Toxic effects of microcystins on the respiratory system

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Abstract: Microcystins (MCs) are produced by cyanobacteria in natural environments, which have more than 90 different congeners. One of the most toxic and thoroughly studied microcystins is microcystin-LR (MC-LR), which can increase the risk of hepatotoxicity, neurotoxicity, reproduction toxicity, cytotoxicity and respiratory diseases by accumulating in the body. This review focuses on respiratory toxicity of microcystins. Pulmonary injury after acute or sub-chronic exposure to different doses of microcystins are summarized. However, further studies are needed to clarify the signaling pathways of respiratory toxicity of microcystins.
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Cyanobacteria are widely existed in the waters around the world, some species of cyanobacteria can produce toxic secondary metabolites microcystins, which more than 90 different congeners have been identified until today. Microcystin-LR(MC-LR) is one of the most frequent and toxic,and hence the most studied variant[1]. The study on the toxicity of MCs involves macrophytes, plankton, fish and animal etc[2-5]. MC-LR is a group of hepatotoxic cyclic peptides, which will elicit hepatotoxic potency via inhibition of hepatic protein phosphatases (PP) 1 and 2A[6]. It leads to over-phosphorylation of vital cellular proteins, cytoskeletal disorder, microfilament decomposition, plasmatorrhexis and even causing hepatic hemorrhage and metabolic disorders in mammals. Long term exposure to low dose MCs can promote the occurrence of skin, colon and liver cancer[7,8].

In 1996, the patients of Brazil occured death during routine haemodialysis treatment. And the major contributing factor was intravenous exposure to microcystins which existed in clinic's water treatment system. For the first time that human deaths associated with MCs[9]. In November 2001, a cyanobacterial bloom occurred in the Funil Reservoir, Brazil. People get in touch with microcystins through drinking water, and serum microcystin concentrations of the hemodialysis patients has the highest values detected one month after initial exposure, which is not completely removed two months later[10]. It was speculated that the MCs had a strong accumulation effect and a longer metabolic period. Water bubbles and waves in the process of entertainment containing MCs into the body through the lungs, which may produce respiratory diseases including shortness of breath, cyanosis, suffocation and even death[11]. The study of MCs's respiratory system toxicity is very important, this paper reviews the toxic effects of MCs in the respiratory system, and puts forward the research direction of our country in this field.

1.Cohort study

It is reported that the British soldiers exposured to toxic cyanobacteria when they training in the Rudyand lake, with the emergence of facial dermatitis, mouth sores, asthma and other symptoms[7]. People in the bath, swimming and other water activities, the skin directly exposed to algae toxins can cause sensitive parts of the body (such as the eye) and skin allergies; The toxic cyanobacteria in water entertainment activities can also generate atomized microcystins into the respiratory tract, which is the main pathway leading to disease of respiratory system[12]. Pilotto reported that participants exposed to cyanobacteria more than 5000 cells/ml for more than one hour had a significant trend to increasing symptoms. The symptom occurrence rate within seven days is significantly higher than the unexposed[13]. In the lake with a high(cell surface area >12.0 mm2/mL) concentration of cyanobacteria, the probability of respiratory symptoms is 2.1 times than those exposed to low levels (cell surface area <2.4 mm^2/mL) of cyanobacteria[14]. In order to research recreational exposure to low concentrations of microcystins in a small lake, Backer recruited 104

people planning recreational activities in that lake or in a nearby bloom-free lake. The results demonstrate low levels of microcystins (2 μg/L to 5 μg/L) in the water and (<0.1 ng/m3) in the aerosol samples. This is the first study to report that the water nature of recreational activities can make people exposed to very low concentrations microcystins via the aerosol[15]. Therefore, microcystins in the pasted water bloom of lakes should be attracted more and more attention.

2.Animal experiment

With the increase of respiratory system disease cases reported by MCs, a study on the upsurge of respiratory toxicity of MCs has been made. The respiratory system toxicity of MCs was mostly used in animal experiments. The toxicant exposures were mainly treated with intraperitoneal injection, intravenous injection, tracheal injection and aerosol inhalation.

2.1 intraperitoneal injection In test groups, Swiss mice were received 40μg MC-LR/kg via intraperitoneal injection. After 2 and 8 h, and 1, 2 and 4 days after toxin injection, the mice were randomly selected from per group for analyses. The results showed that the mice in the exposed group appeared resistive and viscoelastic pressures, static and dynamic elastances after received MC-LR 2 h to 4 days. After exposure to 2h, alveolar atrophy and inflammatory cell infiltration appeared, reaching peak values at 8 h. However, it could not be detected microcystin or inhibition of phosphatases in mice lungs[16]. The young (4 weeks old) mice and adult (12 weeks old) mice were received intraperitoneal injection of MC-LR with sub lethal dose of 48.2μg/kg. The number of polymorphonuclear cell and the percentage of alveolar collapse increased with the exposure times. And the inflammatory response in young mice reached the highest level was earlier than adult mice[17]. It is speculated that the intraperitoneal injection of cyanobacteria toxin and its derivatives can exert toxic effects through the blood to the lungs. Yongding Liu firstly shows the presence of Anabaena species in Lake Dianch and its crude extracts would induce visible symptoms of toxicity. After the mice injected with crude extracts, severe lesions were observed in the livers, kidneys, and lungs. Significant alterations were found in the serum biochemical parameters and the histological lesions were in the same manner[18]. Cyanobacteria strains isolated and cultured from rocky beaches along the Portuguese coast would induce pulmonary interstitial edema, hemorrhage and necrosis[19]. Nakano et al[20] presented that MC-LR and M. aeruginosa were injected into the mice by intraperitoneal injection, and the inflammatory mediators were observed in peritoneal macrophages. Such as TNF-α and IL-1. IL-1 is an important substance to stimulate the migration of neutrophils to the lung parenchyma[21], indicating that microcystin through altering the expression of the inflammatory factors in the lung can play a role in lung toxicity.Some studies have reported that LASSBio 596 was more efficient than dexamethasone in treating the pulmonary injury induced by MC-LR[22], however, the cure effect is not ideal, and the experimental results can not be directly extrapolated to human.

2.2 intravenous injection Ping Xie put forwarded that Wistar rats were injected intravenously with MCs at a dose of 80 mg MC-LR/kg body weight. After 1, 2, 4, 6, 12 and 24 h, the distribution and concentration of MC_S in different tissues were determined by liquid phase chromatography and spectrum determination and the renal accumulation of MC_S highest concentration (0.034-0.295 μ g /g dry weight), followed by lung (0.007-0.067 μ g/g dry weight)[23], indicating that MC_S had a major toxic effect on kidney and lung.

2.3 intratracheal injection The lungs were easily absorbed of MC-LR by the intratracheal injection. It was confirmed that the lethality dose level by intratracheal injection was the same as by intraperitoneal injection. An immunostaining method showed that MC-LR was increased in mice after 1h of injection and could cause liver hemorrhage. It took 2 weeks for the cells to completely remove MC-LR, but the MC-LR can still be detected after 2 weeks of the epithelial cells in the gastrointestinal tract[24]. The excretion of MC-LR by intratracheal injection into the body was slowly and the lung injury is not clear.

2.4 aerosol injection Male BALB/c mice were injected by nose-only inhalation to 260-265 μg microcystin/m^3 for 7 days. The necrosis, atrophy and neutrophil inflammation of the respiratory epithelium were observed, and two protein peaks related to MC-LR were detected in serum[25]. The protein peaks may be useful as biomarkers of microcystin exposure in organism.

3.Cell assay

The study on the respiratory toxicity of cyanobacteria toxins in vitro is very little. Studies have shown that MC-LR stimulated alveolar macrophages to produce inflammatory mediators, such as prostaglandin F2 and PGE2 and

thromboxane B2 and arachidonic acid[26]. It indicated that inflammation is involved in the process of lung injury induced by microcystin. It induced apoptosis of lung and bronchial epithelial cells, and thus induced asthma, respiratory inflammation and other diseases.

4.Summary and Prospect

The chemical properties of the microcapsules are stable with high heat resistance and can be in good condition for several hours in boiling water. The dried MCs can be stored for several years at room temperature[27]. Microcystin is soluble in methanol or acetone, easy to dissolve in water (water solubility of up to 1g/L or above), and conventional drinking water treatment processes such as coagulation, sedimentation, filtration and chlorination can not effectively remove MC_S[28]. The unique physical and chemical properties of MCs lead to the transfer of the food chain and the enrichment of the food chain. MCs is widespread in drinking water and tap water, which can be through drinking water, food, entertainment and health and other ways to enter the human body, and has become one of the main hazards of drinking water safety[29,30]. MCs generates a large amount of oxygen free radicals by oxidative stress reaction, which regulates the expression of related genes and the activity of related proteins and induces apoptosis, and respiratory system damage[29].

Studies have shown that inflammation is also involved in MCs induced lung disease[22,23,28]. Much progress has been made in the study of MCs toxicology, but there are still some problems: (1) MC_S with different exposure routes lead to differences in lung tissue injury. We should try to simulate the human body may be exposed to toxins and select a more representative of the lung cells for the study. Under the condition, the lung injury was observed in order to determine no observed effect concentration; (2) The mechanism of absorption, distribution, metabolism and transport pathways of MCs in the lung is also studied in detail; (3) The role of inflammation and apoptosis in the mechanism of lung injury induced by MCs is not clear, and the leading role of inflammation and apoptosis is not clear; (4) The mechanism of MCs induced pulmonary inflammation needs to be further confirmed at the molecular level. The analysis of the content of pro-inflammatory cytokines and anti inflammatory factors and the pathway of inflammatory reaction can be used as the focus of future research; (5) We should further explore the combined effects of MCs and other substances on lung tissue. That avoiding the enhancement factors and finding the antagonistic factors will be propitious to find possible ways and means for the diagnosis and treatment of MCs.

In summary, the study of respiratory system damage in MC-LR needs to be carried out in vivo and in vitro experiments, molecular and protein levels, apoptosis and inflammation levels, so as to provide clues and theoretical basis for the detection, prevention and treatment of toxic damage. The ultimate goal is to promote human health and achieve harmonious development of human and environment.

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