised the manuscript and contributed to research design, data acquisition and interpretation. All authors have approved the final version of the manuscript.

ACKNOWLEDGEMENT

Declaration of personal interests: None.
Declaration of funding interests: This study was funded by the PSC Partners Seeking a Cure 2009–2010 Research Grant.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Number of patients who experienced side effects from study drugs. (a) Vancomycin groups, (b) Metronidazole groups.

Figure S1. Change in total bilirubin in low- and high-dose vancomycin and metronidazole groups

Figure S2. Change in Mayo PSC risk score in low- and high-dose vancomycin and metronidazole groups

Figure S3. Difference between baseline and 12-week pruritus VAS score for individuals in each study group.

REFERENCES

1. Angulo P, Lindor KD. Primary sclerosing cholangitis. *Hepatology* 1999; **30**: 325–32.
2. Tabiban JH, Lindor KD. Primary sclerosing cholangitis: a review and update on therapeutic developments. *Expert Rev Gastroenterol Hepatol* 2013; **7**: 103–14.
3. Graziadei IW. Live donor liver transplantation for primary sclerosing cholangitis: is disease recurrence increased? *Curr Opin Gastroenterol* 2011; **27**: 301–5.
4. Kugelmas M, Spiegelman P, Osgood MJ, *et al.* Different immunosuppressive regimens and recurrence of primary sclerosing cholangitis after liver transplantation. *Liver Transpl* 2003; **9**: 727–32.
5. Lindor KD. New treatment strategies for primary sclerosing cholangitis. *Dig Dis* 2011; **29**: 113–6.
6. Lindor KD. Ursodiol for primary sclerosing cholangitis. Mayo primary sclerosing cholangitis-ursodeoxycholic acid study group. *N Engl J Med* 1997; **336**: 691–5.
7. Mendes F. Current and future therapy for primary sclerosing cholangitis. *Gastroenterol Hepatol (N Y)* 2008; **4**: 258–60.
8. Lindor KD, Kowdley KV, Luketic VA, *et al.* High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. *Hepatology* 2009; **50**: 808–14.
9. LaRusso NF, Wiesner RH, Ludwig J, MacCarty RL, Beaver SJ, Zinsmeister AR. Prospective trial of penicillamine in primary sclerosing cholangitis. *Gastroenterology* 1988; **95**: 1036–42.

10. Knox TA, Kaplan MM. Treatment of primary sclerosing cholangitis with oral methotrexate. *Am J Gastroenterol* 1991; **86**: 546–52.
11. Olsson R, Broome U, Danielsson A, *et al.* Colchicine treatment of primary sclerosing cholangitis. *Gastroenterology* 1995; **108**: 1199–203.
12. Schramm C, Schirmacher P, Helmreich-Becker I, Gerken G, zum Buschenfelde KH, Lohse AW. Combined therapy with azathioprine, prednisolone, and ursodiol in patients with primary sclerosing cholangitis. A case series. *Ann Intern Med* 1999; **131**: 943–6.
13. Angulo P, Batts KP, Jorgensen RA, LaRusso NA, Lindor KD. Oral budesonide in the treatment of primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**: 2333–7.
14. Bharucha AE, Jorgensen R, Lichtman SN, LaRusso NF, Lindor KD. A pilot study of pentoxifylline for the treatment of primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**: 2338–42.
15. Lindor KD, Wiesner RH, Colwell LJ, Steiner B, Beaver S, LaRusso NF. The combination of prednisone and colchicine in patients with primary sclerosing cholangitis. *Am J Gastroenterol* 1991; **86**: 57–61.
16. Pollheimer MJ, Halilbasic E, Fickert P, Trauner M. Pathogenesis of primary sclerosing cholangitis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 727–39.
17. Tabibian JH, O'Hara SP, Larusso NF. Primary sclerosing cholangitis: the gut-liver axis. *Clin Gastroenterol Hepatol* 2012; **10**: 819.
18. Aron JH, Bowlus CL. The immunobiology of primary sclerosing cholangitis. *Semin Immunopathol* 2009; **31**: 383–97.
19. Wiesner RH, Grambsch PM, Dickson ER, *et al.* Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. *Hepatology* 1989; **10**: 430–6.
20. Oriishi T, Sata M, Toyonaga A, Sasaki E, Tanikawa K. Evaluation of intestinal permeability in patients with inflammatory bowel disease using lactulose and measuring antibodies to lipid A. *Gut* 1995; **36**: 891–6.
21. Murphy MS, Eastham EJ, Nelson R, Pearson AD, Laker MF. Intestinal permeability in Crohn's disease. *Arch Dis Child* 1989; **64**: 321–5.
22. Lepage P, Hasler R, Spehlmann ME, *et al.* Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. *Gastroenterology* 2011; **141**: 227–36.
23. Broome U, Olsson R, Loof L, *et al.* Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. *Gut* 1996; **38**: 610–5.
24. Worthington J, Cullen S, Chapman R. Immunopathogenesis of primary sclerosing cholangitis. *Clin Rev Allergy Immunol* 2005; **28**: 93–103.
25. Lichtman SN, Okoruwa EE, Keku J, Schwab JH, Sartor RB. Degradation of endogenous bacterial cell wall polymers by the muralytic enzyme mutanolysin prevents hepatobiliary injury in genetically susceptible rats with experimental intestinal bacterial overgrowth. *J Clin Invest* 1992; **90**: 1313–22.
26. Lichtman SN, Keku J, Schwab JH, Sartor RB. Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. *Gastroenterology* 1991; **100**: 513–9.
27. Davies YK, Cox KM, Abdullah BA, Safta A, Terry AB, Cox KL. Long-term treatment of primary sclerosing cholangitis in children with oral vancomycin: an immunomodulating antibiotic. *J Pediatr Gastroenterol Nutr* 2008; **47**: 61–7.
28. Cox KL, Cox KM. Oral vancomycin: treatment of primary sclerosing cholangitis in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1998; **27**: 580–3.
29. Tada S, Ebinuma H, Saito H, Hibi T. Therapeutic benefit of sulfasalazine for patients with primary sclerosing cholangitis. *J Gastroenterol* 2006; **41**: 388–9.
30. Elfaki DA, Lindor KD. Antibiotics for the treatment of primary sclerosing cholangitis. *Am J Ther* 2011; **18**: 261–5.
31. Stanich PP, Bjornsson E, Gossard AA, Enders F, Jorgensen R, Lindor KD. Alkaline phosphatase normalization is associated with better prognosis in primary sclerosing cholangitis. *Dig Liver Dis* 2011; **43**: 309–13.
32. Rankin JG, Boden RW, Goulston SJ, Morrow W. The liver in ulcerative colitis; treatment of pericholangitis with tetracycline. *Lancet* 1959; **2**: 1110–2.
33. Boner AL, Peroni D, Bodini A, Delaini G, Piacentini G. Azithromycin may reduce cholestasis in primary sclerosing cholangitis: a case report and serendipitous observation. *Int J Immunopathol Pharmacol* 2007; **20**: 847–9.
34. Kozaiwa K, Tajiri H, Sawada A, *et al.* Three paediatric cases of primary sclerosing cholangitis treated with ursodeoxycholic acid and sulphasalazine. *J Gastroenterol Hepatol* 1998; **13**: 825–9.
35. Broccoletti T, Spaziano M, Capuano G, *et al.* Refractory primary sclerosing cholangitis becoming responsive after sulphasalazine treatment of an underlying silent colitis. *Ital J Pediatr* 2002; **28**: 515–7.
36. Mistilis SP, Skyring AP, Goulston SJ. Effect of long-term tetracycline therapy, steroid therapy and colectomy in pericholangitis associated with ulcerative colitis. *Australas Ann Med* 1965; **14**: 286–94.
37. Kim WR, Therneau TM, Wiesner RH, *et al.* A revised natural history model for primary sclerosing cholangitis. *Mayo Clin Proc* 2000; **75**: 688–94.
38. Farkkila M, Karvonen AL, Nurmi H, *et al.* Metronidazole and ursodeoxycholic acid for primary sclerosing cholangitis: a randomized placebo-controlled trial. *Hepatology* 2004; **40**: 1379–86.
39. Silveira MG, Torok NJ, Gossard AA, *et al.* Minocycline in the treatment of patients with primary sclerosing cholangitis: results of a pilot study. *Am J Gastroenterol* 2009; **104**: 83–8.
40. Harada K, Nakanuma Y. Biliary innate immunity: function and modulation. *Mediators Inflamm* 2010; **2010**: 1–9.
41. Mueller T, Beutler C, Pico AH, *et al.* Enhanced innate immune responsiveness and intolerance to intestinal endotoxins in human biliary epithelial cells contributes to chronic cholangitis. *Liver Int* 2011; **31**: 1574–88.
42. Sasatomi K, Noguchi K, Sakisaka S, Sata M, Tanikawa K. Abnormal accumulation of endotoxin in biliary epithelial cells in primary biliary cirrhosis and primary sclerosing cholangitis. *J Hepatol* 1998; **29**: 409–16.
43. Seki E, De Minicis S, Osterreicher CH, *et al.* TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324–32.
44. Bjornsson E, Cederborg A, Akvist A, Simren M, Stotzer PO, Bjarnason I. Intestinal permeability and bacterial growth of the small bowel in patients with primary sclerosing cholangitis. *Scand J Gastroenterol* 2005; **40**: 1090–4.
45. Lichtman SN, Keku J, Clark RL, Schwab JH, Sartor RB. Biliary tract disease in rats with experimental small bowel bacterial overgrowth. *Hepatology* 1991; **13**: 766–72.
46. Lichtman SN, Sartor RB. Hepatobiliary injury associated with experimental small-bowel bacterial overgrowth in rats. *Immunol Res* 1991; **10**: 528–31.
47. Yamada S, Ishii M, Kisara N, Nagatomi R, Toyota T. Macrophages are essential for lymphocyte infiltration in formyl peptide-induced cholangitis in rat liver. *Liver* 1999; **19**: 253–8.
48. Yamada S, Ishii M, Liang LS, Yamamoto T, Toyota T. Small duct cholangitis induced by N-formyl L-methionine L-leucine L-tyrosine in rats. *J Gastroenterol* 1994; **29**: 631–6.
49. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. *Clin Infect Dis* 1994; **18**(Suppl. 1): S79–83.