REVIEW

Anti-*Mycobacterium paratuberculosis* (MAP) therapy for Crohn's disease: an overview and update

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ABSTRACT

The role of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in the pathogenesis of Crohn's disease (CD) has been strongly debated for many years. MAP is the known aetiological agent of Johne's disease, a chronic enteritis affecting livestock. At present, due to the paucity of high-quality data, anti-MAP therapy (AMT) is not featured in international guidelines as a treatment for CD. Although the much-quoted randomised trial of AMT did not show sustained benefits over placebo, questions have been raised regarding trial design, antibiotic dosing and the formulation used. There are several lines of evidence supporting the CD and MAP association with uncontrolled and controlled trials demonstrating effectiveness, including a retrospective review of cases treated at our own institution. Here, we provide an overview of the evidence supporting and refuting AMT in CD before focussing on updates of the current research in the field, including the ongoing trials with the novel RHB-104 formulation and the MAP vaccine trial. While controversial, gastroenterologists are often asked about long-term combination antibiotic therapy for CD. There has been broadcast and social media coverage surrounding this, particularly with regard to current trials. Although patients should not be deterred from treatments of proven effectiveness, this review aims to help with commonly asked questions and highlights our own approach for the use of anti-MAP in specific circumstances.

Key messages

- The association of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) with Crohn's disease (CD) is a longstanding controversial issue due to paucity of conclusive data.
- Patients with CD are more likely to have MAP present in intestinal tissues, but whether this has a role in pathogenesis or is merely an innocent bystander remains unknown.
- Combination antibiotics targeting MAP have shown therapeutic benefit in a number of studies, but the 2007 Selby study showed disappointing results, although there have been several criticisms of the trial design.
- A multicentre phase III randomised controlled trial (MAP US) showed superiority in response and remission over placebo after 26 weeks of continuous treatment when used as an add-on to conventional therapy.
- The MAP vaccine phase Ib trial, which uses an adenovirus-vectored vaccine to induce an immune response against MAP, is expected to be open to recruitment later in 2020.
- Anti-MAP therapy for a 2-year period can be considered for patients with refractory disease or in patients where immune suppression is not appropriate.

INTRODUCTION

Crohn's disease (CD) is a chronic, relapsing-remitting inflammatory condition of the gut, leading to progressive bowel damage and a poor quality of life. Although it can affect any part of the digestive tract, it has a predilection for the ileocolonic and perianal regions. Cardinal symptoms of CD include chronic diarrhoea and abdominal pain, which is often complicated by systemic features such as malaise and weight loss. CD has no gender predisposition and its usual age of onset lies between the second and fourth decades, with a smaller peak between the

ages of 50 and 60.[1] The exact aetiology of CD remains unclear but it is thought to arise from a combination of genetic predisposition, altered gut microbiota and environmental factors leading to dysregulated immune responses.[2] Indeed, the functional impacts of the genes associated with an increased CD risk are commonly those involved in bacteria-immune interactions. The suggestion that *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is the causative agent of CD, and a potential cause for immune dysregulation, dates back more than a century. MAP is an atypical, non-tuberculous, obligate intracellular pathogen, part of the *Mycobacterium avium* complex (MAC).[3] In 1912, it was identified as the cause of Johne's disease, a chronic granulomatous enteritis affecting domestic livestock.[3] The following year, Dalziel identified a similarity in the clinicopathological features of Johne's disease and a stricturing and fistulising enteritis affecting humans, later identified as CD.[4] Hypotheses surrounding the causal association present today stem from here.

There has been much discussion regarding the environmental presence of MAP and transmission to humans. MAP is excreted in the faeces and milk of infected ruminants and is resistant to standard pasteurisation and other thermal treatments due to its thick, waxy mycolic cell wall.[5–7] This resistance facilitates environmental exposure to humans through a number of sources, particularly the consumption of meat and milk. The prolonged incubation period and asymptomatic, subclinical phase in ruminants also assist spread, as products continue to be farmed from infected livestock. However, this potential public health concern is difficult to acknowledge until the consequence of repeated MAP exposure in humans is clear.

MAP is a very slow growing organism and attempts to culture the organism are notoriously difficult, taking from several weeks to months. A seminal experiment took 18 months for a blood culture to grow MAP in a patient with CD.[8] Furthermore, it is very difficult to microscopically visualise the organism in infected tissue samples. The lack of a cell wall in human infection and its atypical mycobacterial classification renders the Ziehl-Nielsen stain uninformative. Due to the genetic similarities between MAC members, unique markers for the detection of MAP are limited. Molecular-based techniques, including nested PCR and in situ hybridisation, have targeted the Insertion Sequence 900 (IS900), which is found specifically in the MAP genome.[9–11] Ongoing research seeks to standardise methods to detect and isolate MAP and achieve consistent and reproducible conclusions.[12] The inconsistent methods used thus far, generating contradictory results in studies, have questioned the causal relationship and led to some of the controversy surrounding AMT.

Despite studies investigating the link between MAP and CD over the past century, evidence remains circumstantial and the link unproven. Given the genetic and phenotypic heterogeneity of CD, it remains plausible that MAP is relevant in a subset of patients, and therefore, consideration of AMT by both doctors and patients remain commonplace. In this article, we provide a brief overview of the evidence supporting and contesting the use of AMT in CD, followed by an update of the current trials. We highlight our own approach for the use of AMT in a subpopulation of patients with CD for whom this treatment may be beneficial such as those with refractory disease, where conventional treatment has failed, or where immunomodulation is contraindicated or best avoided.

EVIDENCE SUPPORTING ANTI-MAP THERAPY

Together with its clinical and pathological similarities to Johne's disease, the causal association is supported by the higher prevalence of MAP in serum, breast milk and intestinal tissue of patients with CD compared with healthy controls.[13–17] This has been replicated in a blinded study in independent laboratories and summarised in two systematic reviews and meta-analyses.[18 19] Its relatively low pathogenic expression may help, in part, explain its presence in healthy individuals. It is difficult to prove that the presence of MAP preceded gut damage. These findings either highlight an aetiological role of MAP in a subset of genetically predisposed patients with CD or merely highlight selective colonisation of MAP in CD with little role in disease.

There are numerous randomised controlled trials (RCTs) evaluating the therapeutic benefit of long-term antibiotic therapies in CD. These have used differing antibiotic combinations, treatment durations and endpoints, and many of these have used antibiotics that target MAP. Khan *et al* showed that the strongest signal by antibiotic type appeared to be with clofazimine (RR 0.70; 95% CI 0.53 to 0.94) and rifamycin (RR 0.78; 95% CI 0.67 to 0.91), both commonly used in AMT regimens.[20] Feller *et al* demonstrated that long-term treatment with nitroimidazoles showed benefit across three RCTs, with a combined OR of 3.54 (95% CI 1.94 to 6.47), and similarly, the combined OR from four RCTs of clofazimine was 2.86 (95% CI 1.67 to 4.88).[18] For reasons discussed later in this article, standard anti-tuberculosis (TB) therapy was found to be ineffective. These meta-analyses looking at trials of antibiotics in active CD highlight a statistically significant effect above placebo for antibiotics active against MAP . However, with primary endpoints of clinical remission and relapse, concurrent unintended treatment of other diagnoses, for example, irritable bowel syndrome or bacterial overgrowth, which are more frequent in patients with inflammatory bowel disease (IBD), is an important confounder to note.

To address this, a number of studies have shown a therapeutic benefit of AMT in CD with objective measures of response. In a study of 52 patients with severe CD, Gui *et al* demonstrated a significant improvement in Harvey Bradshaw Index, inflammatory markers (C-reactive protein (CRP) and

erythrocyte sedimentation rate (ESR) and a reduction in corticosteroid use after a mean treatment period of 2 years with rifabutin and macrolide antibiotics.[21] In a retrospective analysis of 39 patients with severe CD treated with AMT, Borody *et al* demonstrated endoscopic ulcer healing in 56% of patients.[22] Indeed, a retrospective analysis of 41 patients with follow-up data treated at our own institution over a 7-year period (of a total of 62 patients treated) highlighted a symptomatic benefit in 46% of patients. Importantly, 63% of these patients had improved biochemical markers, radiological or endoscopic indices.[23] Most of these patients had an aggressive disease phenotype and had failed conventional therapy at a time where drugs such vedolizumab and ustekinumab were not available. In our cohort, response was not associated with disease phenotype, prior therapy or use of clofazimine but patients who responded had a longer duration of therapy (median 24 months compared with 14 months; p=0.04) than patients who did not respond.[23] AMT was well tolerated with only five patients (12%) discontinuing therapy due to adverse effects, including cases of a rash, uveitis and arthralgia, secondary to rifabutin. While this study may be limited by flaws inherent to retrospective work in a heterogeneous group of patients with CD, there is a signal of clinical response in a reasonable proportion.

Further and more robust evidence of benefit stems from the preliminary results of the Food and Drug Administration (FDA)'s first, phase III multicentre RCT looking at a novel AMT formulation as an add-on therapy in CD. Although the specific findings will be described later, there were significant improvements in symptoms and biochemical markers of disease activity.[24 25] In a subset of patients who underwent endoscopy, a greater proportion of patients randomised to receive the drug had improved SED-CD 50 scores. Together with the above, these findings also lend further support to the MAP-CD theory.

EVIDENCE AGAINST THE USE OF ANTI-MAP THERAPY

There are several lines of reason that contradict the MAP-CD hypothesis, including epidemiological evidence. Conditions that promote spread of infection, such as overcrowding and poor sanitation, appear to be protective against CD with a higher incidence in developed countries than in developing countries and in urban areas than in rural areas.[1] Immunosuppressive regimens induce and maintain remission in CD rather than worsen outcomes, pointing away from an infectious aetiology. Also, despite significant exposure to MAP through domestic livestock, there is no greater increase in CD in dairy farmers and veterinarians compared with those living in urban areas.[26–29] However, proponents of the MAP-CD theory argue that this may be due to a degree of protective immunity via similar mechanisms to the hygiene hypothesis.[30]

Due to a paucity of conclusive data and the controversy surrounding AMT, the most up-to-date UK, US and European clinical guidelines do not support antimycobacterial treatment regimens in the management of CD.[31–33] As well as evidence of therapeutic benefit of AMT in the aforementioned text, a number of studies looking at the effect of AMT in CD have yielded disappointing results. Four placebo-controlled trials did not show evidence of benefit of AMT.[34–38] This included a study by Swift *et al*, which looked at 138 patients with active CD randomised to receive rifampicin, isoniazid and ethambutol or placebo. Overall, there was no evidence of consistent benefit or disadvantage from AMT up to a 5-year period.[37 38] However, it is worth bearing in mind that these studies used older anti-TB drugs ineffective against MAC with some using less than three drugs in combination, thus promoting antibiotic resistance. A meta-analysis by Borgaonkar in 2000 suggested a role for AMT in maintaining remission but no definite conclusions could be drawn from the small number of heterogeneous clinical trials, some of which also required corticosteroids alongside AMT to show benefit.[39]

The study published by Selby *et al* in 2007 was concluded to be unsupportive of the AMT-CD theory. In this double-blinded, placebo-controlled trial, 213 patients with active CD were randomised to a 2-year course of daily clarithromycin (750 mg), rifabutin (450 mg) and clofazimine (50 mg) or placebo, in addition to a 16-week tapering course of prednisolone.[40] The primary endpoint was relapse at 12, 24 and 36 months. While there was a significant benefit favouring the antibiotic arm at week 16, there was little evidence of prolonged benefit beyond this above placebo.[40]

While the majority of the gastroenterologists community are satisfied that the Selby study has effectively disproven the role of AMT in CD, this trial has also been criticised for two main reasons. First, the antibiotic dosing used was suboptimal for MAP clearance, with clofazimine administered in a double encapsulated form that the authors identified may have hindered bioavailability.[41–43] Second, in a letter to the *Lancet,* Behr *et al* performed a reanalysis with an intention-to-treat analysis, which showed a significant difference favouring the AMT arm at not only week 16 but also after 12 and 24 months (p=0.003 and p=0.005, respectively).[44] Box 1 highlights a summary of the arguments supporting and refuting the use of AMT in CD.

UPDATE OF CLINICAL TRIALS

Redhill Biopharma's RHB-104 phase III RCT of anti-MAP in CD

RedHill Biopharma conducted the first FDA-approved global multicentre phase III RCT studying AMT in CD using a novel oral formulation of combination antibiotic therapy. The MAP US study is a double-blinded, placebo-controlled trial of 331 patients investigating

Box 1 Summary of arguments supporting and refuting the use of AMT in CD

Arguments for;

- CD shares clinical, pathological and immunological[57] similarities to Johne's disease, a chronic granulomatous enteritis affecting livestock, known to be caused by MAP
- MAP is detected more frequently in blood, breast milk and tissue of patients with CD than healthy controls[17–19]
- Numerous case series, uncontrolled and controlled studies of accurate AMT use, demonstrate therapeutic benefit in CD[18 20–23]
- A phase III randomised controlled trial (MAP US) showed superiority in response and remission over placebo when used as add-on therapy[24]

Arguments against;

- A number of studies have yielded disappointing results including the 2007 Selby study, which is the only RCT published to date,[40] however limitations to the study have been identified
- Immunosuppression does not increase spread of MAP or increase clinical symptoms
- Detection of MAP is not exclusive to CD patients and is found in healthy controls
- At-risk populations to MAP for example, farmers, vets do not show a higher rate of CD incidence

AMT, anti-MAP therapy; CD, Crohn's disease; MAP, Mycobacterium avium subspecies paratuberculosis; RCT, randomised controlled trial.

the safety and efficacy of add-on RHB-104 in moderate to severe CD.[45] RHB-104 encapsulates clarithromycin (95 mg), clofazimine (10 mg) and rifabutin (45 mg) administered in a regimen of five capsules twice daily for 12 months, in addition to standard CD treatment. These doses are higher than those used in the Selby study.[40]

The proportion of patients in clinical remission (Crohn's Disease Activity Index (CDAI) <150) at week 16 and at week 24, the primary endpoint, was greater in the RHB-104 add-on arm compared with placebo; week 16 (42.2% vs 29.1%, p=0.015), week 26 (37% vs 23%, p=0.007).[25] The primary endpoint was met in an intention-to-treat population.[46] In the antibiotic arm, there was a statistically significant drop in biochemical markers of disease activity (CRP or faecal calprotectin) at week 16 (25.9% vs 9.7%, p=0.0002) and week 24 (21.1% vs 9.1%, p=0.0003). There was an endoscopic response as evaluated by Simple Endoscopic Score for Crohn's Disease (SES-CD) in the small group of patients that underwent colonoscopy.

Patients that did not enter clinical remission at week 26 were eligible for enrolment in the open-label study with RHB-104 (MAP US2), which has completed recruitment.[47] The primary outcome measure is remission (CDAI <150) at week 16. Importantly, key secondary outcomes include measurement of MAP from blood by PCR at baseline and at several timepoints during treatment. This was not available in the first MAP US study. Data describing outcomes from MAPUS2 and the longer term outcomes from MAP US are eagerly awaited.

Anti-MAP vaccine for CD

The difficulties with treating mycobacterial infection are multifold: their slow reproductive rate, the presence of cell wall deficient forms and their ability to evade host immune detection via a series of evolved mechanisms, such as intracellular residence. Therefore, achieving eradication with anti-mycobacterial chemotherapy is difficult. It is not the mycobacterial presence that is problematic as only 3%–5% of the 2 billion people worldwide with latent TB develop clinical pathology, rather, it is the form or phenotype that triggers pathogenicity, with other contributing steps.[48]

Virally vectored vaccines are a promising tool for foreign antigen delivery to not only protect and initiate the primary immune response but also improve activation of adaptive T-cells to recognise and clear pathogens. This is made possible by replacing the traditional inert plasmid component with an inactivated non-replicating virus. Viral vector vaccines are generally able to produce stronger immune responses than DNA vaccines. There are no viral vector vaccines currently licenced for human use due to difficulties in striking the correct balance between immunogenicity, efficacy (anti-vector immunity vs ability to induce T-cell responses) and safety.[49]

A forthcoming phase Ib clinical trial at Guy's and St Thomas' Hospitals in London aims to assess the safety and immunogenicity of a MAP vaccine in patients with CD. A replication-deficient simian adenovirus-vectored vaccine, expressing four MAP genes, was tested in a phase Ia trial in healthy human volunteers and found to be safe, well tolerated and immunogenic.[50 51] Human adenovirus vectors are not only effective at carrying antigens to host cells to target

Table 1 Guy's and St. Thomas' approach to anti-MAP therapy in Crohn's disease

Drug	Dose	Frequency	Commonly occurring side effects
Clarithromycin	500 mg	BD	Nausea, metallic taste, GI intolerance
Clofazimine	100–150 mg	OD	Skin discolouration, abdominal pain
Rifabutin	300 mg	BD	Discoloured secretions, uveitis, hepatitis, flu-like symptoms, leucopenia, rash

Rifabutin is commenced at 150 mg BD and gradually uptitrated to a total daily dose of 450–600 mg as tolerated and if weight is >50 kg.
BD, twice daily; GI, gastrointestinal; OD, once daily.

intracellular pathogens, but have previously been found to induce a robust immune response and to have an excellent safety profile.[49] Recruitment to the phase Ib aspect of the trial is expected as soon as research activities resume following the COVID-19 pandemic. Whether this results in a clinical response in patients with CD by eliciting cellular immune responses to MAP is of great interest.

ANTI-MAP THERAPY FOR CD: THE GUY'S AND ST THOMAS' APPROACH

Despite advances in therapy over the past two decades, CD remains a chronic, progressive and debilitating illness. Failure on available licenced therapy remains commonplace and sometimes use of immunosuppressive regimens in those with concurrent malignancy, or treated malignancy with high risk of recurrence, is not appropriate. Although patients and clinicians should not be deterred from using CD treatments of proven effectiveness, AMT remains a viable option in those with refractory disease or in those where immunosuppression is best avoided. Unfortunately, the off-label use of these medications means that patients are required to self-fund treatment.

MAP is difficult to treat with standard anti-TB regimens and controlled trials have not shown any meaningful short-term or long-term improvement in CD.[37 38] The lack of a cell wall in human MAP infection means that antibiotics working by disrupting the cell wall are unlikely to be effective. The combination of treatment against several strains of the *Mycobacterium avium* complex was first described in 1996 in a twice daily to treat patients with AIDS.[52] In this study, clarithromycin and clofazimine, with good transmembrane penetration, were most effective for intracellular MAC organisms.[52] Numerous studies have since demonstrated activity against atypical mycobacteria strains.

Various antibiotic regimens have been used to eradicate MAP but the combination of a macrolide, rifabutin and clofazimine has been the most effective in a number of studies.[21 53 54] Also, using this combination, the intention-to-treat analysis of the 2007 Selby trial demonstrated superiority over placebo at 4, 12 and 24 months.[40 44] Unlike clarithromycin, which is widely prescribed, clofazimine and rifabutin are reserved for atypical mycobacterial infections. Clofazimine is an orphan drug and may be difficult to obtain. Combination therapy minimises microbial resistance and also increases effectiveness as the synergism helps disrupt more of the organism's cellular processes.[55]

The Guy's and St Thomas' approach has been to use a prolonged three-drug antibiotic regimen (table 1). Patients are assessed clinically following 3 months of treatment, and if there is evidence of objective improvement in disease activity, treatment is continued for a total of 2 years. It is worth noting that other IBD centres in the world commonly use four antibiotics, incorporating a nitroimidazole such as metronidazole or tinidazole, to enhance effectiveness and reduce resistance. As discussed, preliminary data from the MAP US trial highlight the superiority of RHB-104 was more pronounced in patients receiving concomitant anti-tumour necrosis factor or immunosuppressants, which may change the way AMT is used in the future.

Although AMT is usually well tolerated, patients should be monitored for leucopenia and abnormal liver function tests. Full blood count and liver function tests are recommended at weeks 2, 4, 8, 12 and 3 monthly thereafter, akin to thiopurine monitoring.[56] There is good safety data on the long-term use of AMT in *Mycobacterium tuberculosis* complex, leprosy, MAC and HIV. While there can be common short-term disadvantages such as nausea, intolerability and reversible discolouration of the skin, and significant problems such as renal failure and chronic hepatitis are uncommon.

CONCLUSIONS

At present, the lack of conclusive evidence supporting an aetiological role of MAP in CD has left the IBD community divided. Whether MAP causes CD, has a role in the pathogenesis or is merely an innocent bystander in CD remains unclear. Ongoing research aims to improve diagnostic detection methods to achieve consistent results. The ideal trial would use these methods to determine the effect of AMT in MAP positive CD patients and to see if MAP eradication leads to a meaningful clinical outcome. Harnessing our adaptive immune system to target MAP is the rationale behind the MAP vaccine trial, which is due to begin recruitment at Guy's and St Thomas' in mid to late 2020.

Therapies currently licenced for CD target aspects of the immune response to downgrade and suppress inflammation. AMT, which allows preservation of systemic immune responses, has demonstrated effectiveness in a number of studies to provide another option for treatment refractory patients. Outcomes from the ongoing MAP US phase III trial looking at a novel AMT formulation are awaited.

Patients with CD should be treated with conventional therapies as per international guidelines. However, there are a subset of patients that *may* benefit from AMT when standard therapy is contraindicated or there is a lack of efficacy. The ongoing trials of an AMT 'add-on' therapy and MAP vaccine offer exciting potential additions to the CD treatment armamentarium.

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