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Effects of albendazole on the clinical outcome and immunological responses in helminth co-infected tuberculosis patients: a double blind randomised clinical trial

E. Abate [a,b,*], D. Elias [c], A. Getachew [d], S. Alemu [e], E. Diro [e], S. Britton [f], A. Aseffa [g], O. Stendahl [b], T. Schön [b,h]

[a] Department of Immunology and Molecular Biology, University of Gondar, Gondar, Ethiopia
[b] Department of Medical Microbiology, Linköping University, Sweden
[c] University of Southern Denmark, Institute of Molecular Medicine, Department of Cancer and Inflammation, Odense, Denmark
[d] Department of Radiology, University of Gondar, Gondar, Ethiopia
[e] Department of Internal Medicine, University of Gondar, Gondar, Ethiopia
[f] Department of Infectious Diseases, Karolinska Hospital, Stockholm, Sweden
[g] Armauer Hansen Research Institute, Addis Ababa, Ethiopia
[h] Department of Clinical Microbiology and Infectious Diseases, Kalmar County Hospital, Kalmar, Sweden

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ABSTRACT

Despite several review papers and experimental studies concerning the impact of chronic helminth infection on tuberculosis in recent years, there is a scarcity of data from clinical field studies in highly endemic areas for these diseases. We believe this is the first randomised clinical trial investigating the impact of albendazole treatment on the clinical and immunological outcomes of helminth co-infected tuberculosis patients. A randomised, double-blind, placebo-controlled trial of albendazole (400 mg per day for 3 days) in helminth-positive tuberculosis patients was conducted in Gondar, Ethiopia. The primary outcome was clinical improvement (ΔTB score) after 2 months. Among secondary outcomes were changes in the levels of eosinophils, CD4+ T cells, regulatory T cells, IFN-γ, IL-5 and IL-10 after 3 months. A total of 140 helminth co-infected tuberculosis patients were included with an HIV co-infection rate of 22.8%. There was no significant effect on the primary outcome (ΔTB score: 5.6 ± 2.9 for albendazole versus 5.9 ± 2.5 for placebo, $P = 0.59$). The albendazole-treated group showed a decline in eosinophil cells ($P = 0.001$) and IL-10 ($P = 0.017$) after 3 months. In an exploratory analysis after 12 weeks, the albendazole treated group showed a trend towards weight gain compared with the placebo group (11.2 ± 8.5 kg versus 8.2 ± 8.7 kg, $P = 0.08$)). The reductions in eosinophil counts and IL-10 show that asymptomatic helminth infection significantly affects host immunity during tuberculosis and can be effectively reversed by albendazole treatment. The clinical effects of helminth infection on chronic infectious diseases such as tuberculosis merit further characterisation.

1. Introduction

Similar to tuberculosis (TB), it has been estimated that one-third of the global human population is infected with intestinal parasites (World Health Organization (WHO), 2012; Salgame et al., 2013). Several studies, including some from Ethiopia, have shown an increased rate of helminth infection in TB patients compared with household contacts living in the same room with active TB patients and the healthy population (Tristão-Sá et al., 2002; Abate et al., 2012). The impact of helminth infection on the risk of developing TB and during the course of active TB is not well understood but is believed to involve helminth-induced effects on cell mediated immunity (Bentwich et al., 1999; Borkow et al., 2001; Elias et al., 2006).

Protective immunity in TB has been shown to be dependent on T-helper 1 (Th1) CD4+ T cells producing IFN-γ and TNF-α, as well as cytolytic T cells (CTLs) producing granule-associated cytolytic effector molecules (Brighenti and Andersson, 2012). It has been postulated that helminth co-infection could modulate the protective Th1-response against TB through an effect on the Th1/Th2 balance, with increased Th2 dominance and activity of regulatory

* Corresponding author at: Department of Immunology and Molecular Biology, University of Gondar, P.O. Box 1353, Gondar, Ethiopia. Tel.: +251 911 464024; fax: +251 058 114 1240/058 111 1479.
E-mail address: ebbaabate@yahoo.com (E. Abate).

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T cells (Tregs) (Bentwich et al., 1999; Borkow et al., 2001). Such Th2 and Treg responses induce the production of cytokines including IL-4 and IL-10 which are potent inhibitors of the Th1 response (Rook, 2007; Redford et al., 2011). Indeed, in both humans and mice there is a marked increase in Th2-related cytokines such as IL-4, IL-5 and IL-13 following helminth infection (Anthony et al., 2007). In human volunteers, hookworm infection is associated with strong local and systemic Th2 and Treg responses (Gaze et al., 2012). The vaccine currently used against TB, Bacillus Calmette Guerin (BCG), is not effective against adult pulmonary TB in sub-Saharan Africa (Tameris et al., 2013). One reason could be that helminth infection modulates the immune response by skewing the Th1/Th2 balance and thereby attenuating the effects of the BCG vaccine (Elias et al., 2008). Several experimental studies confirm the immunomodulatory effect of helminths during TB (Bentwich et al., 1999; Borkow et al., 2001; Elias et al., 2005; Potian et al., 2011; Salgame et al., 2013). One of these studies clearly showed that *Nippostrongylus brasiliensis* infection was associated with impaired killing of *Mycobacterium tuberculosis* (Mtb), an effect primarily mediated by a switch to alternatively activated macrophages (AAMs) by IL-4 (Potian et al., 2011).

Among the very limited human studies on the interaction between helminths and active TB, one study showed lower IFN-γ and enhanced IL-10 responses (Resende Co et al., 2007). In our recent study from Ethiopia we found that asymptomatic helminth infection in TB patients showed effects on host immunity in terms of increased IgE and eosinophil levels (Abate et al., 2012). However, clinical studies investigating the impact of deworming during active TB in humans remains very limited. Thus, our aim was to investigate the clinical and immunological impacts of helminth infection in a randomised clinical trial.

2. Materials and methods

2.1. Study participants

Following written informed consent, newly diagnosed patients with pulmonary TB presenting consecutively from 1 March 2009 to 15 October 2012 at the Directly Observed Treatment Short-Course (DOTS) Clinics of the Teaching and Referral Hospital of the University of Gondar, the Gondar Health Centre and at Debark Hospital, Ethiopia were eligible for enrolment. After enrollment, helminth co-infected TB patients were randomly allocated, after 2 weeks of anti-TB treatment, to albendazole treatment (400 mg/day for three consecutive days) or identical placebo tablets. All helminth-positive TB patients, including the placebo group, received anti-helminth treatment at week 12 when the follow-up was completed. The TB treatment consisted of isoniazid, rifampicin, ethambutol and pyrazinamide for the first 2 months followed by 4 months of isoniazid and rifampicin. The inclusion criteria were that patients were 15–60 years old, with a positive smear result for acid-fast bacilli (AFB; smear-positive TB) or with clinical and chest X-ray (CXR) results suggestive of pulmonary TB (smear-negative TB) according to the WHO-based national guidelines (WHO, 2012). The exclusion criteria were that patients required hospital admission, were pregnant, infected with *Schistosoma* spp., displayed symptoms of active helminth infection such as diarrhoea or stomach cramps, or clinical signs or medical treatment indicating any concomitant chronic or infectious disease other than TB/HIV.

The study received ethical clearance from the Ethics Review Board of the University of Gondar, Ethiopia and from the Medical Ethics Board at Linköping University, Sweden. In addition, approval was obtained from the federal Drug Administrative and Control Authority (DACA), Ethiopia. The data collection and patient follow-up were monitored by an independent data and safety monitoring board (DSMB).

2.2. Study outcome

The primary outcome was a TB score change (ΔTB score) at week 8 compared with baseline. The secondary outcomes were sputum smear conversion after 2 months, changes in the CXR pattern from baseline to week 12, CD4+ T cell counts, IgE and eosinophil responses, as well as changes in the frequency of Tregs and IFN-γ, IL-5 and IL-10 producing peripheral blood mononuclear cells (PBMCs) after 3 months.

2.3. Randomization

Random numbers were generated in a block size of eight by the Addis Continental Institute of Public Health, Ethiopia. The treatment allocated for each patient was concealed in an individual envelope. Albendazole and placebo tablets were produced by the Addis pharmaceutical company, Ethiopia which is accredited for Good Clinical Practice (GCP). All tablets looked identical and were assigned a treatment code by the manufacturer. Both the investigators and clinic staff were blinded to the treatment given. The treatment code was kept in a sealed envelope at the manufacturer and opened after the last patient had been to a follow-up visit and the data had been analysed.

2.4. Clinical and laboratory analyses

2.4.1. Socio-demographic characteristics and assessment of the TB score

A structured questionnaire was used to collect socio-demographic and clinical information. As previously described (Wejse et al., 2008; Janols et al., 2012), a clinical score (TB score) which ranges from 0 to 13 points was assessed at baseline and 2 months after anti-TB treatment. The score is composed of signs and symptoms of TB each contributing one point (cough, haemoptysis, chest pain, dyspnoea, night sweating, anaemic conjunctivae, lung auscultation finding, tachycardia ($\geqslant$100/min), temperature ($\geqslant$37 °C), body mass index (BMI) $\leqslant$18 kg/m^2, BMI $\leqslant$16 kg/m^2, mid-upper arm circumference (MUAC) $\leqslant$220 mm, and MUAC $\leqslant$200 mm). As previously described (Wejse et al., 2008), the TB score was divided into three severity classes (SC-I-III) at baseline where SC-I was 0–5 points, SC II 6–7 points and SC-III 8–13 points.

2.4.2. CXR evaluation

Grading of CXR findings of pulmonary TB was done according to the National Tuberculosis Association of the USA as normal, minimal, moderately advanced and far advanced TB (American Thoracic Society, 1961). For statistical evaluation, this grading was translated to a semi-quantitative scale, 0 (normal), 1 (mild), 2 (moderate) and 3 (far advanced TB). During follow-up at 3 months, the initial CXR was used for comparison and the radiological response was classified according to a semi-quantitative scale, (1: normalised, 2: regression, 3: no change, 4: progress). The CXRs were read by the same senior radiologist and reading was blinded for HIV status and treatment assignment.

2.4.3. HIV screening and determination of CD4+ T cell count

Testing for HIV was done at the voluntary counseling and testing clinics, and at the DOTS clinic as part of the clinical routine with HIV rapid test kits (Shanghai Kehua Bio-engineering Co., Ltd. (KHB HIV 1/2 rapid test strip) China), Stat-Pak (HIV 1/2, Chembio Diagnostics Inc., USA) and Unigold (Trinity Biotech, USA). HIV-positive patients were referred to the HIV clinics for further assessment and free antiretroviral treatment (ART) according to the Ethiopian HIV/AIDS