Genotoxicity of testicle cell of mice induced by microcystin-LR☆

Ling Dong, Huizhen Zhang, Liju Duan, Xuemin Cheng, Liuxin Cui*

Department of Environmental Health, College of Public Health, Zhengzhou University, Zhengzhou, Henan 450001*, China*

Received December 10, 2007

Abstract

Objective. To explore potential damage effect of microcystin-LR (MC-LR) on mice genetic material of cells. *Methods.* Male KM mice were treated with different doses of MC-LR by peritoneal injection for 7 days. On the eighth day, the quantity of protein-linked DNA and free DNA in mice testicle cell were detected, the DNA-protein crosslinks (DPC) coefficient was calculated and the degree of DNA and protein crosslinks were evaluated. On the fifteenth day, the micronucleus rate in early stage sperm cells was measured. *Results.* MC-LR of low dose could not increase DPC significantly. DPC formation significantly increased when the dose of MC-LR were elevated to 6 μg/kg·bw and 12 μg/kg·bw. The DPC coefficient reached to the highest by 6 μg/kg·bw MC-LR. The 3 μg/kg·bw MC-LR could not induce the increase of micronucleus number of the early stage sperm cells; but the 6 μg/kg·bw and 12 μg/kg·bw MC-LR could induce the increase of micronucleus number of the early stage sperm cells. *Conclusions.* The 6 μg/kg·bw and 12 μg/kg·bw MC-LR increased the DPC formation in mice testicle cell, and MC-LR also damage the chromosome in early stage sperm cell. [Life Science Journal. 2008; 5(1): 43 – 45] (ISSN: 1097 – 8135).

Keywords: microcystin-LR; DNA-protein crosslinks; micronucleus; genotoxicity

1 Introduction

Blue-green algae are found in lakes, ponds, rivers and brackish waters throughout the world. In case of excessive growth such as bloom formation, these bacteria produce inherent toxins in quantities causing toxicity in mammals, including humans. These toxins include microcystins, which are the most widespread and the most harmful toxins. One of the most commonly found microcystin variants in aquatic systems is microcystin-LR (MC-LR)[1].

Liver is the most important target organ of microcystins[2]. Microcystins also harm heart, kidney, neural system, gastrointestine and geneticity[3]. Some studies have indicated that microcystins are accumulate in the gonad of the invertebrate, and the gonad was regarded as the second target organ of microcystins. Microcystins is genetoxic to the invertebrate[4]. Animal experiment has indicated the toxicity effect of microcystins on the male reproductive system of mice[5], but the research on the genetoxicity of germ cells has not been reported.

The genetoxic effect of microcystins-LR on testicle cell was investigated by the DNA-protein crosslinks (DPC) test and micronucleus test after the mice were injected intraperitoneally of pure microcystins.

2 Materials and Methods

2.1 Animals

Male KM mice (approximal 30 g) were obtained from the Henan Experimental Animals Center (Zhengzhou, Henan, China). Forty-five mice were randomly allocated into 9 groups with 5 mice in each group (4 groups for DPC, 5 groups for micronucleus test). The mice were treated with MC-LR of 0, 3, 6, 12 μg/kg·bw per day respectively, peritoneal injection, for 7 days. The positive control group were treated once with cyclophosphamide of 30 mg/kg·bw, peritoneal injection.

☆Supported by Medicine Innovation Talent Projecting Stake Item in Henan province, No.20031125, and the Japan and Chinese Cooperative Item, No.064SGHH36252-5.

*Corresponding author. Email: clx@zzu.edu.cn

2.2 DPC of the mice testicle cells

The modified KCl-SDS precipitation method[6] was to detect DPC.

2.2.1 Prepared the testicle cell suspension. The animals were poisoned for seven days and killed at the eighth day. The testicle was taken, the tegument was moved in PBS, the surplus part was snipped to rotten status, and filtered by the monolayer lens paper. Filter liquor was centrifuged for 5 minutes, 1500 g/min. The sediment was diluted to 10^5 – 10^6 cells/ml with PBS.

2.2.2 Schizolysis cells. Cells were lysed with 0.5 ml 2% SDS (Sigma, USA), shaked slightly, and warmed for 10 minutes at 65 ºC.

2.2.3 Isolated free DNA. 100 μl Tris-HCl-KCl solution was added in the above solution. Put the samples on ice for 5 minutes and centrifuged at 10000 g/min for 5 minutes at 4 ºC. The sediment was suspended in 1 ml buffer solution (0.1 M KCl, 0.1 mM EDTA, 20 mM Tris-HCl, pH 7.5), warmed for 10 minutes at 65 ºC, chilled on ice for 5 minutes and centrifuged as prior again. The washing step was repeated three times, and the supernatant was transferred together.

2.2.4 Isolated protein-linked DNA. 0.5 ml proteinase K (Amresco, USA) was added in the above solution, digested for 3 hours in 50 ºC water bath, then chilled on ice for 5 minutes and centrifuged, 12000 g/min for 10 minutes at 4 ºC. Collected the supernatant.

2.2.5 Quantitated DNA. A standard curve of DNA concentration was drawn: calf thymus DNA (Sigma, USA) standard solutions were diluted by the buffer solution, and the final concentrations were 0, 100, 300, 500, 750, 1000, 1500, 2000, 3000, 5000 ng/ml. 1 ml 400 ng/ml fresh prepared fluorescent dye Hoechst 33258 (Sigma, USA) was added to each solution, got the final concentration of 200 ng/ml. Put them in the dark place for 30 minutes, then measured the fluorescence value of each concentration solution (provocative wavelength was 353 nm, emission wavelength was 455 nm), and drew the standard curve. The samples were dyed and measured in the same condition.

2.3 Micronucleus test of the sperm cell early stage

Fourteen days after the injection, the testicle was removed from each mouse, snipped to pieces in the 10 ml testicle cell separation medium (TM) solution, and added with diastase vera. The solution was centrifuged for 5 minutes, 1000 g/min. The sediment was suspended with the TM solution, and then fixed with methanol for 10 minutes dried in the air. Dyed for 20 minutes with Giemsa, the solution was diluted 1 : 7 (v/v) with PBS (pH 5.9), washed slowly with the distilled water, and dried in the air. Selected 1000 early stage sperm cells of each animal and calculated the micronucleus.

2.4 Statistical analysis

One-way analysis of variance (ANOVA) and the Dunnett-t analysis were used in the statistical test (SPSS 13.0).

3 Results

3.1 Standard curve of DNA concentration

From the standard curve (Figure 1), the free DNA and protein-linked DNA were quantitated, and the DPC coefficient were calculated. $Y = 265.80 + 0.36x$, $r = 0.9975$, $P < 0.0001$.

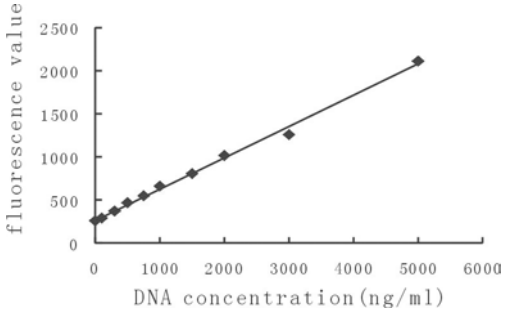

Figure 1. The standard curve of DNA concentration.

3.2 DPC coefficients of the mice testicle cells in different groups

As Table 1 showed, when the dose of MC-LR was 3 μg/kg·bw, the DPC coefficient was of no significant difference, compared with the control group ($P > 0.05$); when the dose of MC-LR were 6 μg/kg·bw and 12 μg/kg·bw, the DPC coefficient was significantly different from control group ($P < 0.05$), and when the dose was 6 μg/kg·bw, the average DPC coefficient value was the maximum.

3.3 Effects of MC-LR on the micronucleus of the sperm cells in early stage

We adopted 1000 cells from each animal, and 5 animals were in each group. Table 2 showed that: compared with the control group, the average micronucleus number of cells in early stage sperm increased. The effect of 3 μg/kg·bw MC-LR was of no significant difference from that of control group ($P > 0.05$). While micronucleus number was significantly increased by 6 μg/kg·bw and 12 μg/kg·bw MC-LR, compared with the control group ($P < 0.05$, $P < 0.01$ respectively).

Table 1. The DPC coefficients under different doses

Dose of MC-LR (μg/kg·bw)	Observed cells	DPC coefficient ($\bar{X}$ ± SD)
0	5000	0.015 ± 0.004
3	5000	0.035 ± 0.005
6	5000	0.056 ± 0.007*
12	5000	0.040 ± 0.024#

Note: *: $P < 0.01$, # $P < 0.05$, *vs.* 0 μg/kg·bw.

Table 2. Comparison of micronucleus number of different groups

Dose of MC-LR (μg/kg·bw)	Observed cells	DPC coefficient ($\bar{X}$ ± SD)
0	5000	0.015 ± 0.004
3	5000	0.035 ± 0.005
6	5000	0.056 ± 0.007*
12	5000	0.040 ± 0.024#
positive	5000	9.60 ± 1.14

Note: *: $P < 0.01$, # $P < 0.05$, *vs.* 0 μg/kg·bw.

4 Discussion

4.1 The DPC effect of the MC-LR on mice testicle cell

As a molecular biomarker of external chemical toxicant, the DPC is focused for many years. In the normal cell, the DPC has a background level, and it is necessary for the growth of the cell. But if the big molecules in the organism are impacted by the external factors, excessive DPC can be induced. The accumulated DPC damages DNA, and leads to the disrupt of DNA copying process. So the DPC has a very important value as the biomarker for the genotoxicity to assess the damage of DNA.

The results of the DPC experiment showed that the 3 μg/kg·bw MC-LR didn't significantly change the DPC coefficient of the mice testicle cells. But the 6 μg/kg·bw and 12 μg/kg·bw MC-LR increased significantly the DPC coefficient of the mice testicle cells. The liver is the primary target organ of MC-LR, and if the poisoning dose of MC-LR is low, most of the toxin congregate in the liver, and only a little toxin is left in the testicle cells. The 3 μg/kg·bw MC-LR is too small to induce significant increase of the DPC in the mice testicle cells. But with MC-LR increased, the toxin amount congregating in the testicle cells also increases, and induce the excessive DPC in the mice testicle cells. In this research, the DPC coefficient value of the 6 μg/kg·bw group was higher than the 12 μg/kg·bw group. It might be that when the dose exceed a certain level, the DPC coefficient may descend. The results indicated that MC-LR has the potential DNA damage on the male mice germ cells.

4.2 Micronucleus test of the germ cell in early stage induced by MC-LR

Observing the damage of the chromosome and the caryocinesia organ is the meaning of the micronucleus test. As a simple, reliable test to measure the aberration rate of the chromosome, the micronucleus test has become a conventional method to screen the chemical mutagen[8]. The micronucleus test for early stage germ cell is good and sensitive to measure the damage of the environmental mutagenic agent on the chromosome of the male germ cell. The result is an important index of the heredity damage. Early in the 1950s, Oakberg[9] has reported that the period from the preleptonema of the first spermatocyte to the early stage germ cell was 2 weeks. The experiment was designed according to the rule. The 3 μg/kg·bw MC-LR didn't increase the micronucleus rate. But the 6 μg/kg·bw MC-LR increased the micronucleus rate ($P < 0.05$). The 12 μg/kg·bw MC-LR also significantly increase the micronucleus rate ($P < 0.01$). The results indicated that MC-LR has the potential mutagenic damage on the chromosome of the male mice germ cells.

5 Conclusion

MC-LR damages DNA and chromosome in germ cell of male mice. Increasing the heredity damaging effect of the carcinogenic pollutant may be one of the promoting cancer mechanisms of MC-LR. The experiment is helpful to the following study of the genotoxicity molecule mechanism of MC-LR.

References

1. Orr PT, Jones GT, Hunter RA, *et al*. Ingestion of toxic *Microcystis aeruginosa* by daily cattle and implications for microcystin contamination of milk. Toxicon 2001; 39(1): 847 – 54.
2. Liu LL, Zhong RG, Zeng Y. Advances in study on microcystins and their toxicology. Journal of Hygiene Research 2006; 35(2): 247 – 9.
3. Lei LM, Song LR. Acute toxicity of MC-LR in BALB/c mice. Journal of First Military Medical University 2005; 25(5): 565 – 6.
4. Chen J. Bioaccumulation of the Hepatotoxic Microcystins in Aquatic Animals of Lakes Chaohu and Taihu with Potential Risk to the Safety of Aquatic Products. Wuhan: Institute of Hydrobiology, Chinese Academy of Science, 2006.
5. Ding XS, Li XY, Duan HY, *et al*. Toxic effects of microcystis cell extracts on the reproductive system of male mice. Toxicon 2006; 48: 973 – 9.
6. Liu YS, Lu ZS, Yang JW, *et al*. Quantification study on the DNA-protein crosslinks of human blood lymphocytes induced by formaldehyde. Journal of Preventative Medicine in Hebei 2004; 15(4): 4 – 7.
7. Wang LM, Ding SM, Wu K, *et al*. Study on cellular DPC induced by di(2 ethylhexyl) phthalate in organs of mice. J of Pub Health and Prev Med 2006; 17(4): 5 – 9.
8. Wang XP, Duan LJ, Zhao Q. Experimental study on the effect of gaseous formaldehyde on micronuclei in mice. Journal of Modern Preventative Medicine 2006; 33(1): 1 – 3.
9. Oakberg EF. Duration of spermatogenesis in the mouse. Nature 1957; 180(23): 1137 – 8.