EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Fatty Acid Metabolism is Associated With Disease Severity After H7N9 Infection

Xin Sun [a,1], Lijia Song [b,1], Shuang Feng [c,1], Li Li [d,1], Hongzhi Yu [d], Qiaoxing Wang [c], Xing Wang [d], Zhili Hou [e], Xue Li [a], Yu Li [a], Qiuyang Zhang [a], Kuan Li [a], Chao Cui [f], Junping Wu [d], Zhonghua Qin [c], Qi Wu [a,b,g,*], Huaiyong Chen [a,g,**]

[a] Department of Basic Medicine, Haihe Clinical College of Tianjin Medical University, Tianjin 300070, China
[b] Department of Respiratory Medicine, Tianjin Medical University General Hospital, Tianjin 300052, China
[c] Department of Clinical Laboratory, Tianjin Haihe Hospital, Tianjin 300350, China
[d] Department of Respiratory Medicine, Tianjin Haihe Hospital, Tianjin 300350, China
[e] Department of Tuberculosis, Tianjin Haihe Hospital, Tianjin 300350, China
[f] Department of Thoracic Surgery, Tianjin Haihe Hospital, Tianjin 300350, China
[g] Key Research Laboratory for Infectious Disease Prevention for State Administration of Traditional Chinese Medicine, Tianjin Institute of Respiratory Diseases, Tianjin 300350, China

ARTICLE INFO

Article history:
Received 15 May 2018
Received in revised form 15 June 2018
Accepted 15 June 2018
Available online xxxx

Keywords:
H7N9 virus
Lung epithelium
Fatty acid metabolism
Palmitic acid
Metabolomics

ABSTRACT

Background: Human infections with the H7N9 virus could lead to lung damage and even multiple organ failure, which is closely associated with a high mortality rate. However, the metabolic basis of such systemic alterations remains unknown.

Methods: This study included hospitalized patients (n = 4) with laboratory-confirmed H7N9 infection, healthy controls (n = 9), and two disease control groups comprising patients with pneumonia (n = 9) and patients with pneumonia who received steroid treatment (n = 10). One H7N9-infected patient underwent lung biopsy for histopathological analysis and expression analysis of genes associated with lung homeostasis. H7N9-induced systemic alterations were investigated using metabolomic analysis of sera collected from the four patients by using ultra-performance liquid chromatography-mass spectrometry. Chest digital radiography and laboratory tests were also conducted.

Findings: Two of the four patients did not survive the clinical treatments with antiviral medication, steroids, and oxygen therapy. Biopsy revealed disrupted expression of genes associated with lung epithelial integrity. Histopathological analysis demonstrated severe lung inflammation after H7N9 infection. Metabolomic analysis indicated that fatty acid metabolism may be inhibited during H7N9 infection. Serum levels of palmitic acid, erucic acid, and phytal may negatively correlate with the extent of lung inflammation after H7N9 infection. The changes in fatty acid levels may not be due to steroid treatment or pneumonia.

Interpretation: Altered structural and secretory properties of the lung epithelium may be associated with the severity of H7N9-infection-induced lung disease. Moreover, fatty acid metabolism level may predict a fatal outcome after H7N9 virus infection.

1. Introduction

The emergence in 2013 of the novel avian-origin influenza A H7N9 virus imposed a great challenge not only to poultry production but also to public health because of the high rates of morbidity and mortality. As of February 2018, H7N9 viruses have caused five seasonal epidemic waves, with the number of laboratory-confirmed human cases increasing appreciably [52]. Travel-related cases have been confirmed outside China, including Canada and Malaysia [26]. Although the occurrence of international spread has not yet been indicated, recent evidence demonstrates that a more highly pathogenic H7N9 variant has evolved, thereby increasing the potential threat of a greater pandemic,

* Correspondence to: Q. Wu, Key Research Laboratory for Infectious Disease Prevention for State Administration of Traditional Chinese Medicine, Tianjin Institute of Respiratory Diseases, Tianjin Haihe Hospital, Tianjin 300350, China.
** Correspondence to: H. Chen, Department of Basic Medicine, Tianjin Institute of Respiratory Diseases, Haihe Clinical College of Tianjin Medical University, Tianjin 300350, China.
E-mail addresses: wq572004@163.com, (Q. Wu), huaiyong.chen@foxmail.com (H. Chen).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ebiom.2018.06.019

especially if it acquires efficient human-to-human transmissibility (Bertran et al., [1]; [28, 54]). Close monitoring of the virological and molecular characteristics of H7N9 viruses is therefore urgently needed. Moreover, clinical severity assessments of H7N9-infected patients are crucial to develop more effective control measures.

In patients with poultry contact, H7N9 virus infection may result in critical illnesses, including rapidly progressing pneumonia, respiratory failure, and acute respiratory distress syndrome (ARDS). Therefore, the administration of steroids, including methylprednisolone, is common in the intensive care unit (ICU) to limit lung inflammation [35, 37]. Hypoxic respiratory failure was reported as the leading cause of death [3]. As a result, endotracheal intubation and protective mechanical ventilation (MV) have become the mainstay of supportive therapy in the ICU [17, 38]. Severe ARDS associated with H7N9 virus infection leads to the use of extracorporeal membrane oxygenation (ECMO), originally applied to support the respiratory function of pediatric patients [24, 36]. Nevertheless, approximately 40% of patients do not survive these treatments, possibly because of the following three factors. First, the clinical prognosis of H7N9-infected patients is closely related to underlying medical complications [51]. Second, the severity is determined by the time interval from disease onset to antiviral treatment. Lastly, the mortality increases when H7N9 virus infection induces multiple organ failure [56].

The key cause of systemic alterations in H7N9 infection is lung damage characterized by impaired mucociliary clearance and disturbed alveolar homeostasis. As regenerating machines, both club and alveolar type 2 epithelial cells are targets of H7N9 viruses, resulting in the inefficient restoration of airway barriers and overproduction of mucus and surfactants [48]. Thus, hospitalized patients confirmed to have H7N9 virus infection are immediately started on antiviral therapy using existing neuraminidase inhibitors, such as oseltamivir and zanamivir, or neutralizing antibodies against hemagglutinin [19, 22, 24, 57]. As the H7N9 virus travels via the circulatory system to target more cell types (Hu et al., 2015), it is hypothesized to elicit systemic alterations that may also contribute to virus-induced multiple organ injuries.

Metabolomics, a rapidly emerging field of "omics" research, presents pathobiological profiles that encompass both microbial and host interactions [42, 45]. Metabolomic approaches have identified novel biomarkers and new pathobiological pathways associated with virus infections [9, 47]. However, to date, no study has applied a metabolomic approach in H7N9-infected patients.

To address this, we used serum samples from four patients who were hospitalized because of H7N9 infection to profile their serum metabolome. We found that the H7N9-infected patients had metabolic disorders possibly associated with fatty acid metabolism. To determine its possible relationship with disease severity, we tried to correlate the abundance of identified serum metabolites with the extent of lung inflammation evaluated using chest digital radiography and absolute serum lymphocyte counts. A three-metabolite set may be associated with the resolution of lung inflammation during H7N9 infection. We also observed that severe inflammation induced by H7N9 causes the altered expression of airway mucin and alveolar surfactants.

2. Materials and Methods

2.1. Ethics Statement

The histopathological features were reported for one patient whose relative consented to limited postmortem biopsy. Full autopsy of the patient could not be performed because of religious and other reasons. The institutional review board of Tianjin Haihe Hospital provided written approval and judged that all methods used in the study met relevant ethical guidelines and regulations (ethical # 2016HHKT-01).

2.2. Subjects

Four H7N9-infected patients were admitted to Tianjin Haihe Hospital between June 13, 2016 and June 20, 2017. During this period, nine consenting healthy volunteers, nine patients with pneumonia (according to the 2007 American Thoracic Society Inclusion Criteria), and ten patients with pneumonia who received steroid treatment were recruited as controls from the same hospital. General participant information, including age, sex, recent poultry exposure, disease duration, and clinical treatments and medication, was collected using a standard form. As described previously [12], for the diagnosis of H7N9 infection, RNA was extracted from throat swabs and sputum of the patients, and a multiplex one-step real-time PCR assay was developed to detect the H7N9 virus by using primers targeting the conserved M and RNase P genes, as well as the hemagglutinin and neuraminidase genes of the H7N9 virus. Serum samples were collected between 6:00 and 8:00 a.m. from all patients, and one part was used for routine clinical measurements and the other part was immediately stored in a −80°C refrigerator for further metabolomic analysis. Tru-cut postmortem biopsy specimens were obtained from the left lung at the sixth intercostal space posteriorly within 18–19 h after death. Two successful biopsies were conducted aseptically according to standard guidelines (Otto et al., 2015), with one specimen being put into a cell lysis medium for gene expression analysis using quantitative PCR and the other into 4% formalin for histopathological examination. For comparison, normal lung tissue adjacent to the tumor was also surgically removed from patients with lung adenocarcinoma.

2.3. Hematoxylin and Eosin Staining

Lung tissues were fixed in 10% neutral-buffered formalin, and then embedded in paraffin. Five-micrometer sections were collected and stained with hematoxylin and eosin (H&E) solution (Solarbio Science & Technology Co, Ltd., Beijing, China). Images were captured using an Olympus IX71 microscope with a DP80 camera (Olympus Corporation, Tokyo, Japan).

2.4. Scanning Electron Microscopy

Lung biopsy specimens were fixed in 2.5% glutaraldehyde for 2 h, and then immobilized in 2% osmium tetroxide. In brief, the tissues were dehydrated through sequential washes in 50, 70, 90, 95, and 100% ethanol followed by immersion in EPON812 (Simga Aldrich, St. Louis, MO, USA). Ultrathin sections were collected onto copper grids and then counterstained with uranyl acetate and lead citrate. Images were acquired using a FEI Tecnai Spirit transmission electron microscope (ThermoFisher Scientific, Hillsboro, Oregon, USA).

2.5. Quantitative Real-Time PCR

RNA was extracted from lung biopsy specimens and normal lung tissue by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The whole RNA was reverse transcribed using oligo (dT) primers for 1 h at 50 °C using the TIANScript RT kit (Tiangen Biotech, Co., Ltd., Beijing, China) according to the manufacturer's protocol. Quantitative PCR analysis was performed using the SYBR Green method. Specific gene primers were designed using the Primer-Quest SM software (http://sg.idtdna.com/PrimerQuest/Home/Index), and then commercially produced (BGI Tech, Shenzhen, China; listed in Table S1). The cDNA amplification reactions were performed using a Light Cycler 96 real-time PCR system (Roche Diagnostics, Indianapolis, IN, USA) with the following reaction conditions: an initial heating cycle of 95 °C for 2 min, and 40 cycles of denaturation at 95 °C for 25 s, primer annealing at 60 °C for 25 s, and extension at 72 °C for 20 s. Melting curves were used for clarifying the identity of amplicons, and the housekeeping gene β-actin was used as an internal control. The relative mRNA expression levels of targeted

Please cite this article as: Sun X, et al, Fatty Acid Metabolism is Associated With Disease Severity After H7N9 Infection, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.06.019