The cell cycle arrest induced by MC-LR in Chinese hamster ovary cells

Yang Li [1], Mingfeng Yang [2], Lijian Xue [1], Guantao Xie [1], Jin Qin [1], Jianxin Liu [1], Liuxin Cui [1], Huizhen Zhang *

[1.] Zhengzhou University, College of Public Health, Zhengzhou, Henan 450001, China
[2.] Chinese medicine hospital of Shouguang, Medical Department, Shandong, 262700, China
*Corresponding Author: huizhen18@126.com

Abstract: In order to explore the possible toxicology mechanism of MC-LR, Chinese hamster ovary (CHO) cells were cultured in vitro to determine the effect of Microcystin-LR (MC-LR) on cell cycle. After treated with MC-LR(0, 2.5, 5, 10μg/ml) for 24h and 48h, the cell cycle distribution profiles were determined by propidium iodide (PI) combined with flow cytometer. The results of fluorescent staining showed that after exposed to MC-LR for 24h, the percentage of CHO cells in G2/M phase accumulated from 3.11% to 15.67%. After exposed to MC-LR for 48h, the percentage of CHO cells in G2/M phase accumulated from 4.9% to 22.24%. CHO cells in G2/M phase accumulated significantly (P<0.05) in the groups of MC-LR compared with the control group (0μg/ml). There was a concentration dependent increase of cells in G2/M phase with a concomitant decrease of the cells in S and G1 phase. In short, MC-LR induced cells cycle arrest in G2/M phase. These results suggest that MC-LR could block the cell mitosis, which may be associated with disorders of apoptosis-related proteins.

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Keywords: Microcystin-LR; cell cycle; Chinese hamster ovary cell

1. Introduction

Nowadays, with the increase of water eutrophication, the reports about occurrences of toxic cyanobacteria called water bloom are increasing all over the world [1]. Microcystins (MCs) produced by several cyanobacterial genera are a family of cyclic peptides. Over 80 variants of the family have been identified [2]. The most abundant ones are MC-LR, MC-RR, MC-YR (L, R, Y represents for leucine, arginine, and tyrosine respectively). MC-LR is the most toxic variant [3], comprising 46-99.8% of the total MCs in natural waters [4, 5].

MCs seriously threaten to both animals and humans health, and causing ecological unbalances and contamination of the environment [6,7]. MCs can be accumulated in a variety of organisms including snails, vivipara and fishes etc. in the rivers or lakes of eutrophication [2]. MCs can enter into the other organisms through food chain or drinking water [8]. In China, epidemiological survey found that the incidences of liver cancer and colorectal cancer significantly increased in residents who drank the water containing MCs for a long time [9]. Recently, in June 2006, on behalf of the WHO International Agency for Research on Cancer (IARC), a working group concluded that MC-LR is ‘‘possibly carcinogenic to humans’’ (group 2B) [10]. Studies on the accumulation of MCs in vivo showed average accumulative magnitude in the digestive tract is the greatest, and gonads rank is the second [11, 12]. Chronic low-concentration treatment with MC-LR resulted in substantial toxicity to male reproduction, causing declines in sperm quality and injury to the testis [13]. Studies have found that MCs could be abounded in eggs and pass on to the offspring, and have estrogen-like effects on fish and mammalian cells [14,15]. Therefore, the study about the toxic effects of MCs on the female reproductive system is of great significance. But so far, in the present experiment cytotoxicity mechanism of MC-LR is not intended to illustrate. In this study, CHO cells were exposed to different concentration of MC-LR for 24h and 48h, respectively. The effect of MC-LR on cell growth was observed to further investigate the toxicity mechanisms of MC-LR in CHO cells.

2. Material and Methods

Chemicals

The chemical MC-LR with purity ≥95% was obtained from Beijing Express Technology Co.,Ltd. RPMI-1640 medium and Trypsin were provided by Beijing Solarbio Science & Technology Co.,Ltd. Cell Cycle and Apoptosis Analysis Kit was supplied by Beyotime Institute of Biotechnology. Other reagents were of analytical grade.

Cell culture

CHO cells were cultured in RPMI-1640 medium containing 10% fetal calf serum. When 80% of confluence was reached, cells were passaged. The culture solution was aspirated, cells were collected, washed with D-Hanks, added to 1mL 0.25% trypsin-EDTA for digesting 1~2min. Then RPMI-1640 medium supplemented with serum was added to

suspend the digestion. Cells were counted with trypan blue staining, and then the cell concentrations were adjusted to 1×10^5 cells/ml. The suspensions were seeded in 6-well plates, retained 200 μL each well, and setted up three parallel samples each dose. The medium was further cultured at 37 ℃ in a humidified incubator with 5% CO_2.

Determination the cell cycle distribution profiles

After treated with 0, 2.5, 5, 10 μg/ml MC-LR for 24h and 48h, cells were harvested and fixed with ice-cold 70% ethanol, treated with 500 μg/mL RNase A, and stained with 25μg/ml PI. Cell-cycle distribution profiles were measured by flow cytometry (Accuri Cytometers, American). The proportion of G0/G1, S and G2/M phase of cell cycle in CHO cells using cell cycle analysis software (MODFIT 4.0).

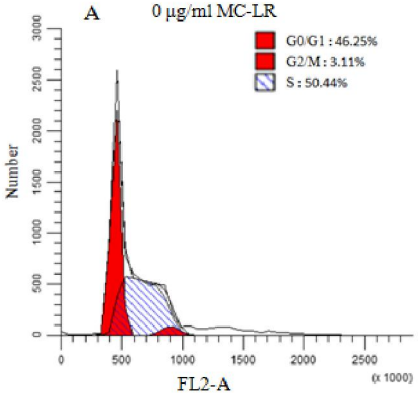
Data analysis

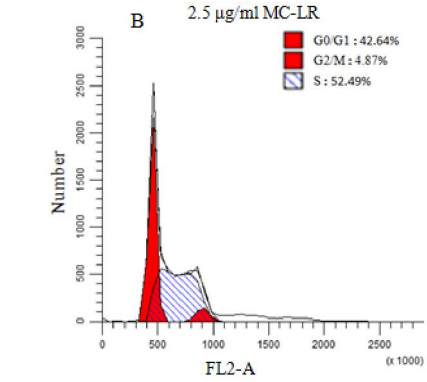
At least three independent experiments were conducted for all analyses. Values are expressed as means ± standard deviation. Student's t-test and Tukey's multiple comparison tests were used to estimate statistical significance, and $P<0.05$ was considered statistically significant.

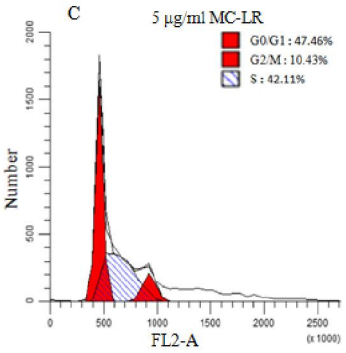
3. Results

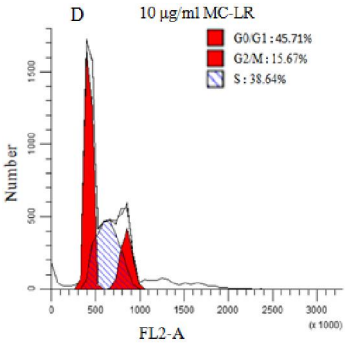
The cell cycle distribution profiles of CHO cells treated with MC-LR for 24 h

When the CHO cells were exposed to 0μg/ml MC-LR for 24 h, the percentage of CHO cells in G0/G1, G2/M and S phase was 46.25%, 3.11% and 50.44% (Figure 1A). As shown in Figure 1B, the percentage of CHO cells in G0/G1, G2/M and S phase was 42.64%, 4.87% and 52.49% in 2.5μg/ml group. The percentage of CHO cells in G2/M phase accumulated from 10.43% in 5μg/ml group (Figure 2C) to 15.67% in 10μg/ml (Figure 2D). MC-LR significantly increased the percentage of CHO cells in G2/M phase, and reduced the percentage of cells in G1 and S-phase. There was a concentration dependent increase of cells in G2/M phase with a concomitant decrease of the cells in S and G1 phase(figure1E).









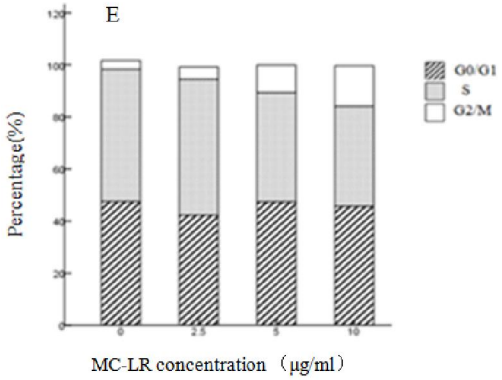
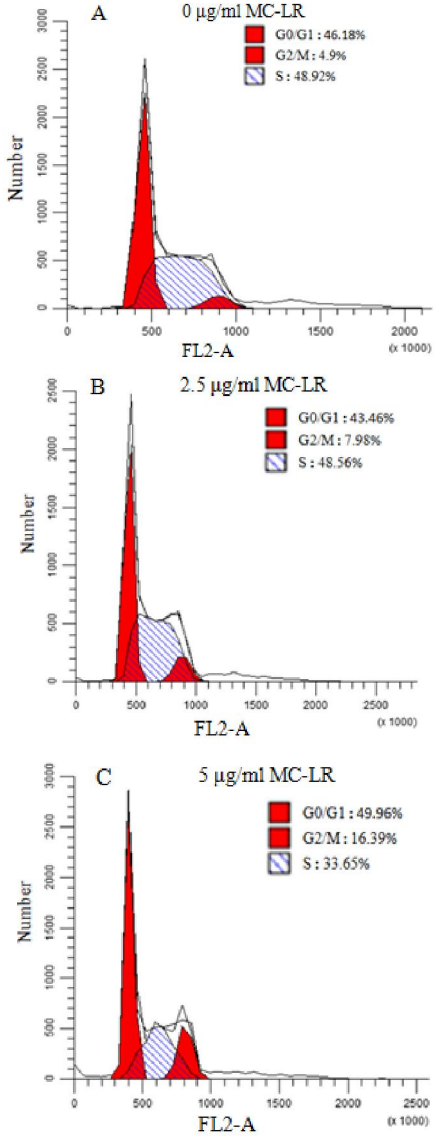


Figure 1 MC-LR induces G2/M phase cell cycle arrest in CHO cells after exposure for 24 h. A, B, C and D showed the cell cycle distribution profiles cells were treated with 0, 2.5, 5, 10 μg/ml MC-LR(FL2-A represents the intensity of PI, and the Y-axis represents the cell counts). E is histograms based on the results of fluorescent dye (Y-axis represents the percentage of CHO cells).

The cell cycle distribution profiles of CHO cells treated with MC-LR for 48 h

Figure 2 showed that the cell cycle distribution profiles after the CHO cells were exposed to MC-LR (0, 2.5, 5, 10 μg/ml) for 48h. The percentage of CHO cells in G0/G1, G2/M and S phase was 46.18%, 4.9%, 48.92% in control group (Figure 2A). The percentage of CHO cells in G0/G1, G2/M and S phase was 43.46%, 7.98%, 48.56% in 2.5μg/ml group (Figure 2B). The percentage of CHO cells in G0/G1, G2/M and S phase in 5μg/ml group was 49.96%, 16.39%, 33.65% (Figure 2C). And 48.24%, 22.24%, 28.52% was the percentage of CHO cells in G0/G1, G2/M and S phase in 10μg/ml group (Figure 2D). There was a decrease in the percentage of CHO cells in the G1 phase and a significant increase in the percentage in the G2/M phase. CHO cells in G2/M phase accumulated with the increase of MC-LR concentration (Figure 2E).



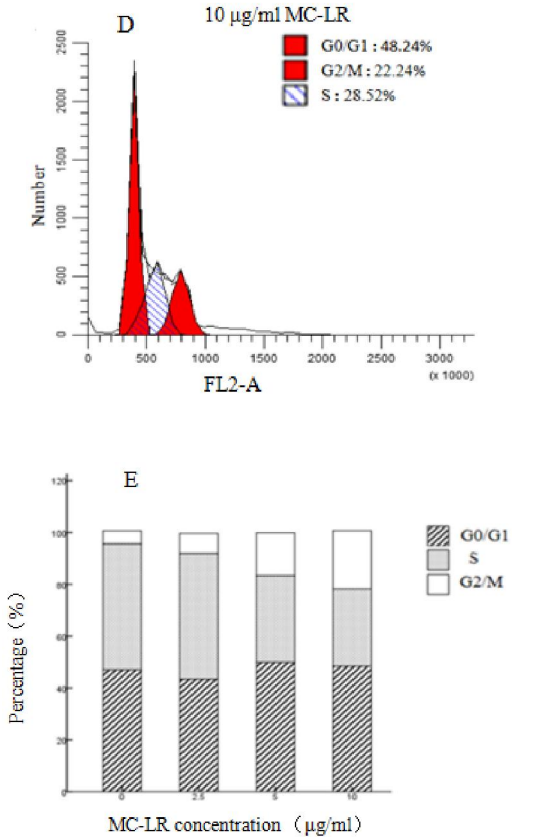


Figure 2 MC-LR induces G2/M phase cell cycle arrest in CHO cells after exposure for 48 h. A, B, C and D showed the cell cycle distribution profiles cells were treated with 0, 2.5, 5, 10 μg/ml MC-LR(FL2-A represents the intensity of PI, and the Y-axis represents the cell counts). E is histograms based on the results of fluorescent dye (Y-axis represents the percentage of CHO cells).

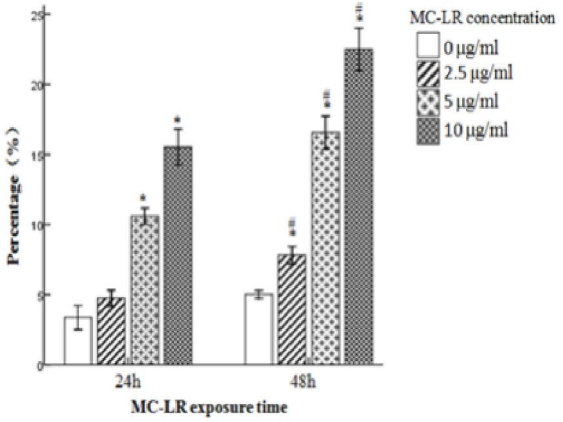


Figure 3 MC-LR induces G2/M phase cell cycle arrest in CHO cells after exposure for 24h and 48h. *represents $p<0.05$ when compared with control; # represents $p<0.05$ when G2/M phase of CHO cells treated with MC-LR for 48h compared with cells in the same concentration of MC-LR for 24h.

MC-LR induced G2/M phase arrest in CHO cells

Based on the results above, the cells were arrest in G2/M phase, the comparison of the G2/M phase after exposed to MC-LR for 24 h and 48 h was shown in figure 3. The percentage of CHO cells in G2/M phase accumulated significantly (P<0.05) in the groups of MC-LR (2.5, 5, 10μg/ml) compared with the control group (0 μg/ml). When exposed to MC-LR a certain time, cells in G2/M phase inceased with the increase of MC-LR concentration. When exposed to a certain concentration of MC-LR, CHO cells in G2/M phase increased steadily in a time-dependent manner.

4. Discussions

Cell proliferation was completed under the action of a series of proliferation signal in vitro or in vivo by regulating the cell cycle. Cell cycle can be divided into four phases based on the cell cycle of DNA synthesis and cell division, the phase of DNA synthesis (S phase), mitotic phase (M phase) and two interval (G1 phase is DNA synthetic prophase, and G2 phase is the late DNA synthesis). Cell growth is dependent on the normal cell cycle, which has two key points, one is the G1/S transition period which regulate DNA replication; another is located in G2/M phase of mitosis and cell differentiation [16]. This study found that G2/M phase of CHO cells can significantly increased after cells were exposed to 2.5, 5, 10 μg/ml MC-LR for 24 h and 48 h. So MC-LR has effect on the mitosis and cell differentiation in CHO cells.

Many studies indicated that the cell cycle regulated by the cyclin, cyclin-dependent kinase inhibitor (CKIs), cyclin-dependent kinase (CDKs) and phosphorylation/dephosphorylation of kinases and phosphatases protein [17]. MC-LR is a strong protein phosphatase inhibitors and can inhibit the vitality of serine/threonine protein phosphatase2A (PP2A) and protein phosphatase1 (PP1), and also can break the balance of intracellular phosphorylation/dephosphorylation protein to exert toxic effects [18, 19]. Thus, we speculated MC-LR may change cell cycle regulation by protein phosphorylation levels, causing cell cycle arrest. Previous studies of our team also have proved that P53 and Bax were up-regulated, and Bcl-2 was down-regulated in CHO cells after MC-LR treatment. The relationships between cell cycle and apoptosis-regulated proteins need further research.

In conclusion, our study showed that MC-LR induced cells cycle arrest in G2/M phase, suggesting that MC-LR could block the cell mitosis and then induce apoptosis of CHO cells, which may be associated with disorders of apoptosis-related proteins.

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

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