Two-Year Combination Antibiotic Therapy With Clarithromycin, Rifabutin, and Clofazimine for Crohn's Disease

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Background & Aims: ***Mycobacterium avium* subspecies *paratuberculosis* has been proposed as a cause of Crohn's disease. We report a prospective, parallel, placebo-controlled, double-blind, randomized trial of 2 years of clarithromycin, rifabutin, and clofazimine in active Crohn's disease, with a further year of follow-up.** ***Methods:*** **Two hundred thirteen patients were randomized to clarithromycin 750 mg/day, rifabutin 450 mg/day, clofazimine 50 mg/day or placebo, in addition to a 16-week tapering course of prednisolone. Those in remission (Crohn's Disease Activity Index ≤150) at week 16 continued their study medications in the maintenance phase of the trial. Primary end points were the proportion of patients experiencing at least 1 relapse at 12, 24, and 36 months.** ***Results:*** **At week 16, there were significantly more subjects in remission in the antibiotic arm (66%) than the placebo arm (50%; $P = .02$). Of 122 subjects entering the maintenance phase, 39% taking antibiotics experienced at least 1 relapse between weeks 16 and 52, compared with 56% taking placebo ($P = .054$). At week 104, the figures were 26% and 43%, respectively ($P = .14$). During the following year, 59% of the antibiotic group and 50% of the placebo group relapsed ($P = .54$).** ***Conclusions:*** **Using combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for up to 2 years, we did not find evidence of a sustained benefit. This finding does not support a significant role for *Mycobacterium avium* subspecies *paratuberculosis* in the pathogenesis of Crohn's disease in the majority of patients. Short-term improvement was seen when this combination was added to corticosteroids, most likely because of nonspecific antibacterial effects.**

A specific bacterial cause for Crohn's disease has been sought since Crohn himself first postulated that the illness was due to a mycobacterial infection. The mycobacterial hypothesis was revived by the isolation of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) from 3 of 11 patients in 1984.[1] Since then, numerous reports have appeared describing its detection in tissue, blood, and even breast milk in patients with Crohn's disease.[2–4] However, others have not found differences between Crohn's disease and controls, and, therefore, the role of the organism remains controversial.[5,6] Conventional antituberculous antibiotics have been ineffective in treating Crohn's disease, even after prolonged treatment.[7] Several open-label studies using agents with activity against MAP have suggested favorable responses to treatment.[8–13] MAP is a slow growing, obligate intracellular organism, which appears to exist as a cell wall-deficient form, characteristics that confer resistance to these antibiotics. The most convincing evidence of a role for MAP in Crohn's disease would be the demonstration of a prolonged benefit from treatment with appropriate antibiotics for sufficient duration to kill the organism. We report a prospective, parallel, placebo-controlled, double-blind, randomized treatment trial using the combination of clarithromycin, rifabutin, and clofazimine for 2 years in patients entering with active Crohn's disease, with a further year of follow-up. In the absence of data about the efficacy of this antibiotic therapy in inducing remission, the study design included an initial 16-week phase in which all patients received prednisolone in addition to trial medications. The predetermined primary end points of the study were the proportions of patients who experienced at least 1 relapse at 12, 24, and 36 months.

Materials and Methods

Subjects

Patients over 18 years of age diagnosed with Crohn's disease according to standard criteria and active disease defined as Crohn's Disease Activity Index (CDAI) ≥200 were enrolled at 20 centers around Australia. Pa-

Abbreviations used in this paper: CDEIS, Crohn's Disease Endoscopic Index of Severity; MAP, *Mycobacterium avium* subspecies *paratuberculosis*; MP, mercaptopurine.

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tients with isolated upper gastrointestinal or isolated perianal disease or a stoma were excluded, as were those requiring intravenous corticosteroids at initial assessment and those thought likely to require surgery during the first 4 months of the study. Permitted medications included corticosteroids at a dose of prednisone of 10 mg or less (or other corticosteroids at an equivalent dosage) over the month prior to enrollment; immunomodulator therapy with azathioprine/6-mercaptopurine (6/MP) at a stable dose for at least 6 months prior to enrolment; and 5-aminosalicylates at a stable dose for at least 4 weeks prior to entry. The use of antibiotics for Crohn's disease within 1 month of entry was an exclusion criterion. No patient had used infliximab.

Study Design

Induction phase. After a screening visit, all subjects were commenced on oral prednisolone 40 mg/day, with a predetermined dosage schedule reducing to zero over 16 weeks, and randomized to receive either the antibiotic combination or matching placebos from week 1. Doses were increased gradually to minimize adverse effects. Clarithromycin was commenced at 250 mg daily for week 1, 250 mg twice daily for weeks 2 and 3, and then 750 mg/day from week 4. Rifabutin dosage was increased at the same time points using daily doses of 150 mg, 300 mg, and building up to 450 mg. Clofazimine was given at a dose of 50 mg daily. Clofazimine capsules were reencapsulated with a second outer gelatine capsule to match the placebo. Subjects were stratified for use of thiopurine therapy. Subjects who had not achieved remission at week 16 (CDAI $\leq$150), and those unable to tolerate full doses of the study medication, were considered treatment failures and were withdrawn.

Maintenance phase. Subjects in remission at week 16 continued trial medications. If a subject had a subsequent relapse (CDAI $>$150 with an increase $\geq$60), he or she could be retreated with "rescue" prednisolone. Failure to respond to this retreatment was an indication for withdrawal. Other rescue treatments for active Crohn's disease were not permitted. Subjects were deemed treatment failures and withdrawn if they were not in remission at the primary end points at week 52 or 104.

Follow-up phase. At 104 weeks, the trial medications were ceased. Subjects in remission at week 104 returned for follow-up visits at weeks 130 and 156.

Assessment. At each visit, subjects underwent clinical assessment, hematologic and biochemical monitoring, and calculation of CDAI. They were instructed to return all unused medication and empty packages at the following clinic visit. Compliance was assessed by reconciliation of subjects' records and pharmacy returns. Subjects who had taken 80% or more of all 3 trial medications were considered to be compliant. Colonoscopy was performed at enrollment and yearly in subjects remaining in the study. The Crohn's Disease Endoscopic Index of Severity (CDEIS) was used to measure disease activity.[14] Quality of life was measured at 3-month intervals using 3 questionnaires (SF-36, Inflammatory Bowel Disease Questionnaire, and Assessment of Quality of Life).

The predetermined primary end points were the proportion of subjects experiencing at least 1 relapse of Crohn's disease at 12, 24, and 36 months. Secondary end points were percentage of subjects in remission at week 16, number of relapses within each study period, time to first relapse, safety profiles of each treatment arm, other clinical outcomes (CDEIS, need for Crohn's-related surgery), changes in laboratory parameters of activity (albumin, C-reactive protein, erythrocyte sedimentation rate), and quality of life.

Analysis and Statistical Method

The size of the sample was determined by assuming that 60% of subjects would achieve remission after 16 weeks of oral corticosteroids and that 20% would not be available for analysis. It was estimated that the relapse rate in the placebo group after 24 months would be 70% and that a difference of 40% in relapse rates between the active treatment and placebo arms would be clinically significant. These estimates were based on published response rates in trials using corticosteroids and from the limited data available from uncontrolled studies of similar antibiotics in Crohn's disease. To provide statistical power of 0.8 for the final analyses to achieve a significance level of 0.017 (allowing for multiple comparisons of primary end points), recruitment of 106 subjects was required per study group, thus leaving a predicted 53 subjects per group for analysis at the beginning of week 16. Regression modelling and analyses were used to compare the percentage of subjects in each treatment group who experienced at least 1 relapse of Crohn's disease during the relevant study period (logistic regression analysis), the number of relapses (Poisson regression analysis), and the time to relapse (Cox proportional hazards model). Multiple regression analysis was used to compare the change in CDEIS and laboratory parameters. Statistical tests were 2-sided, conducted at the 0.017 level of significance for the primary end points and 0.05 for secondary end points. The odds ratios for the treatment comparison of placebo versus antibiotics were calculated with 95% confidence intervals and P values. SAS statistical software (Release 6.12; SAS Institute, Cary, NC) was used for all analyses.

Adverse events were coded using the MedDRA Dictionary (Version 2.1). The percentage of subjects with an adverse event in each treatment group was compared by Pearson χ^2 test.

All data analysis was carried out by Covance Clinical Development Services, Braddon ACT, an independent analyst. The study was initiated by the investigators and supported by Pharmacia, Pharmacia and Upjohn, and

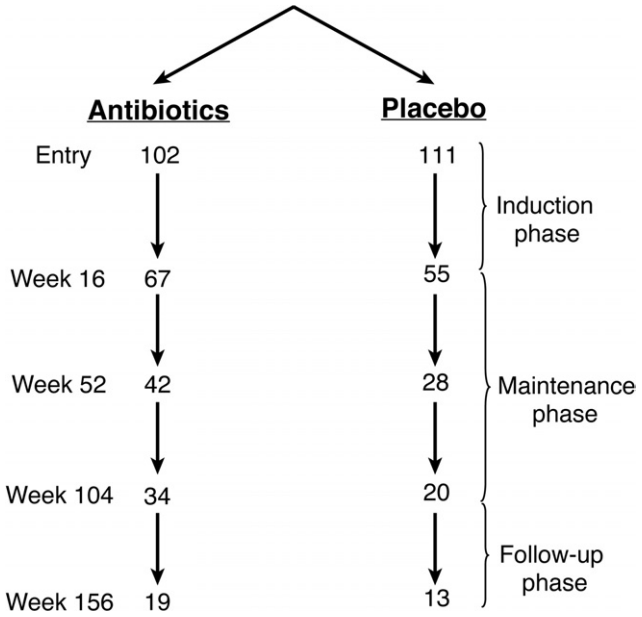

Figure 1. Subject disposition.

Pfizer Pty Ltd. The trial was approved by the relevant Health Research Ethics Committee at all participating centres. Written, informed consent was obtained from all subjects. The trial was registered with the Australian Clinical Trial Register (number 00000611).

Results

Subject Disposition and Induction Phase

Between September 1999 and September 2001, 213 patients were enrolled and randomized to receive either antibiotics (n = 102) or placebo (n = 111) (Figure 1). The characteristics of these 2 groups are shown in Table 1. There were no significant differences between them.

There were significantly more patients in the antibiotics arm who went on to the maintenance phase starting at 16 weeks (P = .02). Ninety-one subjects were withdrawn during the prednisolone-induction phase: 35 from the antibiotic arm and 56 from the placebo arm. In those taking antibiotics, 23 withdrawals were for failure to achieve remission by week 16 and 5 for adverse events, 3 for protocol violations, and 1 for withdrawal of consent; and 3 subjects were lost to follow-up. In the placebo group, 41 withdrawals were for failure to reach remission and 5 for withdrawal of consent, 5 for an adverse event, and 3 for protocol violations; and 2 subjects were lost to follow-up.

One hundred twenty-two subjects entered the maintenance phase of the study comprising 67 on antibiotics and 55 on placebo. A further 48 (25 antibiotics; 23 placebo) subjects were withdrawn by week 52, mostly because of relapse or ongoing disease activity (n = 38), leaving 74 for analysis at the first primary end point (antibiotics 45; placebo 29). Seventy subjects were in remission and continued the study. Eight subjects were withdrawn from each group during the next 52 weeks, 10 because of disease relapse, leaving 54 eligible to continue at week 104, the time point when the trial medications were ceased. At the end of the trial (week 156), a further 22 subjects had been withdrawn, 15 for active disease, which left 32 subjects remaining. Retraction of consent was an indication for withdrawal in 13 subjects in the maintenance phase. This was mostly for active disease. Adverse events (6 subjects), protocol deviations (2 subjects), and loss to follow-up (6 subjects) were less common reasons for withdrawal.

Primary Outcomes

Thirty-nine percent (26/67) of subjects on antibiotics experienced at least 1 relapse between weeks 16 and 52 compared with 56% (31/55) of those on placebo. This difference did not reach the required value of 0.017 to become clinically significant (P = .054; OR, 2.04 [95% CI: 0.84–4.93]). At week 104, the figures were 26% (11/42) for antibiotics and 43% (12/28) for placebo (P = .14; OR, 2.22 [95% CI: 0.62–7.96]). During the following year, after trial medications were ceased, 59% (20/34) of the antibiotic group relapsed compared with 50% (10/20) of the placebo group (P = .54; OR, 0.70 [95% CI: 0.18–2.74]). This left only 14 (13.7%) study subjects on antibiotics and 10 (9.0%) on placebo in remission at the end of the 3-year study period (Figure 2).

Disease site, age, smoking status, use of the oral contraceptive, or a history of surgery for Crohn's disease did not affect response rates. The use of immunomodulator therapy was associated with a significantly greater response in the antibiotic group between weeks 17 and 52 (5 of 21 [24%] of those on azathioprine/6-MP relapsed compared with 21 of 46 [46%] not on this treatment [P = .01]). The same effect was not observed between weeks 53 and 104 (6 of 16 [38%] of those on azathioprine/6-MP vs 5 of 26 [19%] not on this treatment).

Secondary Outcomes

At the end of the 16-week induction period, there was a significantly greater percentage of subjects in re-

Table 1. Clinical Features of the 2 Study Groups

	Antibiotics	Placebo
No. of subjects	102	111
M:F	51:51	50:61
Age, yr	36.5 ± 11.3	34.8 ± 10.0
Smokers	33%	43%
Duration of disease, yr	8.1 ± 7.2	8.7 ± 7.3
Site of disease, n		
Ileum	30	34
Colon	41	36
Ileocolon	31	38
Azathioprine/6-MP, n	35	32
CDAI	291 ± 72.5	282 ± 75.0

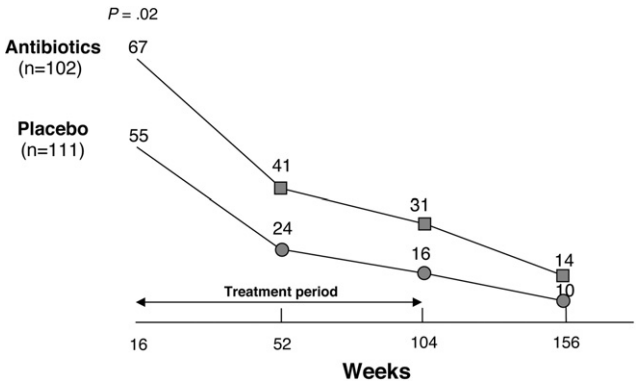

Figure 2. Subjects remaining in remission at each time point.

mission in the antibiotic arm (67/102 [66%]) than in the placebo arm (55/111 [50%]) (P = .02; OR placebo vs antibiotics, 0.51 [95% CI: 0.30–0.90]) (Figure 2). The number needed to treat to achieve 1 additional remission was 6. Neither the mean number of relapses nor the time to relapse differed between the 2 treatment groups at any of the time points (Table 2). Improvements in CDEIS score were slightly greater in the antibiotic group over the first 2 years of the study, but the differences were not statistically significant (Table 3). Of 38 subjects who underwent a final colonoscopy at week 156, only 9 of 23 in the antibiotic group and 4 of 15 on placebo were in endoscopic remission. Few subjects in either group required surgery (6 on antibiotics; 5 on placebo). There was a slightly greater fall in erythrocyte sedimentation rate in those taking antibiotics, but serum albumin and CRP levels were similar in the 2 treatment arms throughout the study. Quality of life was not analyzed because of the negative primary end point results.

Supplementary Analysis

During routine 6-month interval stability testing, at 24 months, it was found that the clofazimine capsules did not rupture in vitro because of hardening of the outer gelatine shell. Because of this, there was a period of approximately 10 months during which it was possible that subjects were not exposed to the correct dose of clofazimine. A supplementary analysis was performed to assess the possible effect this may have had on the study outcomes by excluding 36 subjects who received these clofazimine capsules between weeks 17 and 52 from the week 52 analysis and excluding 20 subjects from the week 16 remission analysis. Performed this way, the rate of relapse at week 52 in the active treatment group was higher at 48% (vs 39% for the entire group). The OR for placebo versus active treatment was 1.41 (P = .45; [95% CI: 0.48–4.17]). Relapse rates at week 104 were not analyzed this way because there were no subjects who were not exposed to the nonruptured clofazimine at some point during that period. The remission rate at week 16 in the antibiotic group was 62%, lower than it was before exclusion of these subjects (66%). The difference between the antibiotic and placebo groups was no longer statistically significant (P = .09; OR, 0.61 [95% CI: 0.30–1.23]).

Adverse Events

Overall, the treatment was well tolerated; only 16 subjects were withdrawn because of an adverse event: 8 in each group, including 5 each in the induction phase. Several adverse events were significantly more common in the antibiotic group than the placebo group during the induction phase: abnormal liver function (2.3% vs 0.3%, respectively), vaginal candidiasis (4.0% vs 0.8%, respectively), abdominal distention (3.4% vs 0.8%, respectively), myalgia (2.3% vs 0.3%, respectively), and urine discoloration (2.8% vs 0.3%, respectively). Between weeks 17 and 52, arthralgia (3.5% vs 1.2%, respectively) and tooth discolouration (2.3% vs 0.2%, respectively) were the only adverse events significantly more common in those on antibiotics than on placebo. The number needed to harm during the induction phase was 77 and, for the whole study, 40.

Blinding

To assess the efficacy of blinding, subjects and investigators were asked to indicate whether they believed the subject had been randomized to antibiotics or placebo. At week 52, 71% of subjects in the antibiotic group and 49% on placebo believed they were receiving active treatment. At the same time, investigators thought that 61% of subjects in the antibiotic group were on active treatment compared with 32% in the placebo group. However, they were unsure about another 54% of subjects on placebo. At week 104, 57% of subjects on antibiotics and 41% on placebo thought they were receiving active treatment. For investigators, the corresponding figures were 48% and 46%, respectively.

Table 2. Ratios Between Placebo and Active Groups of Number of Relapses and Time to First Relapse

	Baseline to week 52	Baseline to week 104	Baseline to week 156
Number of relapses[a]	1.41 (0.86−2.33)	1.47 (0.66−3.27)	0.75 (0.35−1.61)
Time to first relapse[b]	1.41 (0.77−2.58)	1.22 (0.72−2.09)	1.07 (0.64−1.79)

[a]Incidence rate ratio (ratio placebo: active treatment + 95% confidence intervals).
[b]Hazard ratio (ratio placebo: active treatment + 95% confidence intervals).

Table 3. Changes in Values of Endoscopic and Laboratory Parameters

	Baseline to week 52		Baseline to week 104		Baseline to week 156	
	Antibiotics (n = 42)	Placebo (n = 28)	Antibiotics (n = 34)	Placebo (n = 20)	Antibiotics (n = 19)	Placebo (n = 13)
CDEIS	−10.4 ± 15.7	−5.7 ± 13.3	−11.5 ± 16.1	−6.3 ± 12.7	−8.2 ± 16.1	−8.9 ± 22.7
Serum albumin (g/L)	−0.6 ± 4.8	−0.8 ± 4.0	−0.8 ± 4.5	−0.7 ± 3.9	0.6 ± 5.8	−0.5 ± 2.6
CRP (mg/L)	−21.1 ± 57.9	−6.2 ± 29.8	−13.6 ± 35.2	−8.7 ± 30.0	−7.3 ± 14.4	−17.3 ± 36.4
ESR (mm/h)	−12.5 ± 17.7	−3.3 ± 16.0	−12.6 ± 17.8	−5.0 ± 10.5	−13.1 ± 14.2	−2.5 ± 8.7

NOTE. Values expressed as mean change ± SD.
ERS, erythrocyte sedimentation rate.

Compliance

Ninety-five percent of subjects were compliant with all medications between weeks 0 and 16. For weeks 17–52, 82% on antibiotics were compliant as were 87% on placebo. The corresponding figures for weeks 53–104 were 69% and 74%, respectively. At no time point were the differences between the 2 arms statistically significant.

Discussion

This study was designed to show a 40% difference between treatment groups at 24 months, a realistic difference if prolonged combination of clarithromycin, rifabutin, and clofazimine antibiotic therapy was to have a clinically significant impact on Crohn's disease. Although there was a short-term benefit of the antibiotics at 16 weeks additional to the effect of corticosteroid therapy, the study showed no prolonged advantage of the antibiotic combination either during the 2-year treatment phase or, importantly, after therapy was stopped.

The characteristics of MAP and evidence from published studies of antibiotic treatment of Crohn's disease determined the choice, dose, and duration of the antibiotics used in this trial.[8–10] Combination therapy was used to avoid the problem of antibiotic resistance that occurs with mycobacteria. Clarithromycin has both intracellular and extracellular activity.[15] It is effective against *M avium* in patients with acquired immunodeficiency syndrome.[16] Rifabutin is a derivative of rifampicin but appears to have greater activity against MAP.[17] Clofazimine was chosen as the third antibiotic because a small trial suggested a benefit from this agent when combined with ethambutol, although another found no advantage over placebo.[8,9] Other antibiotics that could have been used—azithromycin and ethambutol—were excluded because of their potential for toxicity.

Gui et al described an open label treatment of 46 patients with Crohn's disease using prolonged treatment with clarithromycin and rifabutin (6 to 35 months).[10] Patients were given rifabutin 450 mg/day and either clarithromycin 500 mg/day (43 subjects) or azithromycin (3 subjects). Ten patients also received a quinolone (ciprofloxacin or ofloxacin), and 5 received clofazimine. These authors reported a significant reduction in disease activity within 6 months, which was maintained for up to 24 months of treatment. Overall, 93.5% achieved clinical remission. This response was attributed to the eradication of MAP without any evidence for this being provided. Three small open-label studies have been published since the current trial commenced. All reported a significant benefit using either clarithromycin alone (500 mg/day)[11]; clarithromycin (500 mg/day) plus rifabutin (300 mg/day)[12]; or clarithromycin (750 mg/day), rifabutin (450 mg/day), and clofazimine (2 mg/kg/day).[13] The duration of treatment in these studies varied from 4 weeks to 46 months. Remission rates were 50% or more, but limited information about follow-up after treatment was given. It is clear from these studies that the failure to confirm a long-term benefit in our large, double-blind, randomized controlled trial is not because of inappropriate choice of antibiotics, insufficient dosage, or too short a duration of active treatment because the antibiotics were used at similar or greater dosage and duration. The development of antibiotic resistance is also unlikely to explain the difference between this study and the previous report of a positive response to clarithromycin and rifabutin alone.[10] Consistent with our study, the only other reported controlled trial failed to show a difference between the treatment and placebo groups[18] using similar antibiotics, clarithromycin (1 g/day) and ethambutol (15 mg/kg/day).

Nonrupture of clofazimine capsules observed in vitro during routine stability testing in this study could have influenced the outcome of the study. The bioavailability of clofazimine in subjects receiving these capsules was not tested. However, possible nonrupture in vivo did not affect the conclusion that there was no long-term benefit of the antibiotic combination because a supplementary analysis excluding subjects taking these capsules actually demonstrated a higher relapse rate in the active treatment group.

Blinding was potentially a problem because of the effects of the antibiotics on the color of skin, urine, tears, and teeth. Reassuringly, neither subjects nor investigators were reliably able to predict which treatment subjects were receiving.

The number of subjects remaining in the study fell progressively from 16 weeks. Although this raises the possibility of a type II error, most withdrawals were due to relapse or continuing disease activity, even when repeat courses of prednisolone were used, indicating failure of the trial medications.

The study design randomized subjects with active disease at enrollment, who then remained on their allocated treatment throughout the entire study period unless withdrawn as per the protocol. We decided against the alternative strategy of randomizing patients after they had been brought into remission by a course of prednisolone because we wanted to assess the efficacy of antibiotics in all Crohn's disease patients, not just those responsive to corticosteroids. We also decided against the strategy of randomizing patients brought into remission by a course of both corticosteroids and antibiotics because we wanted to test whether antibiotics have a role in active Crohn's disease rather than base the study on an assumption that they do.

Although no long-term advantage was seen with antibiotics, we show for the first time a significant short-term benefit when they are added to corticosteroids. This was a clinically significant advantage: an additional patient was brought into remission for every 6 patients who received active treatment in addition to a standard 16-week tapering course of prednisolone. The antibiotic response is consistent with a nonspecific antibiotic effect, rather than specific activity against a putative Crohn's pathogen, MAP, given the lack of sustained benefit. A similar effect has previously been demonstrated with antibiotics used without corticosteroids. Metronidazole[19] and ornidazole[20] can reduce postoperative recurrence, and ciprofloxacin was found to be effective in active Crohn's disease, either alone or in combination with metronidazole.[21–23] However, unlike the present study, adding these 2 agents to oral corticosteroids was not shown to add any advantage.[24]

It was not the aim of this study to prove definitively or disprove that MAP causes Crohn's disease. Rather, its purpose was to determine whether, as has been suggested by others,[10,12,13] antibiotics with efficacy against this organism have a long-term effect on a large unselected population of patients with Crohn's disease. Testing for MAP was not performed because the methods available at the time the trial was commenced were not considered sufficiently reliable to detect its presence in humans. However, the absence of specific knowledge of each subject's MAP status does not alter the main conclusion from this adequately powered, double-blind, randomized, controlled trial. If ongoing MAP infection plays a significant role in Crohn's disease, then either the live organism was not present in these patients as frequently as suggested by some or, if it is, then treatment with a prolonged course of appropriate antibiotics does not influence the course of the disease. It could be argued that if MAP causes Crohn's disease in a small minority of patients, then the sample size needed in a trial such as this would need to be substantially larger to show a statistically significant difference. However, this would be unlikely to be significant in a clinical sense, given the large number of patients that would need to be treated for one to receive any benefit. This argument would also be inconsistent with the benefits reported in smaller, uncontrolled studies.[10,12,13]

In summary, this trial has shown that, in a large group of patients with Crohn's disease, there was no evidence that the 2-year combination of clarithromycin, rifabutin, and clofazimine had a prolonged benefit, even in those selected for an initial response to antibiotics. Therefore, the study does not support a significant ongoing pathogenic role for MAP in the majority of patients with Crohn's disease. However, short-term improvement was seen when this combination was added to corticosteroids, most likely because of nonspecific antibacterial effects.

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