A Novel Macrolide Solithromycin Exerts Superior Anti-inflammatory Effect via NF-κB Inhibition

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ABSTRACT

Macrolides are reported to reduce exacerbation of chronic inflammatory respiratory disease, such as chronic obstructive pulmonary disease (COPD), and also show anti-inflammatory effects in vitro and in vivo. However, the anti-inflammatory efficacies of current macrolides are relatively weak. Here we found that a novel macrolide/fluoroketolide solithromycin (CEM-101) showed superior anti-inflammatory effects to macrolides in current clinical use. The effects of solithromycin (SOL) on lipopolysaccharide-induced TNFα (tumor necrosis factor α) and/or CXCL8 (C-X-C motif chemokine ligand 8; interleukin-8) release, phorbol 12-myristate 13-acetate-induced MMP9 (matrix metalloproteinase 9) activity and NF-κB (nuclear factor-κB) activity under conditions of oxidative stress have been evaluated and compared with the effects of erythromycin, clarithromycin, azithromycin, and telithromycin in macrophage-like PMA-differentiated U937 cells and peripheral blood mononuclear cells (PBMC) obtained from COPD patients. We also examined effect of SOL on cigarette smoke-induced airway inflammation in mice. SOL exerted superior inhibitory effects on TNFα/CXCL8 production and MMP9 activity in monocyteic U937 cells. In addition, SOL suppressed TNFα release and MMP9 activity in PBMC from COPD patients at 10 μM, which is 10 times more potent than the other macrolides tested. Activated NF-κB by oxidative stress was completely reversed by SOL. SOL also inhibited cigarette smoke-induced neutrophilia and pro-MMP9 production in vivo, although erythromycin did not inhibit them. Thus, SOL showed better anti-inflammatory profiles compared with macrolides currently used in the clinic and may be a promising anti-inflammatory and antimicrobial macrolide for the treatment of COPD in future.

Introduction

It has long been recognized that macrolides exert not only antibacterial activity but also anti-inflammatory and immunoregulatory effects (Kanoh and Rubin, 2010). Erythromycin was found to induce a dramatic and unexpected improvement in diffuse panbronchiolitis (DPB) (Kudoh et al., 1998), leading to an investigation of its anti-inflammatory actions. Several studies indicate that macrolides inhibit pro-inflammatory cytokine production, such as CXCL8 (interleukin-8) and TNFα (tumor necrosis factor α), as the mechanism of the effects of macrolides on DPB (Sakito et al., 1996). Macrolides also inhibit release of leukotriene B4, a neutrophil chemotactic mediator (Oda et al., 1995), and soluble adhesion molecules (Mukae et al., 1997), resulting in reduction of neutrophil chemotaxis and infiltration into Airways. It has also been reported that macrolides increase antioxidant activity in alveolar macrophages (Morikawa et al., 2000), enhance apoptosis of neutrophils (Inamura et al., 2000), decrease superoxide anion release by neutrophils (Tamaoki et al., 2004), and reduce mucus secretion in the airways (Kaneko et al., 2003).

Macrolides were found to be effective for DPB at doses below the concentrations required for their antibacterial activity by inhibiting not only biofilm-formation and virulence factors produced from Pseudomonas aeruginosa but also quorum-sensing systems in which bacteria coordinate specific virulence genes (Schultz, 2004; Tateda et al., 2004). Thus, macrolides suppress airway inflammation observed in DPB, resulting in an improvement of lung function and prolonged survival (Oda et al., 1995; Kudoh et al., 1998; Kadota et al., 2003).

In addition to DPB, it has been reported that macrolides provide beneficial effects, although to a lesser degree, in other inflammatory airway diseases, such as bronchiectasis, cystic...
fibrosis, sarcoidosis, asthma, and chronic obstructive pulmonary disease (COPD) (Trisolini et al., 2008; Kanoh and Rubin, 2010; Albert et al., 2011). Although DBP is geographically limited to East Asia, COPD is a serious worldwide health problem without any safe and effective anti-inflammatory therapy (Ito and Barnes, 2009). There are few reports investigating the clinical benefit of macrolides for COPD, with some studies showing reduced exacerbations after long-term therapy (Simpson et al., 2008; He et al., 2010; Albert et al., 2011). However, the reduction in exacerbations could be due to the antibiotic action of macrolides rather than any anti-inflammatory effects, and there is no evidence for any significant effects on inflammatory biomarkers in these studies.

The anti-inflammatory effects of macrolides have been previously investigated in vitro and animal model. For example, azithromycin inhibits proinflammatory cytokines from M1 macrophages in cystic fibrosis (Meyer et al., 2009). Clarithromycin and erythromycin inhibit airway inflammation in a cigarette smoke model (Nakanishi et al., 2009; Mikura et al., 2011). One of the molecular mechanisms of anti-inflammatory effects of macrolides is inhibition of pro-inflammatory transcription factors, such as nuclear factor-kB (NF-kB) (Li et al., 2012) and protein-1 (AP-1) (Bosnar et al., 2011).

Solithromycin (CEM-101) was developed as a novel oral and intravenous fluoroalkelide antibiotic agent related to 14-member-ring macrolides and is currently in clinical development for severe community-acquired bacterial pneumonia. Solithromycin exhibits a greater potency and a wider spectrum of in vitro activity against respiratory tract pathogens associated with community-acquired bacterial pneumonia, including macrolide-resistant strains, compared with other available macrolides (Farrell et al., 2010; McGhee et al., 2010). However, the anti-inflammatory efficacies of solithromycin have not yet been explored.

Here we evaluated the anti-inflammatory effects of solithromycin on release of proinflammatory mediators, such as TNFα, CXCL8, and MMP9 (matrix metalloproteinase 9), and activation of NF-kB, and compared with other macrolides currently used clinically, such as erythromycin, clarithromycin, azithromycin, and telithromycin.

Materials and Methods

Reagents. Solithromycin (3aS,4R,7S,9R,10R,11R,13S,15R,15aR,1)-[4R-[4-[3-aminophenyl]-1H-1,2-triazole-1-yl]butyl]-4-ethyl-7-fluorooctahydro-11-methoxy-3a,7a,9,11,13,15-methoxy-10-(3R,4-trideoxy-3-(dimethylamino)-3β-D-xylo-hexopyranosyl)oxy]-2H-oxacyclotetradecine (4,3-day oxazole-2,6,8,1,4'H,7'H,9'H-tetraone) and telithromycin ([2S,5R,7R,8S,11R,13R,14R]-8-[[2S,3R,4S,6R]-4-dimethylamino-3-hydroxy-6-methyl-oxan-2-yloxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-15-oxo-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]-1-oxa-6-azacyclopentadec-13-yl-2,6-dideoxy-3-C-methyl-3-O-methyl-L-rhamnopyranoside], hydrogen peroxide, perboral 12-myrystate 13-acetate (PMA), and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (Poole, UK).

Cells. The human monocytic cell line U937 was obtained from the American Type Culture Collection (American Type Culture Collection, Rockville, MD). PBMCs (peripheral blood mononuclear cells) from COPD patients were obtained from Brompton Hospital and separated with AccuSPIN (Sigma-Aldrich). The characteristics of subjects are shown in Supplemental Table 1. Cells were cultured in complete growth medium (RPMI 1640; Sigma-Aldrich) supplemented with 10% fetal bovine serum and 1% l-glutamine at 37°C in a humidified atmosphere with 5% CO2. U937 cells were differentiated into adherent macrophage-like morphology by exposure to PMA (50 ng/ml) for 48 hours in complete growth medium. Cell viability was assessed microscopically by trypan blue staining, or determined by 3-(4, 5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide (MTT) assay as needed. This study was approved by the ethics committee of the Royal Brompton and Harefield Hospitals, and all subjects gave written informed consent.

Cell Lysis. Whole cell extracts were prepared as previously described (Kobayashi et al., 2011). Briefly, cell protein extracts were prepared using modified RIPA buffer (50 mM Tris-HCl pH 7.4, 0.5% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl) with freshly added complete protease inhibitor cocktail (Roche, Mannheim, Germany). Protein concentration was determined using the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA).

Cytokine Enzyme-Linked Immunosorbent Assay (ELISA). TNFα and CXCL8 concentrations in the supernatant of cell cultures were determined by sandwich ELISA according to the manufacturer's instructions (R&D Systems Europe, Abingdon, UK).

Zymography. MMP9 enzyme activity was measured by gelatin zymography. Cell culture supernatants were diluted with equal amount of Laemmli sample buffer (Bio-Rad, Hertfordshire, UK) and loaded on a Novex 10% zymogram (gelatin) gel (Invitrogen Ltd., Paisley, UK). After electrophoresis, gels were incubated and rinsed with Novex zymogram renaturing buffer (Invitrogen) for 30 minutes at room temperature. The gels were then rinsed in Novex zymogram developing buffer (Invitrogen) for 30 minutes at room temperature prior to overnight incubation in the developing buffer at 37°C. After incubation, the gels were stained using a Colloidal Blue Staining Kit (Invitrogen) to visualize the zymogen band.

NF-kB Activity. The activation of NF-kB (p65 binding activity to NF-kB binding sequence) was determined using a TransAM NF-kB p65 Assay kit (Active Motif, Inc., Carlsbad, CA) according to the manufacturer's instructions. As shown above, whole cell extracts were prepared from PMA-differentiated U937 cells, and 20 µl of each extract was used for this study. Results were determined by measuring the spectrophotometric absorbance at 450 nm with a reference wavelength of 655 nm.

Cigarette Smoke Exposure of Mice. C57BL/6J mice (male, 4 weeks) were purchased from CLEA Japan, Inc. (Tokyo, Japan) and adapted for 1 week. Mice were exposed to cigarette smoke (4% cigarette smoke diluted with compressed air) for 30 minutes/day for 12 days or 4 days with the commercially marketed filtered Peace cigarettes (28 mg of tar and 2.3 mg of nicotine per cigarette; Japan Tobacco Inc., Tokyo, Japan) using a Tobacco Smoke Inhalation Experiment System for small animals (model INH06-CIG020A; MIPS, Osaka, Japan) as described previously (Nakamaru et al., 2009).

In cohort 1, solithromycin (100 mg/kg, orally) was suspended into 0.5% carboxymethylcellulose and administered orally for the last 8 days of 12 days of tobacco smoke exposure (1 hour before each tobacco smoke exposure). In cohort 2, solithromycin (100 mg/kg, orally) or erythromycin (100 mg/kg, orally) was suspended into 0.5% carboxymethylcellulose and administered orally at 1 hour before each tobacco smoke exposure (1 hour before each tobacco smoke exposure).
for 4 times. Bronchoalveolar lavage was performed at 24 hours after the last cigarette smoke exposure as previously described (Nakamaru et al., 2009), and the number of alveolar macrophages and neutrophils was determined from Diff-Quick stained specimens. The pro-MMP-9 protein concentration in Bronchoalveolar lavage (BAL) fluid supernatant was assessed using a commercially available mouse pro-MMP-9 enzyme-linked immunosorbent assay kit (R&D Systems Europe) according to the manufacturer’s instructions. This animal study was approved by The Experimental Animal Ethics Committee in Kyorin University.

Statistical Analysis. The results were expressed as the mean ± S.E.M. Comparisons of data in two groups were performed using the Student’s t test or the Wilcoxon signed rank test. Multiple comparisons were made by one-way analysis of variance with post hoc test (Dunnett’s) as appropriate. The difference was considered significant at P < 0.05. IC50 values (50% inhibitory concentration) of macrolides on production of cytokines or MMP9 were calculated using Prism 4.0 (GraphPad Software Inc., San Diego, CA).

Results

Anti-inflammatory Effects of Macrolides in U937 Cells. LPS significantly increased TNFα and CXCL8 production in PMA-differentiated U937 cells (TNFα, 63.1 ± 2.6 fold in LPS versus nonstimulated and CXCL8, 2.0 ± 0.1 fold in LPS versus nonstimulated cells, n = 3). Solithromycin significantly inhibited both TNFα and CXCL8 at 100 μM (Figs. 1A and 2A). Although clarithromycin showed modest effects on both TNFα and CXCL8 production at a higher concentration (333 μM) (Figs. 1C and 2C), erythromycin and azithromycin had no inhibitory effect (Figs. 1B, D, and 2B, D). Telithromycin (100 μM) was similarly without effect on TNFα and CXCL8 (Figs. 1E and 2E). The IC50 values for solithromycin on TNFα and CXCL8 release were 41.6 ± 1.9 μM and 78.2 ± 9.5 μM, respectively, and were superior to those of clarithromycin (IC50, 426.3 ± 63.9 μM for TNFα and 506.5 ± 44.0 μM for CXCL8) (Table 1). None of compounds affected cell viability evaluated by 3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay at maximal concentrations used in this test.

We also investigated the effects of macrolides on MMP9 activity, which was clearly elevated by PMA stimulation in U937 cells (9.9 ± 2.0 fold in PMA versus non-stimulation, n = 3). Solithromycin markedly reduced MMP9 activity, with an IC50 of 14.9 ± 3.1 μM (Fig. 3A; Table 1). In contrast, clarithromycin and azithromycin showed 10-fold lower inhibitory effects than solithromycin, whereas erythromycin showed no effect (Fig. 3, B–D; Table 1). Telithromycin also inhibited MMP9 activity, although to lesser extent than solithromycin, with an IC50 of 83.4 μM (Fig. 3E).

Anti-inflammatory Effects of Macrolides in PBMC from COPD Patients. Anti-inflammatory effects of solithromycin were evaluated in PBMC from COPD patients. Solithromycin (10 μM) slightly but significantly inhibited LPS-induced TNFα release from PBMC of COPD patients, whereas erythromycin, clarithromycin, and azithromycin failed to suppress TNFα release, even at a higher concentration (100 μM) (Fig. 4, A–D). Furthermore, solithromycin clearly reduced MMP9 activity in PBMCs from COPD patients at 10 μM. Although clarithromycin slightly but significantly inhibited MMP9 activity at a higher concentration (100 μM), erythromycin and azithromycin showed no effect (Fig. 4, E–H). Telithromycin showed cytotoxicity at less than 100 μM, and could not get meaningful data.

Solithromycin Inhibited NF-κB Activity under Conditions of Oxidative Stress. As the secretion of TNFα, CXCL8, and MMP9 are reported to be NF-κB dependent, we investigated the effects of solithromycin on NF-κB activity as defined by p65 binding activity to NF-κB DNA binding

Fig. 1. Effects of macrolides on LPS-induced TNFα release in PMA-differentiated U937 cells. Cells were pretreated with solithromycin (A) (10 to 100 μM) or erythromycin (B), clarithromycin (C), azithromycin (D) (33 to 333 μM), telithromycin (E) (10 to 100 μM) for 1 hour, followed by LPS (100 ng/ml) stimulation for 4 hours. LPS-induced TNFα release was evaluated by enzyme-linked immunosorbent assay. Values represent means of three experiments ± S.E.M. *P < 0.05, **P < 0.01 (versus nontreatment control), *P < 0.05, **P < 0.01 (versus treatment with LPS only).
sequence under conditions of oxidative stress. Basal NF-κB activity was high in PMA-differentiated U937 cells, and 4 hours exposure of H2O2 (200 μM) slightly but significantly enhanced NF-κB activity (114.1 ± 1.1% of nontreatment control, n = 4). Clarithromycin and azithromycin restored H2O2-induced enhancement of NF-κB activity at a high concentration (100 μM) (Fig. 5, C and D), whereas solithromycin provided remarkable inhibitory effects even at a lower concentration (10 μM) (Fig. 5A). Erythromycin did not show any effects (Fig. 5B).

Inhibition of Inflammatory Cell Accumulation in Cigarette Smoke-Exposed Mice In Vivo by Solithromycin. C57BL/6J mice were exposed to cigarette smoke (4%) for 12 days (30 minutes/day) and treated with solithromycin at 100 mg/kg orally once daily 1 hour before each smoke exposure during the last 8 days of the 12-day smoking course. At 24 hours after the last cigarette smoke exposure, bronchoalveolar lavage fluid was collected. Cigarette smoke exposure significantly increased the number of alveolar macrophages (air: 48,828 ± 2847 cells/ml, smoke vehicle: 240,712 ± 22,917) (Fig. 6A), neutrophils (air: 30.9 ± 20.3 cells/ml, smoke vehicle: 1024.0 ± 138.1) in bronchoalveolar lavage fluid. Solithromycin did not inhibit alveolar macrophage accumulation (smoke vehicle: 28,060 ± 40121) (Fig. 6A), but significantly inhibited neutrophil accumulation by 49% (smoke vehicle: 2635.0 ± 829.3, P < 0.05) (Fig. 6B) and lymphocyte accumulation by 75% (smoke vehicle: 276.5 ± 202.2, P < 0.05). In addition, although cigarette smoke exposure decreased body weight, solithromycin significantly restored the body weight (Fig. 6C).

The effects of erythromycin were also compared with that of solithromycin. Head-to-head comparison was conducted in a 4-days smoking model. C57BL/6J mice were exposed to cigarette smoke (4%) for 4 days (30 minutes/day) and treated with vehicle, solithromycin at 100 mg/kg, or erythromycin at 100 mg/kg orally once daily 1 hour before each smoke exposure. At 24 hours after the last cigarette smoke exposure, bronchoalveolar lavage fluid was collected. As shown in Fig. 6D, although solithromycin significantly

### TABLE 1
Effect of macrolides on inhibition of LPS-induced CXCL8 and TNFα release, and PMA-induced MMP9 activation in U937 cells

<table>
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<tr>
<th></th>
<th>Solithromycin</th>
<th>Erythromycin</th>
<th>Clarithromycin</th>
<th>Azithromycin</th>
<th>Telithromycin</th>
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<tr>
<td>LPS-induced CXCL8 release (μM)</td>
<td>78.2</td>
<td>NE at 333 μM</td>
<td>506.5</td>
<td>NE at 333 μM</td>
<td>NE at 100 μM</td>
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<tr>
<td>LPS-induced TNFα release (μM)</td>
<td>41.6</td>
<td>NE at 333 μM</td>
<td>426.3</td>
<td>NE at 333 μM</td>
<td>NE at 100 μM</td>
</tr>
<tr>
<td>PMA-induced MMP9 activation (μM)</td>
<td>14.9</td>
<td>NE at 333 μM</td>
<td>118.0</td>
<td>212.1</td>
<td>83.4</td>
</tr>
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</table>

CXCL8, C-X-C motif chemokine ligand 8 (interleukin-8); LPS, lipopolysaccharide; MMP9, matrix metalloproteinase 9; NE, no effect; PMA, phorbol 12-myristate 13-acetate; TNFα, tumor necrosis factor α.
inhibited airway neutrophilia, erythromycin did not inhibit it (air: 46.3 ± 25.7 cells/ml, smoke + vehicle: 13,500 ± 1360, smoke + SOL: 8320 ± 1160, *P < 0.05, smoke + erythromycin: 15,100 ± 1140, not significant). In addition, pro-MMP9 (not active form) was also elevated in BAL by smoke exposure and significantly inhibited by solithromycin, but not by

Fig. 3. Effects of macrolides on PMA-induced MMP9 activation in U937 cells. Cells were pretreated with solithromycin (A) (10 to 100 μM) or erythromycin (B), clarithromycin (C), azithromycin (D) (33 to 333 μM), or telithromycin (E) (10 to 100 μM) for 1 hour, followed by PMA (50 ng/ml) treatment. After 48 hours, supernatants were collected for zymography. MMP9 enzyme activity was measured by gelatin zymography. Data are expressed relative to standard. Values represent means of four (A) or three (B, C, and D) experiments ± S.E.M. ##P < 0.01 (versus nontreatment control), *P < 0.05, **P < 0.01 (versus treatment with PMA only).

Fig. 4. Effects of macrolides on TNFα release and MMP9 production by PBMC from COPD patients. PBMC were incubated with macrolide compounds [solithromycin (SOL) (A and E), 10 μM; erythromycin (EM) (B and F), clarithromycin (CLM) (C and G), and azithromycin (AZ) (D and H), 100 μM] or vehicle (non-treatment; NT) overnight. TNFα release was evaluated by enzyme-linked immunosorbent assay. MMP9 enzyme activity in supernatants was measured by gelatin zymography. Seven or eight individual values are shown. ##P < 0.01, *P < 0.05 (versus NT control).
erythromycin (air: 1.6 ± 0.21 ng/ml, smoke + vehicle: 3.9 ± 0.29, smoke + SOL: 2.5 ± 0.17, P < 0.05, smoke + erythromycin: 3.9 ± 0.70, not significant).

Discussion

In this study, we demonstrated that a novel macrolide/fluoroketolide, solithromycin, has beneficial anti-inflammatory effects in addition to the antimicrobial ability previously shown. The inhibitory activities on production of TNFα, CXCL8, and MMP9, as well as NF-κB activation, were superior to those of other macrolide antibiotics currently used in the clinic. Even more importantly, solithromycin also inhibited proinflammatory mediators released by PBMC obtained from COPD patients and inhibited neutrophilia and pro-MMP9 production in cigarette smoke-exposed mice in vivo, which are known to be corticosteroid resistant.

TNFα and CXCL8 were increased in bronchoalveolar lavage, sputum, and serum in COPD (Stankiewicz et al., 2002; Vernooy et al., 2002) and are believed to play an important role in the pathogenesis of COPD (Barnes, 2009). Several in vitro studies indicated that macrolides inhibit proinflammatory mediators, such as CXCL8 and TNFα released from monocytes (Iino et al., 1992; Kikuchi et al., 2002), neutrophils (Tsukihashi et al., 2002), bronchial epithelial cells (Takizawa et al., 1997), and induced sputum cells (Marjanovic et al., 2011). Macrolides also exerted inhibitory effects on neutrophil-dominant inflammation in the in vivo models (Leiva et al., 2008; Bosnar et al., 2009). Furthermore, macrolides inhibited release of inflammatory mediators including TNFα and CXCL8 from sputum cells obtained from patients with COPD (Marjanovic et al., 2011). In addition to these in vitro results, clinical benefits by long-term macrolide therapy have been reported in COPD patients. Although short-term treatment did not provide any advantages for patients with stable COPD (Banerjee et al., 2005), long-term (more than 6 months) macrolide therapy reduces exacerbation of COPD (Seemungal et al., 2008; Blasi et al., 2010; He et al., 2010).

Macrolides have also been reported to exert beneficial effects in asthma. Low-dose erythromycin therapy in children with asthma for 6 months improved their symptoms and serum CXCL8 levels (Korematsu et al., 2010). Telithromycin showed beneficial effects on acute exacerbations of adult asthma (Johnston et al., 2006). Even short-term (8 week) clarithromycin treatment reduced sputum CXCL8, neutrophils, neutrophil elastase, and MMP9 and also improved quality of life scores in patients with refractory neutrophilic asthma (Simpson et al., 2008). Some in vitro studies have supported these clinical findings. Erythromycin and clarithromycin reduced CXCL8 release from human eosinophils in patients with allergic diseases, including asthma (Kohyama et al., 1999). Moreover, azithromycin inhibited IL-5 release from Th0 and Th2 cells in atopic asthmatic children (Lin et al., 2011). Thus, macrolides have shown great promise in their ability to reduce airway inflammation, including improvements in quality of life and exacerbation rates in airways diseases such as COPD and asthma (Simpson et al., 2009), but the efficacies are relatively moderate.

Excessive or inappropriate expression of MMP is closely associated with pathogenesis of tissue destructive processes in a wide variety of diseases, including lung diseases such as COPD (Ohbayashi, 2002). In peripheral lung from COPD patients, MMP9 expression and activity are upregulated and related to disease severity (Nakamaru et al., 2009). Furthermore, overexpression of human MMP9 in macrophages induced adult onset emphysema in mice and was associated with the loss of alveolar elastin (Foronjy et al., 2008). MMP9 produced from macrophages might be important in the development of emphysema (Russell et al., 2002). Ribeiro
et al. (2009) demonstrated that azithromycin prevented the upregulation of MMP9 genes, which were triggered by inflammatory stimuli. In addition, there were a few in vivo studies evaluating effect of macrolide on MMP9 activity (Simpson et al., 2008; Ogawa et al., 2009). Simpson and colleagues showed that MMP9 concentrations were reduced in sputum from patients with refractory asthma receiving clarithromycin. As shown in Figs. 3 and 4, the effect of solithromycin on MMP9 production was more potent than that of clarithromycin, promising higher efficacy of solithromycin. The inhibition of MMP9 by solithromycin was also confirmed in smoke exposed mice (Fig. 6E).

The inflammation in COPD is amplified by increased oxidative stress (Rahman and Adcock, 2006). Oxidative stress activates NF-κB, a transcription factor, that orchestrates the expression of multiple inflammatory genes, including TNFα, CXCL8, and MMP9 (Barnes, 2009). In fact, NF-κB is reported to be regulated by macrolides. For example, azithromycin inhibited proinflammatory mediators, such as CXCL8, via inhibition of NF-κB in vitro models (Aghai et al., 2007; Matsumura et al., 2011), although it inhibited LPS-induced pulmonary neutrophilia and IL-1β with concomitant suppression of AP-1, but not NF-κB in vivo (Bosnar et al., 2011). In addition, erythromycin and clarithromycin also inhibited CXCL8 with inactivation of NF-κB and/or AP-1 in human bronchial epithelial cells in vitro (Abe et al., 2000; Desaki et al., 2000) as the effects were moderate. In this study, we also confirmed that solithromycin inhibited activated NF-κB under oxidative stress, and the efficacy was superior to those of other macrolides currently used clinically. As the mechanism of NF-κB inactivation by macrolides, it is reported that macrolides are able to suppress the degradation of IκBα, an inhibitor of NF-κB, and/or to affect downstream of the dissociation from IκBα in the NF-κB signaling pathway (Desaki et al., 2004; Aghai et al., 2007).

There have been some negative findings on the anti-inflammatory effects of macrolides. For example, neither erythromycin nor azithromycin directly inhibited inflammatory mediators such as CXCL8 and MMPs in human gingival cells (Kamemoto et al., 2009). Neither clarithromycin nor azithromycin had beneficial effects on CXCL8 release in human whole blood cells and alveolar macrophages (Kurdowska et al., 2001). These inconsistent results on anti-inflammatory effects could be due to time-dependent responses to macrolides via cross talk of signaling pathway (Shinkai et al., 2006); due to weak/moderate efficacies or variable cellular intake, the results became variable.

Telithromycin, a commercially available ketolide, has also been reported to exert anti-inflammatory effects through NF-κB inhibition (Leiva et al., 2008). We therefore compared the effect of telithromycin with that of solithromycin. We found that telithromycin inhibited MMP9 activity (IC₅₀ 83.4 μM), although to a lesser extent than solithromycin (IC₅₀ 14.9 μM). However, it did not inhibit TNFα or CXCL8 release in the macrophage system. In PBMC, because telithromycin showed cytotoxicity (significant at 100 μM), we could not evaluate the
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efficacies properly. In addition, the clinical use of telithromycin is limited due to its side effects that include liver failure, exacerbation of myasthenia gravis, and visual disturbances. The pyridine-imidazole-containing moiety, which is a part of telithromycin, could induce inhibition of the nicotinic acetylcholine receptors at the vagus nerve innervating the liver, the neuromuscular junction receptors, and the ciliary ganglion, resulting in these side effects, whereas solithromycin does not contain this moiety (Bertrand et al., 2010).

In conclusion, our findings revealed that a novel macrolide/ketolide, solithromycin, exerts potent anti-inflammatory effects compared with other macrolides used clinically, possibly through NF-κB inhibition. This suggests that with its superior antimicrobial and anti-inflammatory activities, solithromycin might be a potential therapy for refractory chronic inflammatory respiratory diseases such as COPD.

Acknowledgments

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Authorship Contributions

Participated in research design: Kobayashi, Goto, Wada, Barnes, Ito. Conducted experiments: Kobayashi, Wada, Rossios, Takagi, Higaki, Mikura, Ito. Performed data analysis: Kobayashi, Wada, Ito. Wrote or contributed to the writing of the manuscript: Kobayashi, Barnes, Ito.

References


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**Supplemental Table 1.**

Study subjects for PBMCs

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