Journal of Hazardous Materials 303 (2016) 131–136

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Inhibition of lipopolysaccharide induced acute inflammation in lung by chlorination

Jinshan Zhang[a], Jinling Xue[a], Bi Xu[a], Jiani Xie[a], Juan Qiao[b,*], Yun Lu[a,*]

[a] Environmental Simulation and Pollution Control State Key Joint Laboratory, School of Environment, Tsinghua University, Beijing 100084, China
[b] Department of Chemistry, Tsinghua University, Beijing 100084, China

HIGHLIGHTS

- Chlorination is effective to reduce the inflammation inducing capacity of LPS in lung.
- LAL-detected endotoxin activity is not correlated to the potency of inflammation induction.
- Alkyl chain of LPS was chlorinated in chlorination process.
- LPS aggregate size decreases after chlorination.

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form
25 September 2015
Accepted 12 October 2015
Available online 22 October 2015

Keywords:
Lipopolysaccharide
Chlorine
Inhalation exposure
Aggregate size
Reclaimed water

ABSTRACT

Lipopolysaccharide (LPS, also called endotoxin) is a pro-inflammatory constituent of gram negative bacteria and cyanobacteria, which causes a potential health risk in the process of routine urban application of reclaimed water, such as car wash, irrigation, scenic water refilling, etc. Previous studies indicated that the common disinfection treatment, chlorination, has little effect on endotoxin activity removal measured by Limulus amebocyte lysate (LAL) assay. However, in this study, significant decrease of acute inflammatory effects was observed in mouse lung, while LAL assay still presented a moderate increase of endotoxin activity. To explore the possible mechanisms, the nuclear magnetic resonance (NMR) results showed the chlorination happened in alkyl chain of LPS molecules, which could affect the interaction between LPS and LPS-binding protein. Also the size of LPS aggregates was found to drop significantly after treatment, which could be another results of chlorination caused polarity change. In conclusion, our observation demonstrated that chlorination is effective to reduce the LPS induced inflammation in lung, and it is recommended to use health effect-based methods to assess risk removal of water treatment technologies.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Wastewater reclamation and reuse is important in sustainable development and water resource conservation strategy. The ratio of wastewater reuse is increasing rapidly and it has been used for flushing toilet, car wash, irrigation, refilling scenic water body, etc, which are closely related to human health [1]. However, there are many risk factors identified from reclaimed water contact, in which the microbial pathogens and their derivatives are the most commonly discussed [2]. Lipopolysaccharide (LPS) as a pro-inflammatory constituent of gram negative bacteria and some cyanobacteria, is released by cell multiplication, death, and lysis. It is relatively heat stable and similar in structure regardless of source [3,4], which can cause serious health issues including fever, inflammation, asthma, diarrhea, shortness of breath, intravascular coagulation and even death [5–7].

LPS is composed of lipid A, core oligosaccharide and O antigens. The toxicity of LPS is associated with the structure of lipid A whereas the immunogenicity is dependent on polysaccharide component [6,8,9]. The size of LPS monomer is ranging from 10 to 20 kDa, and it can form aggregates with high stability like micelle or vesicle with the size over 1000 kDa due to their hydrophobic interaction in aquatic systems [10,11]. It is known that only aggregates of LPS are biologically active [12]. The exposure routes to LPS include inhalation, intravenous injection and oral intake. In the urban use of reclaimed water, inhalation of aerosolized water droplets is the main exposure. Endotoxin activity in the secondary

* Corresponding authors.
E-mail addresses: qjuan@tsinghua.edu.cn (J. Qiao), luyun@tsinghua.edu.cn (Y. Lu).

http://dx.doi.org/10.1016/j.jhazmat.2015.10.024
0304-3894/© 2015 Elsevier B.V. All rights reserved.

effluent from a multi-country investigation report ranges from 201 to 20100 EU/mL, and the average level is 2994 EU/mL. Although the average endotoxin activity reduced to 1584 EU/mL after advanced treatments, the endotoxin activity still ranges 3–19700 EU/mL in the reclaimed water [13].

In order to minimize the health risk of exposure to reclaimed water containing LPS, microbial pathogens and other chemical compounds, the tertiary treatments are crucial to the safety of wastewater reuse [14], such as chlorination, ozone disinfection, UV irradiation, ultrafiltration and so on. Chlorination is one of the most widely used disinfection methods in wastewater reclamation to guarantee water quality due to its easiness to operate and low cost [15]. Endotoxin inactivation by chlorine had been studied previously. Anderson et al. [4] examined chlorination effect on tap water spiked with LPS standard (*Escherichia coli* strain O55:B5) and found that chlorine can hardly remove the endotoxin activity of LPS with the maximum inactivation rate of 1.4(EU)/mLh at residual chlorine from 2 mg/L up to 100 mg/L with reaction time more than 100 h. Rapala et al. [3] indicated that chlorination had no effect on endotoxin activity by studying endotoxin removal during drinking water treatment. Gehr et al. [16] found that endotoxin activity in low endotoxin-containing water samples (7–10 EU/mL) can both be moderately decreased or increased by chlorination. Can et al. [17] investigated endotoxin activity following different treatment process steps and indicated that endotoxin increased after chlorination, these were caused by bacteria lysis and endotoxin release. Similar results were also found in secondary effluent [9]. However, all these studied used LAL assay to assess treatment effects. One thing, which is worth of attention, is that the validity of using the in vitro LAL assay for assessing the inactivation efficiency of LPS has not been verified, since chemical treatment of LPS can cause changes at molecular level.

In this study, we measured the chlorinated LPS activities by LAL assay and a mouse inhalation exposure model simultaneously. LAL assay showed moderate increase of endotoxin activity, while lung inflammation was significantly reduced by chlorination, which indicates that chlorination can reduce the health effect caused by LPS inhalation and LAL is not suitable to evaluate the treatment efficiency. The mechanism of LPS inactivation by chlorine was also investigated.

2. Materials and methods

2.1. Animals

The study protocol was approved by the Institutional Animal Care and Use Committee of Tsinghua University and was performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals. We made our best efforts to minimize suffering and the number of experimental animals. Male ICR mice aged 8–9 weeks were purchased from Vital River company (Beijing, China) and kept in a barriered facility for experimental animals in Tsinghua University.

2.2. Inhalation exposure

HOPE-med 8050 dynamic inhalation system (HOPE Ltd, Tianjin, China) was used to provide an exposure chamber with a constant concentration of water aerosol flow. The aerosol concentration versus samples fluid rate was calibrated by an atmospheric sampler (Casella, UK) with drying columns. The water pumping rate was set to 0.7 mL/min, and the water aerosol concentration in the chamber was about 9 mg/L. Other parameters are as follows: carrier gas flow velocity 3.00 m^3/h, temperature 25 °C, and exposure time 6 h.

2.3. Bronchoalveolar lavage fluid (BALF) and cells counting

Three hours after inhalation exposure, mice were sacrificed by cervical dislocation. In our previous study, three-hour has been found to be a time point for a peak of cellular response induced by LPS. BALF was collected by cannulating the upper part of trachea using syringe in 2 mL PBS (pH7.4) containing 0.5% bovine serum albumin. 300 μL BALF was centrifuged at 800 rcf for 5 min at 4 °C, and resuspended in 1.5 mL dilution reagent (supplementary reagent of animal hematology analyzer, Mindray, China) for the concentration of white blood cells (WBC) analysis by animal hematology analyzer (Mindray, China). The rest BALF was also centrifuged, and resuspended again in 10 μL PBS containing 0.5% BSA for smear.

For smear, every 5 μL sample was spread on a slide (Shitai Ltd., China), dried in the hood, and then stained by Wright–Giemsa method. Briefly, the slides were soaked into methanol for 15 min and dried in the air. Then they were stained by Wright solution for 3 min, Wright/PBS (PBS pH 6.6, volume ratio 1:1) solution for another 3 min, rinsed thoroughly with PBS (pH 6.6). Next, the slides were stained by Giemsa solution for 10 min, rinsed thoroughly by deionized water. Finally, the slides were dried and mounted for microscopy (Leica, Germany). 300 to 600 white cells were counted for each sample to calculate the proportion of polymorphonuclear neutrophils (PMNs).

2.4. LAL assay

Standard Limulus Amebocyte Lysate assay kits were purchased from Xiamen Houshiji Ltd., China, and performed according to the manufacturer's instructions.

2.5. Chlorination of LPS

LPS (L2637, L2880) purchased from Sigma–Aldrich was extracted from *E. coli* serotype O55:B55, and dissolved in pyrogen-free water at high concentration, and stored at 4 °C for further use.

Synthetic LPS containing water was prepared by spiking LPS stock solution to make the concentration about 2000 EU/mL, and was adjusted to pH 7.0 by adding phosphate buffer. Chlorination was carried out by adding sodium hypochlorite stock solution (15 g/L as available chlorine) except for the chlorine-free control and concentration of available chlorine was detected by chlorine-Spectrophotometric method (HANNA, Italy) before use. At the end of the pre-determined reaction time, excessive sodium thiosulfate (2 times the maximal consumption of chlorine) was added to neutralize residual chlorine, the whole process was performed in the dark. The chlorine dosage ranged from 0 to 50 mg/L and the reaction time ranged from 5 to 240 min were selected. Then, the water samples were ready for LAL assay and inhalation exposure experiments. The chlorination and quenching process did not change the pH, which was maintained at 7.17 ± 0.02.

2.6. Scanning electron microscopy (SEM) and ^{1}H NMR spectroscopy

The concentrations of LPS in synthetic water samples were 0.5 mg/mL for SEM and 1 mg/mL for NMR spectroscopy. Two samples before and after chlorination (50 mg/L free chlorine, reaction time was 4 h for SEM and 15 min for NMR) were prepared and dialyzed to deionized water by 7KD dialysis cassette (Pierce Biotechnology, USA). After dialysis, the samples were freeze-dried for SEM (Hitachi, Japan). For NMR spectroscopy, 2 mg freeze-dried samples were dissolved in 0.5 mL D_2O, and performed in the Ana-

Download English Version:

https://daneshyari.com/en/article/575655

Download Persian Version:

https://daneshyari.com/article/575655

Daneshyari.com