Antiviral Research 144 (2017) 48–56

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Anti-inflammatory effects of adjunctive macrolide treatment in adults hospitalized with influenza: A randomized controlled trial

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ARTICLE INFO

Article history:
Received 27 February 2017
Received in revised form
16 May 2017
Accepted 18 May 2017
Available online 20 May 2017

Keywords:
Macrolide
Anti-inflammatory effects
Influenza

ABSTRACT

Introduction: – Macrolides can ameliorate inflammation in respiratory diseases, providing clinical benefits. Data in influenza is lacking.
Method: – A randomized, open-label, multicenter trial among adults hospitalized for laboratory-confirmed influenza was conducted. Study treatments of oseltamivir and azithromycin (500 mg/day), or oseltamivir alone, both for 5 days, were allocated at 1:1 ratio. The primary outcome was plasma cytokine/chemokine concentration change over time (Day 0–10); secondary outcomes were viral load and symptom score changes. Generalized Estimating Equation (GEE) models were used to analyze longitudinal data.
Results: – Fifty patients were randomized to the oseltamivir-azithromycin or oseltamivir groups, with comparable baseline characteristics (age, 57 ± 18 years; A/H3N2, 70%), complications (72%), and viral load. Pro-inflammatory cytokines IL-6 (GEE: β −0.037, 95%CI-0.067,-0.007, P = 0.016; reduction from baseline −83.4% *vs* −59.5%), CXCL8/IL-8 (β −0.018, 95%CI-0.037,0.000, P = 0.056; −80.5% *vs* −58.0%), IL-17 (β −0.064, 95%CI-0.117,-0.012, P = 0.015; −74.0% *vs* −34.3%), CXCL9/MIG (β −0.010, 95%CI-0.020,0.000, P = 0.043; −71.3% *vs* −56.0%), sTNFR-1, IL-18, and CRP declined faster in the oseltamivir-azithromycin group. There was a trend toward faster symptom resolution (β −0.463, 95%CI-1.297,0.371). Viral RNA decline (P = 0.777) and culture-negativity rates were unaffected. Additional *ex vivo* studies confirmed reduced induction of IL-6 (P = 0.017) and CXCL8/IL-8 (P = 0.005) with azithromycin.
Conclusion: – We found significant anti-inflammatory effects with adjunctive macrolide treatment in adults with severe influenza infections. Virus control was unimpaired. Clinical benefits of a macrolide-containing regimen deserve further study.
[ClinicalTrials.gov NCT01779570]

1. Introduction

Influenza continues to be a major cause of morbidity and mortality worldwide. Patients hospitalized with severe lower respiratory tract infections caused by seasonal, pandemic ($H1N1_{pdm09}$), or avian influenza (e.g. H5N1, H7N9) viruses may develop acute respiratory failure, acute respiratory distress syndrome (ARDS) and

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http://dx.doi.org/10.1016/j.antiviral.2017.05.008
0166-3542/

multi-organ dysfunction, with high fatality risk. Antiviral treatment alone, given after such complications have developed, could be ineffective in reversing the clinical course (Dunning et al., 2014; Hui et al., 2013; Lee et al., 2015). Current understanding on influenza pathogenesis suggests that in addition to direct viral actions, host immune responses can play a major role in impelling disease manifestations (Baskin et al., 2009; Herold et al., 2015; Hui et al., 2013). Evidently, the pro-inflammatory cytokines/chemokines (e.g. IL-6, CXCL8/IL-8, TNF-α) are highly expressed in these infections, inducing systemic physiological responses, recruiting and activating the innate immune cells, and directly/indirectly mediating tissue inflammation (Baskin et al., 2009; Lee et al., 2007, 2011a, 2011b; Wang et al., 2012). In clinical studies, such 'cytokine storms' have been shown to correlate significantly with clinical severity and outcomes (e.g. pneumonia, respiratory failure, ICU admission, death) (Bermejo-Martin et al., 2009; Hui et al., 2013; Lee et al., 2007, 2011a, 2011b). As a result, adjunctive therapy using agents with anti-inflammatory and/or immunomodulatory properties have been proposed (Dunning et al., 2014; Hui et al., 2013; Ison, 2014). Systemic corticosteroids, studied in septic shock and ARDS to expedite recovery however, are found to impair viral clearance, cause secondary infections, and increase mortality (Dunning et al., 2014; Hui et al., 2013; Ison, 2014; Lee and Hui, 2011).

Macrolides (e.g. erythromycin, clarithromycin, azithromycin) are antibiotics known to have anti-inflammatory effects beyond their antibacterial properties; *in vitro*, they are shown to down-regulate pro-inflammatory cytokines/chemokines, inhibit signal transduction and adhesion molecules expression, and regulate inflammatory cell functions (Kanoh and Rubin, 2010; Parnham et al., 2014). In animal and human studies, macrolides are shown to alleviate symptoms and improve outcomes of a range of infective and non-infective conditions (e.g. community-acquired pneumonia, exacerbations of COPD/asthma, cystic fibrosis, diffuse panbronchiolitis, acute lung injury, sepsis), independent of their antibacterial actions (Asadi et al., 2012; Essilfie et al., 2015; Kanoh and Rubin, 2010; Parnham et al., 2014; Verleden et al., 2006; Walkey and Wiener, 2012). For influenza, limited data from murine models suggest cytokine inhibition and reduced severity of pneumonia with macrolide treatment (Hui et al., 2013; Kanoh and Rubin 2010; Min and Jang, 2012). Clinical studies are scarce and largely confining to mild infections; and apart from fever duration, effects on host inflammatory/immune responses, viral load, and clinical progress have not been fully characterized (Dunning et al., 2014; Hui et al., 2013; Kakeya et al., 2014; Min and Jang, 2012). In this randomized trial, we aimed to investigate anti-inflammatory effects of macrolide therapy in severe influenza infections in adults. Patients receiving oseltamivir and adjunctive azithromycin were compared with those receiving oseltamivir alone on cytokines/chemokines and pro-inflammatory mediator changes, viral clearance, and symptom resolution; safety profiles were also assessed. Such information if available may reveal the potential of macrolide for the management of severe influenza, and provide important insights into future adjunctive therapy research.

2. Methods

2.1. Study design and case recruitment

An investigator-initiated, randomized, open-label, multicenter trial was conducted. The primary objective was to assess the anti-inflammatory effects of macrolide treatment in influenza. The secondary objectives were to assess effects on viral clearance and symptom resolution. The clinical settings, viral diagnostic methods and procedures for case identification were identical to our previous antiviral trials (Lee et al., 2007, 2013a, 2015, 2016). Briefly, patients presenting with acute respiratory infections were hospitalized if they had developed potentially serious complications (e.g. pneumonia), exacerbation of underlying cardiorespiratory illnesses (e.g. COPD, asthma), or severe constitutional and lower respiratory (cough, sputum production, dyspnea, chest discomfort) symptoms. Nasopharyngeal samples were collected for virus testing, regardless of perceived etiology. During the influenza seasons beginning from 2013–2014 through 2015–2016, adults confirmed with influenza virus infection at three participating hospital study sites in Hong Kong were prospectively assessed for study eligibility. Inclusion criteria were age ≥18 years, influenza A and B virus infections confirmed by PCR and/or immunofluorescence assays, hospitalized for the management of severe manifestations of influenza, presenting within 4 days from illness onset, and ability to provide written informed consent. Exclusion criteria were systemic corticosteroids or other immunosuppressant use, pregnancy/lactation, end-stage renal failure, hepatic failure, cardiac failure/arrhythmia, prolonged corrected QT interval >450 msec on electrocardiogram, and allergic history or other medical contraindications to macrolides (Parnham et al., 2014). The numbers of influenza patients screened and excluded based on these criteria were provided in Supplementary-1. Informed written consent was obtained from all recruited subjects. Ethical approval was obtained from Clinical Research Ethics Committee of the Chinese University of Hong Kong-New Territories East Cluster Hospitals. The study was conducted in accordance with the Declaration of Helsinki [ClinicalTrials.gov NCT01779570].

2.2. Description of study treatments

Recruited patients were randomly allocated, at 1:1 ratio, to one of the study treatments: (1) azithromycin 500 mg daily and oseltamivir 75 mg bid for 5 days, both given orally, or (2) oral oseltamivir 75 mg bid alone for 5 days. Renal dosage adjustment for oseltamivir, if required, was performed according to current recommendations (Lee et al., 2013a). Patients were otherwise managed and discharged according to standard hospital procedures, with follow-up outpatient visits scheduled until Day 30.

2.3. Sampling and data collection

Peripheral blood samples (10 ml EDTA) were collected at baseline (Day 0), at time of completion of treatment (Day 5) and at convalescence (Day 10) for cytokine/chemokine and pro-inflammatory mediator assays. These time-points were chosen based on our previous cytokine data in hospitalized adults (Lee et al., 2007; 2011a, 2011b, 2013b). Serial nasopharyngeal flocked swabs (NPFS) were collected at similar time points with standardized techniques for viral load assays (Day 0, 2, 5, and 10) (Lee et al., 2011a, 2013a, 2016). Urinary antigen tests for *L. pneumoniae* and *S. pneumoniae*, PCR tests for *M. pneumoniae* and *C. pneumoniae*, and bacterial cultures were performed in all recruited cases (Lam et al., 2007; Lee et al., 2013a, 2016). Chest radiographs, electrocardiogram, and liver function tests were also performed. A standard questionnaire was used to collect baseline and serial clinical data, as previously described. These included clinical manifestations/complications, symptom severity score, vital signs (e.g. temperature, respiratory rate, oxygen saturation), fever duration (defined as time to reach <37.5 °C), requirements for supplemental oxygen therapy and invasive/non-invasive ventilation, duration of hospitalization, death, and occurrence of adverse events (see Table 1 footnotes for definitions) (Lee et al., 2007, 2011a, 2011b, 2013a, 2015).