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Research Paper

Adjunctive Phosphodiesterase-4 Inhibitor Therapy Improves Antibiotic Response to Pulmonary Tuberculosis in a Rabbit Model

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ABSTRACT

Objectives: Adjunctive host-directed therapy is emerging as a new potential approach to improve the outcome of conventional antimicrobial treatment for tuberculosis (TB). We tested the ability of a phosphodiesterase-4 inhibitor (PDE4i) CC-11050, co-administered with the first-line anti-TB drug isoniazid (INH), to accelerate bacillary killing and reduce chronic inflammation in the lungs of rabbits with experimental *Mycobacterium tuberculosis* (Mtb) infection.

Methods: A rabbit model of pulmonary TB that recapitulates the pathologic manifestations seen in humans was used. Rabbits were infected with virulent Mtb by aerosol exposure and treated for eight weeks with INH with or without CC-11050, starting at four weeks post infection. The effect of CC-11050 treatment on disease severity, pathology, bacillary load, T cell proliferation and global lung transcriptome profiles were analyzed.

Results: Significant improvement in bacillary clearance and reduced lung pathology and fibrosis were noted in the rabbits treated for eight weeks with INH + CC-11050, compared to those treated with INH or CC-11050 only. In addition, expression of host genes associated with tissue remodeling, tumor necrosis factor alpha (TNF-α) regulation, macrophage activation and lung inflammation networks was dampened in CC-11050-treated, compared to the untreated rabbits.

Conclusions: Adjunctive CC-11050 therapy significantly improves the response of rabbits with experimental pulmonary TB to INH treatment. We propose that CC-11050 may be a promising candidate for host directed therapy of patients with pulmonary TB, reducing the duration and improving clinical outcome of antibiotic treatment.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb) is a common infectious disease among humans, accounting for 9 million new cases and 1.5 million deaths in 2013 (http://www.who.int/tb/publications/factsheet_global.pdf?ua=1). The current anti-TB drug regimen used for directly observed therapy, short course (DOTS) is ineffective at completely eliminating the infecting Mtb and disease associated lung pathology (Mitchison, 2000; Sirgel et al., 2000). Since most of the antibiotics currently in use for TB treatment under DOTS primarily target actively growing Mtb, non- or slow replicating and dormant bacilli, present in the granulomas of active TB cases, are not efficiently killed (Mitchison, 2000; Zhang et al., 2012). Such dormant bacillary population can resume growth and cause symptomatic, active disease in the infected host in response to conditions that suppress immunity (Cardona, 2010; Ehlers, 2009). Moreover, after successful completion of DOTS therapy, lung function and quality of life of TB patients can be significantly compromised by the fibrotic scars and irreversible tissue damage, rendering them more susceptible to recurrent TB disease and other pulmonary infections (den Boon et al., 2008; Verver et al., 2005). Therefore, alternate approaches for better bacterial elimination and treatment outcome are urgently needed to control the TB epidemic and improve the quality of life of TB patients after treatment (Zumla et al., 2014).

Progression of initial pulmonary Mtb infection to chronic active TB versus establishment of latent Mtb infection (LTBI) involves a shift in the regulation of inflammatory responses of host immune cells (Dorhoi & Kaufmann, 2014; Behar et al., 2014). During progressive pulmonary TB, the onset of early and exacerbated inflammation, associated with tissue necrosis and cavity formation impairs host resistance

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and, promotes dissemination of Mtb within and outside the lung (Kaufmann & Dorhoi, 2013; Sugawara, 2009). Disease pathology during Mtb infection is driven by reactive oxygen and nitrogen species as well as cytokines, chemokines and other inflammatory mediators such as TNF-α, interferon gamma-induced protein 10 (IP-10) and C-reactive protein (CRP) produced by activated macrophages, neutrophils and other immune cells (Kaufmann & Dorhoi, 2013; Schlesinger, 1996). The mycobacterial population thriving in an elevated host inflammatory environment adapts to a dormant phenotype with slow or no replication that is refractory to antibiotic killing (Chao & Rubin, 2010). Thus, adjunctive host targeted immune therapies that improve disease pathology by modulating the inflammatory response to Mtb infection and that shorten the duration of standard antibiotic treatment by increasing macrophage anti-microbial activity, is an important emerging concept in treatment of TB (Wallis & Hafner, 2015; Hawn et al., 2015). Phosphodiesterases (PDE) are enzymes that hydrolyze cyclic nucleotides, such as cyclic adenosine and guanosine monophosphates (cAMP and cGMP) (Francis et al., 2011; Conti et al., 2003). In higher mammals at least 11 different PDEs (PDE1–PDE11) have been reported; many are implicated in modulating host cell functions, including cytokine and chemokine produced in response to various diseases (Keravis & Lugnier, 2012; Bender & Beavo, 2006). PDE4 is a cAMP-specific hydrolase present predominantly in host leukocytes, including macrophages, neutrophils and lymphocytes (Spina, 2003; Houslay et al., 1998). Since accumulation of cAMP, through blocking its hydrolysis, negatively regulates inflammation, several PDE4is have been tested and found to be useful for treating human inflammatory diseases including asthma and psoriasis (Spina, 2003; Azam & Tripuraneni, 2014; Maurice et al., 2014). Over the past several years, we have investigated the therapeutic effects of the immune modulatory drugs (IMiDs®), PDE4i, and other small molecules as adjunctive therapies with anti-TB antibiotics in different animal models (Moreira et al., 1997; Tsenova et al., 2002; Koo et al., 2011; Subbian et al., 2011a; Subbian et al., 2011b). Our central hypothesis is that reducing but not fully blocking TNF-α production by host cells would alleviate inflammatory responses and improve the outcome of antibiotic treatment during Mtb infection. Recently, we showed that treatment with the PDE4i CC-3052, together with INH, significantly reduced Mtb growth and disease pathology in murine and rabbit models of pulmonary TB (Koo et al., 2011; Subbian et al., 2011a; Subbian et al., 2011b). In the present study, we report the "proof-of-concept" for the activity of another PDE4i (CC-11050) with improved therapeutic properties. Moreover, CC-11050 has an investigational new drug (IND) application and has been in clinical trials for other indications. We chose INH, since this drug predominantly kills actively replicating bacteria and ineffective in killing against dormant bacilli. In addition, INH monotherapy has been used as a prophylactic treatment against reactivation of latent Mtb infection in humans. Our results show that CC-11050 dampens infection-induced inflammation and improves bacterial clearance as well as clinical outcome in INH treated rabbits with pulmonary TB. The present work confirmed that PDE4 inhibition with CC-11050 gives similar effects as CC-3052 and other PDE4is. Results from this study will serve as a proof of concept to guide a human clinical trial that will be conducted soon (JBZ personal communication).

2. Materials and Methods

2.1. Bacterial Culture and Chemicals

M. tuberculosis HN878 (a strain of W-Beijing lineage) was grown to mid-log phase (OD_{600} = 0.5–0.7) in Middlebrook 7H9 media containing 0.5% glycerol, 0.25% Tween 80 and 10% oleic acid dextrose catalase (OADC) supplement (BD Biosciences, MD) and aliquots were stored at −80 °C. The bacterial inoculum for rabbit aerosol infection was prepared as described previously (Subbian et al., 2011c). Ketamine, acepromazine and euthasol used to sedate and euthanize rabbits were purchased from Henry Schein Animal Health, OH. CC-11050 was supplied by Celgene Corporation, NJ. All other chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich, MO), unless mentioned otherwise.

2.2. Animal Welfare and Ethical Statement

Specific pathogen free, female New Zealand white rabbits (Millbrook Farms, MA) of 2.3 to 2.6 kg body weight were used in this study. Each rabbit was housed individually without any restriction on food and water consumption (ad libitum) and was handled humanely according to the United States Department of Agriculture (USDA) policies. All the procedures with Mtb-infected rabbits, including infection, gavage, post-infection and treatment monitoring were performed in bio safety level-3 facilities according to the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Rutgers University and were in compliance with institutional, national and international guidelines governing the use of experimental animals.

2.3. Pharmacokinetics of CC-11050

2.3.1. Structure of CC-11050

CC-11050 (N-[2-[(1S)-1-(3-ethoxy-4-methoxyphenyl)-2-(methylsulfonyl) ethyl]-2,3-dihydro-3-oxo-1H-isoindol-4-yl]-(9Cl) cyclopropanecarboxamide) is a PDE4i with anti-inflammatory activities. CC-11050 has an empirical formula of $C_{24}H_{28}N_2O_6S$ with one chiral center in its structure (Supplementary Fig. 1A).

2.3.2. Sample Collection

Uninfected rabbits were divided into two experimental groups (n = 3 per group) and treated by oral gavage with either 25 mg per kg or 50 mg per kg of CC-11050. Venous blood was collected in vacutainer tubes (BD & Co, NJ) at time 0 (pre-treatment), 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h after a single gavage administration of CC-11050. In another experiment, groups of rabbits (n = 3 per group) were treated by oral gavage with CC-11050 at 25 or 50 mg per kg per day for four days and blood was collected as before. Plasma was collected after centrifuging the blood samples at 1100 ×*g* for 10 min. To determine whether INH and CC-11050 interact with each other in the Mtb-infected rabbits, blood samples were collected after 2 weeks of treatment and plasma was collected as mentioned above for liquid chromatography–mass spectrometry (LC–MS) analysis.

2.3.3. Bioanalytical Method for CC-11050

The standard curve (1 to 4000 ng per ml) and quality control samples (3 to 3200 ng per ml) were prepared by diluting 1 mg per ml stock CC-11050 solution in rabbit plasma diluted 1:1 with Sorenson's citrate buffer (25 mM; pH 1.5). The internal working standard solution (25 ng per ml) was prepared by diluting 1 mg per ml internal standard stock solution of $^{13}CD_3$–CC-11050. Briefly, 100 μl of the working internal standard solution was added to 25 μl of each standard, quality control or test samples, and mixed well. After centrifugation at 4000 rpm for 10 min, 75 μl of the supernatant was transferred to a clean 96-well plate for analysis. Samples were analyzed by LC–MS using a Sciex API 4000 Triple Quadrupole Mass Spectrometer (Sciex, Division of MDS Inc., Canada) coupled to a Shimadzu HPLC System (Shimadzu Scientific Instruments, MD) with a Phenomenex Gemini column (5 μm, 2.0 × 50 mm) (Phenomenex, CA). Samples were analyzed using the following chromatographic conditions: mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient conditions were: 0 min for 10% B, 0.0–2.0 min from 10% B to 75% B, 2.1–3.0 min for 95% B, and 3.05–4.0 min for 10% B. A positive ion mode with turbo spray, an ion source temperature of 450 °C, and a dwell time of 100 ms were utilized for mass spectrometric detection. Quantification of analytes was performed using multiple reactions monitoring (MRM) at the following transitions: *m/z* 473.1 to *m/z* 178.1 and *m/z* 477.1 to *m/z* 182.1 for CC-11050