Effects of MC-LR on inflammatory cytokines in human bronchial epithelial cells

Jinxia Wu, Shenshen Zhang, Chuanrui Liu, Haohao Liu, Chu Chu, Yang Li, Xuemin Cheng, Huizhen Zhang*

College of Public Health, Zhengzhou University, Zhengzhou, Henan, 450001, China
Huizhen18@126.com

Abstract: Microcystins-LR (MC-LR) produced by cyanobacteria is responsible for toxicity in humans and animals. To study the effect of MC-LR on the expression of inflammatory cytokines in human bronchial epithelial cells (HBE), we detected the levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) in culture supernatant of HBE cells exposed to 20 μg / ml MC-LR solution at 0 h, 6 h, 12 h, 24 h, 36 h and 48 h by ELISA kits. The results showed that the levels of TNF-α, IL-6 and IL-8 in the supernatant of HBE cells increased with the prolongation of MC-LR exposure ($p < 0.05$ vs Control group). Therefore, we deduced that direct expose to MC-LR could induce inflammation in HBE cells and lead to higher generation and release of inflammatory cytokines like TNF-α, IL-6 and IL-8.

[Wu JX, Zhang SS, Liu CR, Liu HH, Chu C, Li Y, Cheng XM, Zhang HZ. **Effects of MC-LR on inflammatory cytokines in human bronchial epithelial cells.** *Life Sci J* 2017;14(1):74-77]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 10. doi:10.7537/marslsj140117.10.

Keywords: Microcystin-LR; Human bronchial epithelial cells; inflammatory factor

1. Introduction

The frequency occurrence of red tide and cyanobacteria bloom increased as Chinese water eutrophication gradually intensified. 80% of cyanobacteria bloom can be detected secondary metabolites, microcystins (MCs), which has become a worldwide concern because of its harm to water environment and human health.

MCs, the most widely distributed hepatotoxin, are a class of biologically active cyclic heptapeptides, which are mainly produced by algae microcystis aeruginosa in freshwater ecosystems [1]. They possess considerable stability for their ring structures and spacer double bonds. MCs are cyclic heptapeptides with two variable amino acids, of which more than 100 kinds of different structural variants have been identified. The most toxic MC is microcystin-LR (MC-LR), which is considered to be the most commonly occurring, distributed and abundant [2-5]. Recent studies have suggested that Adda region plays an important role in the toxicity of MC-LR [6, 7]. Many people died every year after drinking water from lakes or eating seafood contaminated by MC-LR, therefore, the World Health Organization (WHO) has stipulated that the provisional safety guideline for MC-LR in drinking water was 1.0 μg/L [5].

Cyanobacterial toxins, released from the ruptured algae cells into the water, can be taken through different ways such as oral, pulmonary respiration, skin contact and so on, posing a threat to human health [1]. Tang baolian et al. demonstrated that cyanobacteria toxins may enter the body via the lungs due to water bubbles and the spray during entertainment, which would induce respiratory diseases [8].

Respiratory diseases like airway inflammation and airway remodeling have a common pathophysiological characteristic of a variety of lung diseases such as chronic obstructive pulmonary disease, bronchial asthma and so on, which are caused by the interaction between a variety of inflammatory cells and cytokines [9]. Macrophages play a crucial role in the pathophysiology processes, which can produce and release a variety of cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-1(IL-1). These cytokines are released from the macrophages when they are stimulated by irritants such as bacteria, smoke and other harmful components like formaldehyde, NO_2 and other oxidation products. In turn, these cytokines released from macrophages can promote neutrophils, T cells, eosinophils to migrate to lung tissues. These cells and inflammatory cytokines are the important factors of chronic airway inflammation and airway serious damage [9, 10]. Previous studies have shown that MC-LR can stimulate the release of inflammatory cytokines from alveolar macrophages, destruct mouse lung parenchyma, and cause interstitial pulmonary edema and inflammatory cells aggregation, bringing about damage to respiratory system, but the specific mechanism is unclear. In this study, we detect the levels of IL-8, IL-6 and TNF-α in the supernatant of the HBE cells treated with MC-LR to investigated the effects of MC-LR on HBE cells.

2. Materials and Methods

Cell source and reagents

HBE cells were donated by Professor Xiuli An (New York Blood Center). MC-LR (purity ⩾95%,

Beijing Yip Reese Technology Co., Ltd.). Fetal bovine serum (Hangzhou Sijiqing Biological Engineering Co., Ltd.). RPMI-1640 culture solution (Beijing Solaibao Technology Co., Ltd.). Preparation of MC-LR solution: MC-LR was accurately weighed and then dissolved in serum-free RPMI-1640 medium to prepare 20μg/mL MC-LR stock solution stored at -20 °C.

Cell culture

HBE cells are cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS), when the cells are approximately more than 80%, adjusting the cells at a density of 1 x 10^5 cells/ ml, and inoculate into 12-well cell culture plates, in CO_2 incubator at 37 °C for 24h.

Determination of cytokines

HBE cells were cultured with of 20 μg/mL of MC-LR for 0 h, 6 h, 12 h, 24 h, 36 h and 48 h respectively. Then the culture supernatants were collected to detect the levels of TNF-α, IL-6 and IL-8 respectively following the ELISA kit instructions.

Statistical analysis

SPSS 21.0 software was used for statistical analysis and the results were expressed as mean ± stand error (SE). All groups were compared with control group. Statistical significance was determined by Student's t-test. $P<0.05$ was regarded as statistically significant differences.

3. Results

Effects of MC-LR on TNF-α in HBE Cells

HEB cells were incubated with MC-LR for 0h, 6h, 12h, 24h, 36h, 48h respectively, then the contents of TNF-α in the culture supernatant were measured by ELISA kits according to the manufacturer's instructions. As shown in table 1 and Fig.1, TNF-α was gradually increased with the prolongation of MC-LR exposure, and the difference was statistically significant at 36h and 48h ($P<0.05$).

Table 1. Levels of TNF-a in the supernatant of HBE cells (pg / ml)

Exposure time (h)	Number of samples(n)	OD value	TNF-α concentration (pg/ml)
0	3	0.087±0.008	7.020±1.303
6	3	0.094±0.006	7.989±1.262
12	3	0.107±0.005	10.637±1.040
24	3	0.116±0.007	12.628±1.502
36	3	0.133±0.009*	16.257±1.989*
48	3	0.174±0.013*	25.415±2.432*

Effects of MC-LR on IL-6 production in HBE cells

IL-6 in the culture supernatant was measured using ELISA kits according to the manufacturer's instructions. As shown in table2 and Fig.2, the concentration of IL-6 increased from 11.530 ± 0.038 pg / ml to 16.401 ± 0.939 pg / ml, and the difference was statistically significant at 24 h, 36 h and 48 h ($P<0.05$).

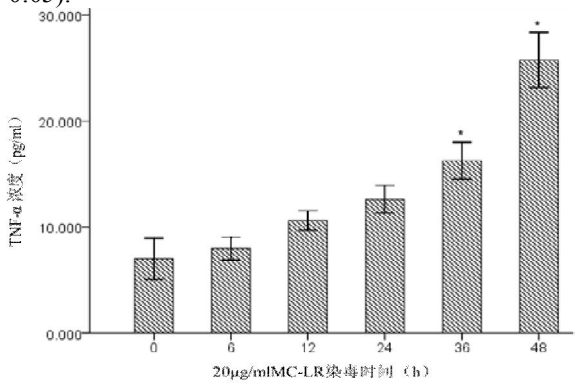

Fig.1 Contents of TNF-α in supernatant of HBE cells treated with 20μg/ml MC-LR for different time point. Asterisk (*) indicates that compared with the control group, the difference was statistically significant (P <0.05).

Table 2. Levels of IL-6 in the supernatant of HBE cells (pg/ ml)

Exposure time(h)	Number of samples(n)	OD value	IL-6 concentration(pg/ml)
0	3	0.078±0.001	11.530±0.038
6	3	0.080±0.002	11.706±0.153
12	3	0.897±0.002	12.332±0.142
24	3	0.105±0.002*	13.401±0.123*
36	3	0.126±0.012*	15.003±0.881*
48	3	0.144±0.012*	16.401±0.939*

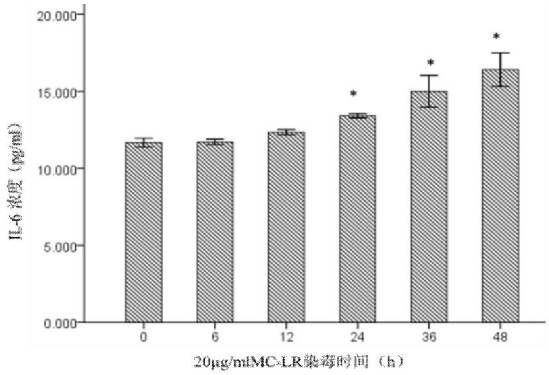

Fig. 2 Levels of IL-6 in the supernatant of HBE cells (pg / ml). Asterisk (*) indicates that compared with the control group, the difference was statistically significant (P <0.05).

Effects of MC-LR on IL-8 in HBE Cells

After incubating with MC-LR for 0h, 6h, 12h, 24h, 36h and 48h, the contents of IL-8 in culture supernatant of HBE cells were measured using ELISA kits. As shown in table 3 and Fig.3, we observed that

the levels of IL-8 was 3.92 times higher than that of control group at 48h after exposure to 20 μg/mL MC-LR, and the difference was significant at 12h, 24h, 36h and 48h (P <0.05).

Table 3. Levels of IL-8 in the supernatant of HBE cells (pg / ml)

Exposure time(h)	Number of samples(n)	OD value	IL-8 concentration (pg/ml)
0	3	0.368±0.006	67.793±1.917
6	3	0.388±0.006	74.973±2.101
12	3	0.472±0.010*	108.543±4.348*
24	3	0.534±0.009*	137.724±4.243*
36	3	0.644±0.002*	197.745±1.035*
48	3	0.749±0.024*	265.797±16.782*

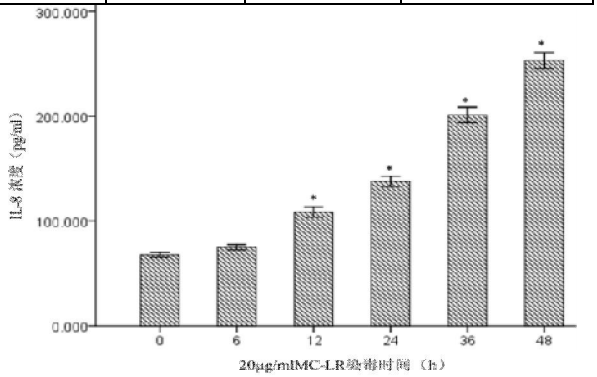

Fig.3 The levels of IL-8 in the supernatant of HBE cells (pg / ml). Asterisk (*) indicates that compared with the control group, the difference was statistically significant, P <0.05

4. Discussion

The incidence of respiratory diseases has increased year by year with the increasingly serious environmental pollution. The airway injury is not only related to the proliferation of smooth muscle cells and the infiltration of inflammatory cells, but also to the damage of airway epithelium cells, which is one of the important factors of airway injury [11]. Therefore, this study aims to investigate the effect of MC-LR on the expression of inflammatory cytokines in human bronchial epithelial cells (HBE) to provide a scientific basis for the prevention and control of respiratory diseases.

TNF-α, one of the major inflammatory cytokines after various stimuli, is mainly secreted by macrophages and can induce the activation of pulmonary endothelial cells, migration of leukocyte, degranulation of granulocyte and the leakage of capillary, which can reflect the body's inflammatory state and determine the degree of severity of lung injury [12, 13]. The accumulation of edema fluid further blocked the alveolar cell perfusion and oxygen exchange, which led to the acute lung injury [14]. In this experiment, TNF-α was increased in the culture supernatant of HBE cells with the prolongation of MC-LR exposure, suggesting that the damage of airway epithelial gradually increased.

The inflammatory mediators IL-6 is a multi-functional inflammatory cytokine who acts as a cytoprotective and anti-inflammatory agent in bacterial endotoxin-induced experimental lung injury by inhibiting production of IL-1 and TNF in macrophagethe [15]. In inflammatory response, IL-6 can also work together with other cytokines to stimulate fibroblast proliferation, promote collagen deposition, inhibit extracellular matrix decomposition and involve in the remodel of the chronic obstructive pulmonary disease airway [16]. IL-6 also has context-dependent anti- and pro-inflammatory effects and too much of it can cause tissue damage. In our research, IL-6 was gradually increased with the prolongation of MC-LR exposure, causing the occurrence of inflammation and leading to lung injury.

IL-8 belongs to the family of chemokines which is mainly produced by macrophages and other cell types like epithelial cells, airway smooth muscle cells and endothelial cells. Previous study found that IL-8 levels were closely related to the occurrence and progression chronic obstructive pulmonary disease [17]. Some study has pointed out that the main pathophysiological basis of chronic obstructive pulmonary disease is pulmonary vascular, pulmonary parenchyma and the inflammatory response in the course of the airway disease, therefore the number of neutrophils, T lymphocytes and alveolar macrophages in different locations in the lungs increased, which led to the acute exacerbation of chronic obstructive pulmonary disease due to the coexist of non-specific inflammation and acute inflammation caused by pulmonary infection. This led to the activation of inflammatory cells and the release of a variety of media such as interleukin IL-8 and so on [18]. IL-8 is implemented by the activation and chemotaxis of neutrophils being the main endogenous chemokines in the body [18]. Therefore, IL-8 can promote neutrophil-associated inflammation, so that a large number of T lymphocytes and neutronphils infiltrate, proliferate and aggregate in patient' s respiratory tract mucous membrane. [19]. In present study, IL-8 increased with the increase of exposure time to MC-LR in HBE cells, indicating that MC-LR may cause lung disease via inducing the cytokine IL-8 production.

In this study, the levels of TNF-α, IL-8 and IL-6 in supernatants of HBE cells increased with the increase of exposure time to MC-LR(20 μg/ml), suggesting that MC-LR have a time-dependent effect on the cytokines production in HBE cells. The contents of TNF-α, IL-8 and IL-6 increased coupling with the increase of secretion in airway inflammatory substances, which could result in airway obstruction

[13]. Therefore, MC-LR stimulation is one of the important factors to the respiratory disease. In conclusion, MC-LR can induce the production of inflammatory cytokines in HBE cells such as TNF-α, IL-6 and IL-8, causing respiratory diseases.

Acknowledgements:

This study was supported by the National Natural Science Foundation of China (Grant No.81472948) and the Scientific and Technological Project of Henan Province (Grant No.142102310344).

Corresponding Author:
Dr. Zhang Huizhen
College of Public Health, Zhengzhou University,
Zhengzhou, Henan, 450001, China
E-mail: huizhen18@126.com

References

1. Xue L, Li J, Li Y, Chu C, Xie G, Qin J, Yang M, Zhuang D, Cui L, Zhang H, Fu X. N-acetylcysteine protects Chinese Hamster ovary cells from oxidative injury and apoptosis induced by microcystin-LR[J]. Int J Clin Exp Med, 2015, 8(4): 4911-4921.
2. Ma J, Feng Y, Liu Y, Li X. PUMA and survivin are involved in the apoptosis of HepG2 cells induced by microcystin-LR via mitochondria-mediated pathway [J]. Chemosphere, 2016, 157: 241-249.
3. Lone Y, Bhide M, Koiri R K. Microcystin-LR Induced Immunotoxicity in Mammals [J]. J Toxicol, 2016, 2016: 8048125.
4. Huang X, Chen L, Liu W, Qiao Q, Wu K, Wen J, Huang C, Tang R, Zhang, X. Involvement of oxidative stress and cytoskeletal disruption in microcystin-induced apoptosis in CIK cells [J]. Aquat Toxicol, 2015, 165: 41-50.
5. Liu W, Chen C, Chen L, Jin J, Kawan A, Zhang, X. Sex-dependent effects of microcystin-LR on hypothalamic-pituitary-gonad axis and gametogenesis of adult zebrafish[J]. Sci Rep, 2016, 6: 22819.
6. Zhou M, Tu W W, Xu J. Mechanisms of microcystin-LR-induced cytoskeletal disruption in animal cells[J]. Toxicon, 2015, 101: 92-100.
7. Svircev Z, Baltic V, Gantar M, Jukovic M, Stojanovic D, Baltic M. Molecular aspects of microcystin-induced hepatotoxicity and hepatocarcinogenesis[J]. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 2010, 28(1): 39-59.
8. Liu J, Sun Y. The role of PP2A-associated proteins and signal pathways in microcystin-LR toxicity [J]. Toxicol Lett, 2015, 236(1): 1-7.
9. Giannuzzi L, Sedan D, Echenique R, Andrinolo, D. An acute case of intoxication with cyanobacteria and cyanotoxins in recreational water in Salto Grande Dam, Argentina [J]. Mar Drugs,2011,9(11):2164-2175.
10. Emami Ardestani M, Zaerin O. Role of Serum Interleukin 6, Albumin and C-Reactive Protein in COPD Patients[J]. Tanaffos, 2015, 14(2): 134-140.
11. Crisafulli E, Menendez R, Huerta A, Martinez R, Montull B, Clini E, Torres A. Systemic inflammatory pattern of patients with community-acquired pneumonia with and without COPD[J]. Chest, 2013, 143(4): 1009-1017.
12. Hu J, Ma P, Chen Y, Xu C. Preliminary study on establishing inflammation model of human bronchial epithelial cells in vitro [J]. Hunan J Tradit Chin Med, 2016, (10): 170-173.
13. Zhao F, Wang W, Fang Y, Li X, Shen L, Cao T, Zhu H. Molecular mechanism of sustained inflation in acute respiratory distress syndrome[J]. J Trauma Acute Care Surg, 2012, 73(5): 1106-1113.
14. Zhang W, Wang H, Shao X. The level of TNF -α and GM-CSF in Patients with Acute Lung Injury Serum and significance [J]. Guangdong Medical Journal, 2013, (18): 2834-2835.
15. Lin L, Zhang H, He S. Advance in the research of correlation of IL-6 and its receptors with inflammation diseases [J]. China Tropical Medicine, 2008, (04): 680-682.
16. Lu J J, Zheng Y, Yi Y, Guo Z, Ren T. Relationship between interleukin-6, interleukin-17 and chronic obstructive pulmonary disease[J]. Journal of Clinical Pulmonary Medicine, 2010, (07): 940-941.
17. Gao J, Zhan B. The effects of Ang-1, IL-8 and TGF-beta1 on the pathogenesis of COPD[J]. Mol Med Rep, 2012, 6(5): 1155-1159.
18. Haczku A. The dendritic cell niche in chronic obstructive pulmonary disease[J]. Respir Res, 2012, 13: 80.
19. Chiba T T, Ueki S, Ito W, kato H, Takeda M, et al. 15-Deoxy-Delta(12,14)-prostaglandin J2 induces IL-8 and GM-CSF in a human airway epithelial cell line (NCI-H292). Int Arch Allergy Immunol, 2009,149 (09):77-82.

1/25/2017