MC-LR induces Reproductive Toxicity

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Abstract: Cyanobacterial blooms and the detrimental effects of their toxins were seriously threaten to both animals and humans health, and causing ecological unbalances and contamination of the environment. So far, microcystin-LR (MC-LR) was considered as the most common and toxic Microsystems. In vitro and vivo experiments have been undertaken to address the impact of MC-LR. In this regard, the aim of this work was to present the toxicity of microcystin-LR (MC-LR) on the reproductive system. There are two parts including animal tests and vitro experiments. The details are outlined in this review.
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1. Introduction

Nowadays, with the increase of water eutrophication extent，the reports about occurrences of water bloom and red tide are increasing all over the world[1]. Microcystins (MCs) are a group of hepatotoxin resulting from algal blooms. The hazards of MCs to water environment and human health has touched off a heated concerns of public, and the detection of cyanotoxins is aroused interest of scientists, clinicians, and public health officials because MCs can cause apoptosis or promote tumor development in both animals and humans[2]. MCs are cyclic heptapeptides which are consisted of seven amino acids, and have quite stable properties. Over 80 variants of MCs have been identified [3], and the most abundant and toxic ones are MC-LR, MC-RR, MC-YR (L, R, Y represents for leucine, arginine, tyrosine respectively), of which MC-LR is the most toxic variant.

In the lakes and rivers of eutrophication, MCs can accumulate in a variety of organisms including snails, vivipara and fish [4]. MCs can also enter into the other organisms through drinking water or food chain. Human beings′ intake of MCs can occur through contaminated water or aquatic animals (such as fish, shrimp, etc). Epidemiology investigation showed that long-term exposure to MCs can increase the incidence of HCC and colon cancer significantly.

A study on the accumulation of MCs in vivo showed average accumulative magnitude in the digestive tract is the greatest, and gonads rank is the second [4, 5], so the gonads may be potential target organs for MCs. MCs arouse detrimental effects to reproductive system of human beings and animals.

2. Animals test – through mouth ingestion

In a test of teratogenesis, after pregnant female mouse were exposed to MC-LR (0, 200, 600 and 2000 μg/kg bw) for over 13 weeks, 2000 μg/kg bw MC-LR can cause maternal toxicity and lead to the death of 6 mice in total 26 rats. It is more serious that 2 mice were dead at the first 6 days and 15 days of pregnancy after MC-LR treatment. However, surviving female mice livers were damaged, and fetal alloplasty-body weight and bone development delayed [6].

Another study showed Microcystin-LR can effect loach embryo-larval and juvenile development. The results of this study showed that loach embryos were more sensitive when exposed to microcystin-LR at a later than at an earlier stage of development. Juveniles were far less sensitive to MC-LR than were embryos and larvae. Mortality and developmental abnormality were proven to be dose-dependent and to be stage-specific sensitive[7]. In addition, one study was used to evaluate the effects of various fractions of cyanobacterial biomass with different composition and microcystin content on embryolarval development of carp (Cyprinus carpio) . The data indicated that cyanobacterial water blooms as well complex biomass extracts induce significant embryolarval toxicity in common carp[8].

In summary, when aquatic animals were exposed to MCs, MCs can also elevate the rate of embryo deformity and affect embryo incubation and development.

3. Animals test –through intraperitoneal injection

The effects of MC-LR on embryonic development and offspring growth were observed by Chernoff et al. It was found that MC-LR did not cause harmful effect on embryonic and offspring growth. In the test, the pregnant mice were randomly grouped into equal team of 8 mice. In the first part test, mice were exposed at 0, 32, 64 and 128 μg MC-LR/kg bw through intraperitoneal injection respectively, and in another part test, mice were exposed at 128 μg MC-LR/kg bw for consecutive two days in the different gestation (7~8 days, 9~10 days and 11~12 days).

Pregnant mice were sacrificed at the first 17d of pregnancy, and the mice fetus were weighed and used to calculate the fetal malformation rate. The first part results showed that MC-LR did not cause the detrimental effects of fetal number, weight and vitality. The second part test make fetus born and allow fetal growth for 4 days, and the results displayed that MC-LR did not result in the adverse effects [9].

After intraperitoneally injected with MCs at 3.33 and 6.67 μg/kg bw for 14 days, toxic effects of Microcystis cell extracts on the reproductive system of male mice were studied by Ding et al. The study results showed that mean the body weight, the mean absolute weight of the testes and epididymides decreased compared to the control group. However, compared to the control group, the mean relative weight of the testes increased. In addition, histological detection of microcystin-treated mice indicated that the testes were damaged, and the space among the seminiferous tubules was broader compared to controls. The quality of mature sperm in the seminiferous tubules was decreased and the viability of the sperm was reduced in the mice treated with MCs. However, there was no significant difference in the concentration and abnormality of the sperm between the treatment and control. This study suggested that MCs could cause the numerous toxic effects on the reproductive system of male mice [10].

LiYan et al investigated whether microcystin-LR has toxic effects on reproductive system of SD Male rats. Male rats were treated with MC-LR (i.p.) at a dose of 0, 5, 10 or15 μg/(kg bw day) for 28 days. The study showed that exposure to 5 μg /(kg bw day) of MC-LR decreased the sperm motility and increased the sperm abnormality rate, and exposure to 15 μg /(kg bw day) of MC-LR decreased testis weight, sperm concentration, the levels of serum testosterone, FSH and LH. The histological detection showed that the seminiferous tubules atrophied and obstructed [11].

The changes of ultrastructures and biochemical index in rabbit testis were observed after i.p. injection with 12.5 μg /kg microcystin (MC) extracts. Ultrastructural observation results showed intercellular junction widened, swell of mitochondria, endoplasmic reticulum and Golgi apparatus at 1, 3, and 12 h. The levels of MDA and H_2O_2 increased significantly at 1 h, suggesting MC can cause oxidative stress. The level of H_2O_2 recovered to the normal levels at last, while MDA kept at high levels still. The antioxidative enzymes (CAT, SOD, GPx, GST) and antioxidants (GSH) also increased rapidly at 1 h. Finally, the activity of CAT, SOD and GPX decreased to the normal levels, but the GST activity and GSH maintained at a high level, suggesting antioxidative systerm plays an importance role in MCs detoxification. In conclusion, MCs can cause toxicity to reproductive system of the male rabbit and the underlying mechanism might to be related to the oxidative stress caused by MCs [12]. To investigate the reproductive toxicity and the underlying mechanism, BALB/c mice were intraperitoneally injected with different injection duration (1, 4, 7 and 14 days) and injection concentration (3.75, 7.5, 15 and 30 μg kg /bw day). The results showed that MC-LR decreased the Gnrh expression in a dose- and duration-dependent manner. Moreover, MC-LR increased and subsequently decreased the expressions of FSH, LH and testosterone, implying that MC-LR affected male mice serum hormones and mRNA expressions by damaging the hypothalamic-pituitary systems, and then caused the reproductive system damage [13].

Recently, the effects of chronic low-dose exposure to microcystins on sperm quality and testicular function were studied in male mice that orally administered at 0, 1, 3.2, and 10 μg/L for 3 and 6 months. The preliminary study showed that sperm quality declined at 3.2 and 10 μg/L, testosterone levels decreased at 10 μg/L, levels of LH and FSH increased, and Leydig cells apoptosis occurred in three-month group. The changes of sperm abnormality rate and testosterone level in six-month group were similar to the three-month group, but these changes were more marked. It was noted that the testis structural impairment was observed at 10 μg/L dose in six-month group. Therefore, these results implied that chronic low-dose MC-LR treatment can lead to toxicity to testis and affect the hormone level [14].

Although the acute and chronic test showed the different result of some hormone levels, these results still proved that MCs can cause the detrimental effects on the reproductive system. The inconformity of results may be related to the different experiment cycle and animals.

4. In vitro experiment

After embryos were exposed to microcystin-LR (10-100 microM), changes of morphology and cytoskeletal elements in the stages of early embryonal development were studied by Frangez et al. The results showed MC-LR did not affect embryonal cytoskeleton organization, growth and development, but the whole embryo cell is very sensitive to MC-LR. Actin and microtubule organization were even affected by low concentrations (10-20 microM) of MC-LR. After treated with high concentrations (100 microM) of MC-LR, embryo cells were detached and destroyed [15].

Leydig cells is an important endocrine cell in the testis and responsible for androgen production that is essential for spermatogenesis and sperm maturation.

A previous study was performed to investigate whether microcystin-LR has toxic effects on Leydig cells. After treated with various concentration of 0, 0.5, 5, 50 or 500 nM MC-LR, Leydig cells decreased testosterone production and SOD activity. In the 50 and 500 nM MC-LR groups, the cell viability significantly decreased, DNA fragmentation and apoptosis increased, the ratio of necrotic cells increased, the lipid peroxidation enhanced. In the 500 nM group, ROS production increased. Therefore, MC-LR might cause Leydig cells oxidative stress and lead to cytotoxicity, even cell apoptosis, resulting in the reproductive toxicity in male rats [12]. However, in another vitro test, after Leydig cells isolated from mice were exposed to MC-LR at 1, 10, 100, 250, 500, 750 and 1000 nmol/L for 24 h, MC-LR did not enter Leydig cells and had no cytotoxicity on Leydig cells [15]. The two tests results indicated that proper culture condition and isolation methods were critical to investigate the effects of MC-LR on Leydig cells. Our previous results also suggested that MC-LR can induce oxidative stress in primary cultured rat sertoli cell, but can not lead to lipid peroxidation[16].

The toxicity effects of MC–LR on Sertoli cells were studied. Sertoli cells isolated from rats were treated with 0, 0.5, 5, 50 or 500 nmol/L MC–LR. The results showed the increase of reactive oxygen species (ROS), lipid peroxidation and apoptosis rate, the decline of mitochondrial membrane potential (MMP) and superoxide dismutase (SOD) activity, and the up-regulated expression of caspase-9 and caspase-3. Therefore, MC-LR could induce oxidative stress, depressed cellular viability, and caused cells to apoptosis in Sertoli cells, leading to the reproductive toxicity in male rats [17].

Other study investigated the toxic effects of MC-LR on spermatogonia in vitro. Spermatogonia were treated with 0, 0.5, 5, 50, and 500 nmol/L MC-LR for 6h. The results showed that cell viability and total antioxidant capacity significantly declined, meanwhile, the ratio of apoptotic cells, reactive oxidative species (ROS) generation, mitochondrial membrane potential (MMP), and intracellular free Ca(2+) enhanced after exposure to 5, 50, and 500 nmol/L MC-LR. This study demonstrated that MC-LR can lead to cytotoxicity of spermatogonia , affecting the the reproductive systerm [18].

Sertoli cells play an essential role in the development and maturation of sperm cells. To investigate the effect of MC-LR on apoptosis of Sertoli cells, Sertoli cells were isolated from healthy immature rats, and then cultured with MC-LR. The viability of Sertoli cells was decreased after treatment with MC-LR at 10 μg/ml for 24 h ($P<0.05$). Moreover, the MC-LR-treated cells exhibited condensed chromatin and fragmented nuclei, features of apoptosis. We also analyzed the mRNA and protein levels of three apoptosis-related genes, p53, bax and bcl-2, using reverse transcription-polymerase chain reaction and Western blot analyses, respectively. Both p53 and bax function as promoters of apoptosis, while bcl-2 is an apoptotic suppressor. The mRNA and protein expression levels of p53 and bax were increased in Sertoli cells treated with MC-LR at 10 μg/ml compared with the control group ($P < 0.05$), while the bcl-2 protein levels were decreased in cells treated with MC-LR at 10 μg/ml ($P < 0.05$). Moreover, the activity of caspase-3, which is involved in the induction of apoptosis, was significantly increased in Sertoli cells treated with MC-LR. These results indicate that MC-LR induces apoptosis of Sertoli cells [19].

When mammalian cells were treated with 2.01~60.1 nM MC-LR, it was found that MC-LR could play estrogenic activity by Oziol et al. A maximal-induced response occurred at 10.1 nM, which was approximately 25% of the maximal effect obtained with 1 nM E2 [20]. Another recent study also confirmed that MC-LR has the estrogenic-like effects. After Zebrafish was exposed to the 0~1000μg/L MC-LR, vitellogenin genes were up-regulated, suggesting that MCs may be a natural source of environmental estrogens. Therefore, MC-LR may affect the secretion of hormone, and cause the toxicity to the reproductive system [21].

5. Conclusion

It is known that MC is a cyanotoxin, of which MC-LR is the most widely distributed and the most hazardous toxin. So there are many researchers studying the harmfulness of MC-LR on Human beings. In this paper, we mainly described the effect of MC-LR on the reproductive system in animal experiment with various methods, including in vitro and in vivo test, acute and chronic exposures. Consequently, MC-LR could not only induce fetal alloplasty-body weight and bone development delays, enhance the rate of embryo deformity, and affect embryo incubation and development, but also lead to oxidative stress, decrease the testis weight and sperm concentration, and reduce the levels of serum hormone in vivo tests. In vitro experiments, MC-LR could destroy embryo cells, lead to cytotoxicity of spermatogonia, oxidative stress of Leydig cells and apoptosis of Sertoli cells. Therefore, MC-LR has the potential reproductive toxicity, so we should pay more and more attention to the study on microcystin-LR.

6. Prospects of Future Study

With the frequent occurrence of cyanobacterial blooms, MCs continuously released into many freshwater bodies, so the hazards of Microcystins toxins have aroused more attention in the world. With the deep study of Microcystins,

people have had a better understanding on MCs types and structures. In addition, the study on the extent and the mechanisms of MCs toxicity are carried out in a deep-going way. Because of the varieties of algae toxins, there have been not effective methods to eliminate it. Meanwhile, the study on the reproductive toxicity is limited, therefore, this aspect need to be further studied.

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References

1. Zhang H Z, Guo H X, Liu X H, Duan L J, Cheng X M, Cui L X. Assessment and prediction on the eutrophic state of a drinking water source. Life Science Journal 2011,8(1):86-92.
2. Funari E & Testai E. Human health risk assessment related to cyanotoxins exposure. Crit Rev Toxicol 2008, 38(2):97-125.
3. Hoeger S J, Hitzfeld B C & Dietrich D R. Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants. Toxicol Appl Pharmacol 2005, 203(3):231-242.
4. Xie L, Yokoyama A, Nakamura K & Park H. Accumulation of microcystins in various organs of the freshwater snail Sinotaia histrica and three fishes in a temperate lake, the eutrophic Lake Suwa, Japan. Toxicon 2007, 49(5):646-652.
5. Chen J & Xie P. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, Palaemon modestus and Macrobrachium nipponensis, from a large shallow, eutrophic lake of the subtropical China. Toxicon 2005, 45(5):615-625.
6. Fawell J K, Mitchell R E, Everett D J & Hill R E. The toxicity of cyanobacterial toxins in the mouse: I microcystin-LR. Hum Exp Toxicol 1999, 18(3):162-167.
7. Liu Y, Song L, Li X & Liu T. The toxic effects of microcystin-LR on embryo-larval and juvenile development of loach, Misguruns mizolepis Gunthe. Toxicon2002, 40(4): 395-399.
8. Palikova M, Krejci R, Hilscherova K, Babica P, Navratil S, Kopp R & Blaha L. Effect of different cyanobacterial biomasses and their fractions with variable microcystin content on embryonal development of carp (Cyprinus carpio L.). Aquat Toxicol2007, 81(3): 312-318.
9. Chernoff N, Hunter E S, 3rd, Hall L L, Rosen M B, Brownie C F, Malarkey D, Marr M & Herkovits J. Lack of teratogenicity of microcystin-LR in the mouse and toad. J Appl Toxicol 2002, **22**(1):13-17.
10. Ding X S, Li X Y, Duan H Y, Chung I K & Lee J A. Toxic effects of Microcystis cell extracts on the reproductive system of male mice. Toxicon 2006, 48(8):973-979.
11. Li Y, Sheng J, Sha J & Han X. The toxic effects of microcystin-LR on the reproductive system of male rats in vivo and in vitro. Reprod Toxicol2008, 26(3-4): 239-245.
12. Liu Y, Xie P, Qiu T, Li H Y, Li G Y, Hao L & Xiong Q. Microcystin extracts induce ultrastructural damage and biochemical disturbance in male rabbit testis. Environ Toxicol 2010, 25(1):9-17.
13. Wang X, Ying F, Chen Y & Han X. Microcystin (-LR) affects hormones level of male mice by damaging hypothalamic-pituitary system. Toxicon 2012, 59(2):205-214.
14. Chen Y, Xu J, Li Y & Han X. Decline of sperm quality and testicular function in male mice during chronic low-dose exposure to microcystin-LR. Reprod Toxicol 2011, 31(4):551-557.
15. Frangez R, Zuzek M C, Mrkun J, Suput D, Sedmak B & Kosec M. Microcystin-LR affects cytoskeleton and morphology of rabbit primary whole embryo cultured cells in vitro. Toxicon 2003**,** **41**(8):999-1005.
16. Yi D, Liu X H, Zhang F Q, Wang J, Zhao Y, Sun D J, Ren J W, Zhang H Z. Oxdative stress on sertoli cells of rats induced by Microcystin-LR. Life Science Journal2011, 8(2) :249-253.
17. Li Y & Han X. Microcystin-LR causes cytotoxicity effects in rat testicular Sertoli cells. Environ Toxicol Pharmacol 2012, 33(2):318-326.
18. Zhou Y, Yuan J, Wu J & Han X. The toxic effects of microcystin-LR on rat spermatogonia in vitro. Toxicol Lett 2012, 212(1):48-56.
19. Zhang H Z, Zhang F Q, Li C F, Yi D, Fu X L & Cui L X. A cyanobacterial toxin, microcystin-LR, induces apoptosis of sertoli cells by changing the expression levels of apoptosis-related proteins. Tohoku J Exp Med 2011, 224(3):235-242.
20. Oziol L & Bouaicha N. First evidence of estrogenic potential of the cyanobacterial heptotoxins the nodularin-R and the microcystin-LR in cultured mammalian cells. J Hazard Mater 2010, 174(1-3):610-615.
21. Rogers E D, Henry T B, Twiner M J, Gouffon J S, McPherson J T, Boyer G L, Sayler G S & Wilhelm S W. Global gene expression profiling in larval zebrafish exposed to microcystin-LR and microcystis reveals endocrine disrupting effects of Cyanobacteria. Environ Sci Technol 2011, 45(5):1962-1969.

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