J Infect Chemother 23 (2017) 598–603

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Tedizolid inhibits MUC5AC production induced by methicillin-resistant *Staphylococcus aureus* in human airway epithelial cells

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ARTICLE INFO

Article history:
Received 3 February 2017
Received in revised form
14 March 2017
Accepted 28 April 2017
Available online 17 July 2017

Keywords:
Mucin
MUC5AC
Oxazolidinones
Staphylococcus aureus

ABSTRACT

The innate immune system plays an important role in early immunity against respiratory tract infection. Although airway epithelial cells produce mucus to eliminate pathogens and irritants, hypersecretion of mucus is harmful for the host as it may cause airway obstruction and inhibit influx of antimicrobial agents. It has been reported that several antimicrobial agents have an immunomodulatory effect *in vitro* and *in vivo*, but little is known about whether tedizolid, a novel oxazolidinone, can modulate immune responses. In this study, we evaluated whether tedizolid can suppress MUC5AC production in human airway epithelial cells stimulated by methicillin-resistant *Staphylococcus aureus* (MRSA). Compared with the control, tedizolid significantly inhibited MUC5AC protein production and mRNA overexpression at concentrations of both 2 and 10 μg/mL (representative of trough and peak concentrations in human epithelial lining fluid). Among the mitogen-activated protein kinase inhibitors tested, only extracellular signal-regulated protein kinase 1/2 (ERK1/2) phosphorylation was inhibited by tedizolid as indicated by western blot analysis. These results indicate that tedizolid inhibits the overproduction of MUC5AC protein by inhibiting phosphorylation of ERK1/2. This study revealed that tedizolid suppresses excessive mucin production in human airway epithelial cells. The immunomodulatory effect of tedizolid may improve outcomes in patients with severe respiratory infectious diseases caused by MRSA.

1. Introduction

The innate immune system plays an important role in early immunity against respiratory tract infections. Airway epithelial cells produce mucus to protect epithelial cell surfaces, and to trap pathogens and irritants. Foreign particles are then eliminated from respiratory tract by ciliary movement. In this way, mucus is an indispensable part of airway host defense mechanisms.

Mucus consists mainly of mucin proteins and water. MUC5AC and MUC5B are two members of the mucin protein family that are strongly expressed in airway epithelial cells [1], and MUC5AC overexpression is observed in patients with chronic respiratory diseases such as diffuse panbronchiolitis and asthma [2,3]. Mucin overexpression is also observed in patients with acute respiratory infectious diseases such as ventilator-associated pneumonia (VAP) [4]. Excessive mucus induced by these diseases is harmful for the host because it results in airway obstruction, atelectasis, inhibition of oxygenation, and reduction of drug permeability. Hence, it is important to control MUC5AC production.

Several pathogens such as *Pseudomonas aeruginosa*, *Haemophilus influenzae*, *Acinetobacter baumannii*, *Fusobacterium nucleatum*, *Chlamydophila pneumoniae*, and methicillin-resistant *Staphylococcus aureus* (MRSA), stimulate airway epithelial cells and induce MUC5AC overexpression *in vitro* [5–10]. Several studies have reported that direct immunomodulatory effects of antibiotics on airway epithelial cells can reduce excessive MUC5AC production [6–10]. MRSA is the major causative microorganism associated with VAP, and isolation of MRSA from the respiratory tract of patients with cystic fibrosis is associated with the poorest survival

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http://dx.doi.org/10.1016/j.jiac.2017.04.015
1341-321X/

[11,12]. MRSA is associated with both acute and chronic severe respiratory infections, and therefore it is important to evaluate whether anti-MRSA drugs have an immunomodulatory effect. It has been reported that linezolid, the first member of the oxazolidinones to be introduced, can suppress the MUC5AC overexpression induced by supernatant from MRSA cultures, but it is unclear whether other anti-MRSA drugs also have an immunomodulatory effect [10].

Tedizolid, a novel oxazolidinone, is approved for treatment of bacterial skin and soft tissue infections caused by MRSA and vancomycin-resistant enterococci. Tedizolid possesses several advantages over linezolid such as fewer adverse events, longer half-life, and greater *in vitro* susceptibilities [13,14]. Because the treatment efficacy of tedizolid is equivalent to linezolid in a murine pneumonia model, tedizolid is expected to become the main therapeutic modality for MRSA pneumonia and VAP [15]. Linezolid, the first-line drug for MRSA pneumonia, has been shown to have several immunomodulatory effects such as suppression of inflammatory cytokine production, and inhibition of MUC5AC overexpression [10,16–18]. However, the immunomodulatory effect of tedizolid has not been demonstrated *in vitro*. In this study, we evaluated the immunomodulatory effect of tedizolid on MUC5AC overexpression in human airway epithelial cells.

2. Materials and methods

2.1. Materials

Tedizolid was supplied by Bayer HealthCare AG. Tedizolid was diluted in dimethyl sulfoxide (DMSO). The following antibodies were used: mouse anti-MUC5AC monoclonal (clone 45M1; Monosan); goat anti-mouse horseradish peroxidase-conjugated secondary (Bio-Rad); and anti-ERK, anti-phospho-ERK, anti-p38 MAPK, anti-phospho-p38 MAPK, anti-JNK, anti-phospho-JNK, anti-IκBα, and anti-phospho-IκBα (Cell Signaling Technology). The following inhibitors were diluted in DMSO and used: extracellular signal-regulated protein kinase (ERK) inhibitor (U0126; Promega); and p38 mitogen-activated protein kinase (MAPK) inhibitor (SB203580), c-JUN N-terminal kinase (JNK) inhibitor II (SP600125), and caffeic acid phenethyl ester, a specific NF-κB inhibitor (CAPE; Calbiochem).

2.2. Bacterial strains

The MRSA strain NUMR101 was obtained from a blood sample isolated clinically at the Nagasaki University Hospital [19]. The strain was stored at −80 °C in a Microbank bead-based preservation system (Pro-Lab Diagnostics) until use. NUMR101 was characterized genetically by real-time polymerase chain reaction (PCR) using a previously described method [20]; the staphylococcal cassette chromosome *mec* (SCC*mec*) was type II, and the strain carried virulence genes such as *sec* and *tsst*, but did not carry *etb* and *pvl* genes.

2.3. Preparation of MRSA supernatant

To avoid direct antibiotic effects of tedizolid on MRSA, we used an MRSA culture supernatant as a stimulator. The MRSA supernatant was prepared according to our previously published method [6]. NUMR101 strain was cultured in Mueller-Hinton II medium (BD) at 37 °C with shaking at 250 rpm for 72 h. After incubation, the bacteria were centrifuged at 10,000× *g* for 10 min at 4 °C, and the supernatant was filtered using a 0.22-μm Millex-GP filter (Millipore). The MRSA supernatant was stored at −80 °C until use.

2.4. Cell culture

NCI-H292 human airway epithelial cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were grown at 37 °C with 5% CO_2. When the cells reached confluence, they were serum-starved for 24 h and then stimulated with the MRSA supernatant. During inhibition studies, the cells were treated with tedizolid simultaneously with MRSA supernatant stimulation. Based on previously reported trough and peak concentrations of tedizolid in the epithelial lining fluid (ELF) of healthy volunteers, tedizolid was used at concentrations of 2 and 10 μg/mL [21]. Because there is a possibility that LB broth induces MUC5AC overexpression, controls were incubated with a volume of LB broth equivalent to the MRSA supernatant. Cells were also pretreated with signal transduction inhibitors at a concentration of 10 μM for 30 min before stimulation. Cells in controls were incubated with medium plus the same amount of DMSO without the inhibitors.

2.5. Enzyme-linked immunosorbent assay (ELISA)

MUC5AC protein levels were measured using ELISA [5]. NCI-H292 cells were cultured in 24-well plates until confluent. After stimulation with MRSA supernatant for 24 h as described above, the culture medium (cell supernatant) was collected and incubated at 40 °C in a 96-well plate until dried. The plates were blocked with 2% FBS for 1 h at room temperature and then incubated with anti-MUC5AC antibody diluted in phosphate-buffered saline (PBS) containing 0.05% Tween 20 for 1 h. Horseradish peroxidase-conjugated anti-goat immunoglobulin G was then dispensed into each well. After 1 h, immunoreactivity was detected colorimetrically using 3,3′,5,5′-tetramethylbenzidine peroxidase (TMB) solution before the reaction was stopped by adding 1 N H_2SO_4, and the absorbance read at 450 nm.

2.6. RNA extraction and real-time quantitative reverse transcription-PCR (qRT-PCR)

NCI-H292 cells were stimulated for 6 h before the level of MUC5AC mRNA was assayed by qRT-PCR according to a previously published method [10]. Total RNA was extracted from NCI-H292 cells cultured in 6-well plates, using the ISOGEN II (Nippon Gene) and the PureLink RNA micro scale kit (Invitrogen) according to the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed into cDNA using oligo (dT) primers and SuperScript III reverse transcriptase (Invitrogen) and then treated with RNase H. To quantify expression of the MUC5AC gene, PCR primers and TaqMan probes were designed and used as reported previously (forward primer, 5′–CAGCCACGTCCCCTTCAATA–3′; reverse primer, 5′–ACCGCATTTGGGCATCC–3′: TaqMan probe, 5′–6–carboxyfluorecein [6-FAM]–CCACCTCCGAGCCCGTCACTGAG–6–carboxytetramethylrhodamine [TAMRA]–3′) [9]. The *MUC5AC* transcript was amplified for 40 cycles (each cycle consisted of 15 s at 95 °C and 30 s at 60 °C), using a LightCycler system (Roche Diagnostics). To normalize *MUC5AC* expression, human porphobilinogen deaminase (*PBGD*) expression was also measured using specific PCR primers and TaqMan probes ([22]; forward primer, 5′–AACCAGCTCCCTGCGAAGA–3′; reverse primer, 5′–6–FAM –ACTCCTGAACTCCAGATGCGGGAACT–TAMRA–3′).

2.7. Western blot analysis

NCI-H292 cells were harvested at 0, 60, and 120 min after MRSA stimulation and then washed and homogenized at 4 °C in lysis buffer (0.1% sodium dodecyl sulfate, 1% Igepal CA-630, 0.5% sodium deoxycholate). Cell lysates (40 μg) were resolved by electrophoresis