Poster A127

Solithromycin Reduces Inflammation In Mice Caused By Bleomycin-Induced Lung Injury

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Abstract

Introduction: Solithromycin (SOLI) is a fourth generation oral and intravenous macrolide, the first fluoroketolide, which is in Phase III clinical development for the treatment of community-acquired bacterial pneumonia. SOLI has been reported to show strong effects on cytokine release and superior anti-inflammatory effects on cigarette smoke-induced airway inflammation in mice (*Kobayashi, J. Pharmacol. Exp. Ther. 2013; 345:76-84).*

Aim: To explore the effects of SOLI on the ability to prevent lung inflammation and fibrosis, it was tested in a bleomycin induced-lung inflammation and fibrosis model.

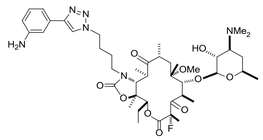
Methods: Lung inflammation was induced in female mice by a single intratracheal administration of bleomycin. Twenty mice were divided into two groups. From Day -2 to Day 6, one group was administered vehicle and the other was administered SOLI orally at a dose of 100 mg/kg. Animals were sacrificed on Day 7.

Results: The total numbers of cells in the bronchoalveolar lavage fluid (BALF), especially those of lymphocytes, neutrophils and eosinophils, were significantly decreased in the SOLI group compared to the vehicle group. BALF MMP-9 levels showed a decreasing trend in the SOLI group. Masson's Trichrome staining revealed focal fibrotic lesions in the interstitial region of the lung in the vehicle group and SOLI treated group with no significant differences between the groups.

BALF Analysis	Vehicle treated	SOLI treated
Lymphocytes	7.9 ± 3.9 X 10^4	3.9 ± 1.9 X 10^4 **
Macrophages	22.6 ± 7.3 X 10^4	16.9 ± 9.6 X 10^4
Neutrophil	2.4 ± 1.3 X 10^4	0.7 ± 0.4 X 10^4 ***
Eosinophil	0.8 ± 0.5 X 10^4	0.3 ± 0.2 X 10^4 **
MMP-9	0.62 ± 0.25 ng/mL	0.37 ± 0.30 ng/mL
Values are means ± SD. **p <0.01, ***p <0.001		

Introduction

SOLI is a 4th generation macrolide, the first fluoroketolide which is in Phase 3 clinical development for the treatment of community acquired bacterial pneumonia (CABP).



It has been shown to be well tolerated in the oral and intravenous form and SOLI has been demonstrated to have strong anti-inflammatory properties (1,2). SOLI has greater anti-inflammatory properties than the older macrolides, like clarithromycin and azithromycin (1,2). It has been tested in a smoking mouse model and shown to inhibit neutrophil numbers, as well as decrease MMP9 in cigarette smoke exposed mice.

Pulmonary fibrosis is a problem in the management of patients who have received chemotherapy for malignancies particularly with regimens that contain bleomycin (BLM), methotrexate, cyclophosphamide, and many new agents (3). These patients are susceptible to pulmonary infections as well as to inflammation from pulmonary injury.

The objective of this study was to evaluate the potential of SOLI in decreasing the damage caused by inflammation, keeping in mind that solithromycin could have a double use in controlling secondary infections.

Materials and Methods

Test Substance
Solithromycin (SOLI; formerly CEM-101) (Cempra Pharmaceuticals, Inc.) powder was dissolved in vehicle (0.5% methycellulose + 0.2% Tween 80).

Preparation and Randomization of BLM-induced Pulmonary Fibrosis Model

On Day 0, 20 mice were intratracheally administered BLM (Nippon Kayaku, Japan) in 0.9% saline in a volume of 50 µL per animal using a Microsprayer® (Penn-Century, USA).

Treatment schedules: SOLI and vehicle were administered by oral route in a volume of 10 mL/kg to seven-week-old female C57BL/6 mice (17~21 g) (CLEA Japan). The dose was 100 mg/kg once daily (Table 1).

Materials and Methods (cont.)

BALF Collection and Analyses

BALF samples were collected by flushing the lung via the trachea with sterile PBS three times (0.8 mL each). The first lavage was kept separate from the other two. BALF was centrifuged at 1,000 xg for 3 minutes at 4˚ C and the supernatant was collected and stored at -80˚ C until use. The cell pellet from the first fraction and the remaining fractions of lavage fluid were pooled. Total cell number of BALF was counted with a hemocytometer and the cell differentials were determined by cytospin preparation stained with Diff-Quick (Sysmex, Japan). A differential cell count was performed on up to about 200 cells.

MMP-9 in the BALF was quantified by the Mouse Total MMP-9 Quantikine ELISA Kit (R&D Systems, USA). The immunoassay had a detection limit of 0.078 ng/mL

Histopathological Analyses

Right lung tissues prefixed in 10% neutral buffered formalin were sectioned and used for hematoxylin and eosin (HE) staining and Masson's Trichrome staining. The degree of pulmonary fibrosis was evaluated using the Ashcroft score (4) for the quantitative histological analysis.

Quantitative RT-PCR

Total RNA was extracted from lung samples using RNAiso (Takara Bio, Japan). One µg of RNA was reverse-transcribed using a reaction mixture containing 4.4 mM $MgCl_2$ (Roche, Switzerland), 40 U RNase inhibitor (Toyobo, Japan), 0.5 mM dNTP (Promega, USA), 6.28 µM random hexamer (Promega), 5 x first strand buffer (Promega), 10 mM dithiothreitol (Invitrogen) and 200 U MMLV-RT (Invitrogen) in a final volume of 20 µL. The reaction was carried out for 1 hour at 37˚ C, followed by 5 minutes at 99˚ C. Real-time PCR was performed using real-time PCR DICE and SYBR premix Taq (Takara Bio). To calculate the relative mRNA expression level, the expression of each gene was normalized to that of reference gene GAPDH.

Statistical Tests

Statistical analyses were performed using Student's t-test on GraphPad Prism 4 (GraphPad Software, USA). *P* values < 0.05 were considered statistically significant. A trend or tendency was assumed when a one-tailed t-test returned *P* values < 0.10. Results were expressed as mean ± SD.

Table 1. Treatment schedule

Group	No. mice	Mice	Test substance	Dose	Volume	Regimen	Sacrifice
1	10	BLM	Vehicle	-	10 mL/kg	Oral, QD Day -2 – day 6	Day 7
2	10	BLM	Solithromycin	100 mg/kg	10 mL/kg	Oral, QD Day -2 – day 6	Day 7

Animal Monitoring

The viability, clinical signs and behavior were monitored every day. Body weight was recorded daily after the day of starting the treatment (day -2).

Results

Body Weight Changes and General Condition

Body weight was expressed as percentage body weight change from baseline (Day 0). In the Vehicle group, the body weight was gradually decreased through the study period. There were no significant differences in the body weight changes at any day and on the day of sacrifice between the Vehicle group and the SOLI group (Vehicle: 95 ± 10%, SOLI: 99 ± 6%)

BALF (cellular) Analysis

The total number of cells in the BALF was significantly decreased in the SOLI group compared to the Vehicle group (Vehicle: 3.4 ± 1.1x10^5 cells, SOLI: 2.2 ± 1.2 x10^5 cells) (Figure 1).

In the differential count of the cells in the BALF, the numbers of lymphocytes, neutrophils and eosinophils were significantly decreased in the SOLI group compared to the Vehicle group.

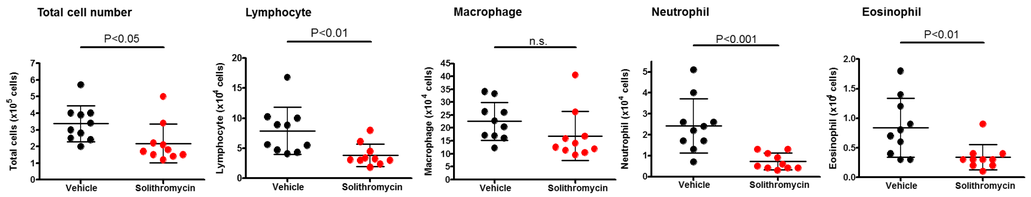
There was no significant difference in the number of macrophages between the Vehicle group and the SOLI group.

BALF MMP-9

BALF MMP-9 levels in the Solithromycin group showed a decreasing trend compared to the Vehicle group (Vehicle: 0.62 ± 0.25 ng/mL, SOLI: 0.37 ± 0.30 ng/mL) (Figure2).

Results (cont.)

Figure 1. Cell analysis of BALF



Histological analysis: Masson's Trichrome staining and Ashcroft score

In the Vehicle group, Masson's Trichrome staining revealed focal fibrotic lesions in the interstitial space of the lung. There was no significant difference in the Ashcroft score between the Vehicle group and the SOLI group (Vehicle: 1.6 ± 0.2, SOLI: 1.4 ±0.5) (Figure 3).

HE-staining

In the Vehicle group, HE staining revealed alveolar wall thickening, diffuse alveolar destruction with collapse and obliteration of alveolar spaces, and inflammatory cell infiltration in the alveolar and interstitial space of lung. There were no obvious differences in the alveolar wall thickening, diffuse alveolar destruction and inflammatory cell infiltration between the Vehicle group and the SOLI group (Figure 4).

Figure 2. BALF MMP-9

Figure 3. Representative photomicrographs of Masson's Trichrome-stained lung sections

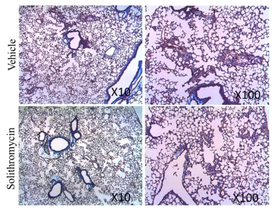
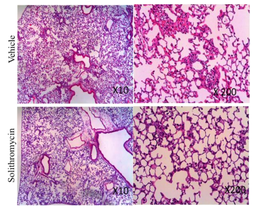


Figure 4. Representative photomicrographs of HE-stained lung sections



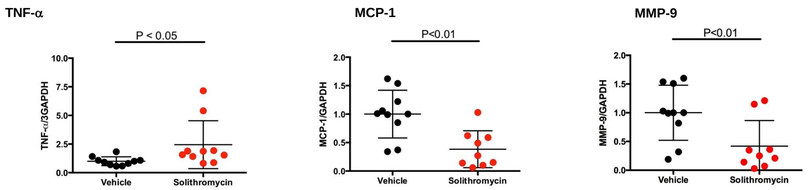
Gene expression Analysis

TNF-α mRNA expression levels were significantly up-regulated in the SOLI group compared to the Vehicle group (Vehicle: 1.00 ± 0.39, SOLI: 2.45 ± 2.10). However, this difference was driven primarily by increases in TNF-α expression in 2 out of 10 mice (Figure 5).

MCP-1 mRNA expression levels were significantly decreased in the SOLI group compared to the Vehicle group (Vehicle: 1.00 ± 0.42, SOLI: 0.38 ± 0.32).

MMP9 mRNA expression levels were significantly decreased in the SOLI group compared to the Vehicle group (Vehicle: 1.00 ± 0.48, SOLI: 0.42 ± 0.45).

Figure 5. Relative Gene Expression



Discussion

SOLI is in Phase 3 clinical trials for CABP. SOLI has 4-16 fold greater activity than azithromycin and has activity against macrolide-resistant strains. SOLI achieves high concentrations in the lung, in the epithelial lining fluid, as well as in pulmonary macrophages in human studies (3). It has been shown to be effective and well tolerated in a Phase 2 CABP study (4).

In this study SOLI showed significant decreases in lymphocytes, eosinophils and neutrophils of the BALF in mice with BLM-induced lung injury. SOLI decreased MCP-1 and MMP-9 mRNAs in the lung and increased TNF-α mRNA, although the latter only occurred in 2 out of 10 mice.

MCP-1 and MMP-9 are involved in the recruitment of inflammatory cells, whilst TNF-a has been reported to repress disease development via inducing apoptosis of inflammatory cells (5). These results confirm and extend previous studies that have demonstrated the strong anti-inflammatory properties of SOLI, including in the smoking mouse model (1, 2). Therefore, reduction of BALF cells by SOLI may be attributable to increased apoptosis of and reduced recruitment of inflammatory cells. These results suggest that SOLI could prevent the disease progression of pulmonary fibrosis.

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