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Effects of microcystin-LR on hippocampal N-acetylaspartate and neurobehaviors in rats

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Abstract: The effects of low-doses of microcystin-leucine-arginine (MC-LR) exposure on neurobehaviors and N-acetylaspartate (NAA) expression in the hippocampus of rats were investigated. After male Sprague-Dawley (SD) rats were treated intra-gastrically with different doses of MC-LR for 90 d, the locomotor activity, spatial learning and memory function were evaluated in the rats after treatment using open field tests and Morris water maze tests. The results show that MC-LR exposure can lead to impairment of the spatial learning capacity and locomotor activity in rats at the dose of 2.00 μg/kg. The levels of NAA in the hippocampus were measured by magnetic resonance spectroscopy (MRI). A significant decrease of NAA/Cr ratio ($P < 0.05$) was observed in the hippocampous. This study indicates that intra-gastrical exposure to low-doses of MC-LR has adverse effects on neuronal behavior and NAA levels in the hippocampous.

Key words: low-doses of microcystin-leucine-arginine (MC-LR); N-acetylaspartate (NAA); neurobeha-viors; magnetic resonance spectroscopy; neuro

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The toxicity of microcystins (MCs) has attracted much attention in public health research[1]. Microcystin-LR (MC-LR) is a widely studied toxic peptide among 80 variants of MCs that have been identified[2]. Most toxicity studies for MC-LR mainly focus on hepatotoxicity[3] and other multi-organ injury[4], while neurotoxicity has received limited attention. Fisher et al.[5-6] reported that the organic anion transporting polypeptides (Oatps) which are responsible for hepatic uptake of MCs were expressed in the brain, and detected low concentrations of MC-LR in the brains of rats after intravenous MC-LR injections were administered[7]. This implies that MCs can cross the blood-brain barrier. Intra-hippocampal injections of MC-LR causes histological damage to rat brains, indicating that MC-LR may be a neurotoxic peptide[8]. Although the neurotoxicity of the MC-LR has recently received much attention, little is known concerning the adverse effects of MC-LR on neuronal functions.

N-acetylaspartate (NAA) is one of the most prominent metabolites in the brain and it is considered to be an important marker of neuronal health, number and function[9]. The hippocampus plays a role in encoding and processing information for learning and memory in animals and reduced NAA levels in the hippocampus have been associated with impaired cognition[10]. We have previously demonstrated that exposure to 1 μg/L MC-LR can reduce the number of GABAergic neurons and decrease locomotor behavior in Caenorhabditis elegans[11]. We also reported inflammation in the hippocampus of rats induced by MC-LR[12]. In this paper, we examine the effects of MC-LR exposure on NAA levels in the hippocampus and investigate the consequences on locomotinor activity and spatial learning in rats.

1 Materials and Methods

1.1 Animals and chemicals

Prague-Dawley (SD) rats (28 d old) were purchased from Shanghai SLRC Laboratory Animal Co. Ltd., China. MC-LR (purity ≥ 95%) was purchased from Enzo Life Sciences International (USA). MC-LR (1 mg) was dissolved in 0.1 mL of methanol and diluted with 0.9% saline solution to prepare the stock solution. The final concentration of methanol was adjusted to 2×10^{-5}.

1.2 Animal treatment

Rats were housed in the animal facility in Southeast University, China, at the room temperature of 18 to 23 ℃, with a relative humidity of 45% to 55% and 12/12 h light/dark cycle. All the procedures were conducted in conformity with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Rats were divided into four groups randomly and administered intragastrically with 0.13, 0.50, and 2.00 μg/kg MC-LR once a day for 90 d. The controlled rats were given with methanol dissolved in 0.9% saline solution at a concentration of 2×10^{-5}. During the 90 d experiment, all the rats were feeding and drinking water freely.

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1.3 Morris water maze test

The Morris water maze test was analyzed by the Noldus developed animal behavior tracking analysis system (Ethovision TX 7.0) as previously described[13]. The Morris water maze consisted of a circular pool (diameter: 160 cm), the image acquisition system and analysis of the computer operating system. The test was performed for 5 consecutive days, once a day. The sequence of starting positions was randomly selected. The time that the rat found the hidden platform from one of the four designated starting points (escape latency) was recorded by a computerized video-tracking system. Within 120 s, it was gently guided to and placed on the platform where it had to remain for 10 s before being returned to its home cage. For the probe test, swim distance, velocity, time in platform position and the number of cross platforms were measured and analyzed.

1.4 Open field test

The open field test was performed according to the description by Frankland et al[14]. Rats were placed in the center of the square arena. Then, locomotor activity data reflecting qualities of movement were collected at 5-min intervals, including total distance, central region duration, edge region duration and edge region duration. Behavioral duration refers to time spent on movement when the speed of rats is greater than 1.5 m/s.

1.5 Levels of NAA in hippocampous

MRI scans were carried out with four rats from each group at the end of the experiment. MRI and 1H-MRS studies were performed on a 7.0 T animal MRI scanner (70/16 PharmaScan, Bruker Biospin GmbH, Germany) equipped with a quadrature transmit-receive coil. During the MRI scan, the respiration and ECG of rats were monitored. To obtain routine MRI data, the following parameters are used: repetition time T_R is 2 500 ms; echo time T_E is 33 ms; the field of view is 3.5 cm × 3.5 cm; the matrix size is 256 × 256; the slice thickness is 1.0 mm; and the slice gap is 1.5 mm. The frequency of water peaks was calculated. MR spectroscopic data were collected via point resolved spectroscopy (PRESS) sequence, with $T_R = 2\,500$ ms, $T_E = 20$ ms, and an average of 512 times in a scan duration. The spectral lines were treated by baseline calibration and phase adjustment. NAA/Cr was evaluated as previously described[15].

1.6 Statistical analysis

Data were expressed as means ± standard deviations (SD). Statistical analyses were performed using the SPSS 13.0 software. Differences among these means were done by a one-way or repeated-measures ANOVA followed by SNK post hoc tests. The level of statistical significance was taken at $P < 0.05$.

2 Results and Discussion

2.1 Effects of MC-LR on spatial learning and memory ability in rats

The results of escape latencies are shown in Fig. 1. Repeated-measures ANOVA revealed that the escape latency of rats exposed to 2.00 μg/kg MC-LR showed a significant increase in the third and fourth day. The results of the spatial probe test indicate that, after 90 d treatment, the times for crossing over the platform area of rats exposed to 2.00 μg/kg MC-LR was shorter than that of the vehicle, while the swim distance of the rats was significantly longer than that of the vehicle ($P < 0.05$) (see Fig. 2).

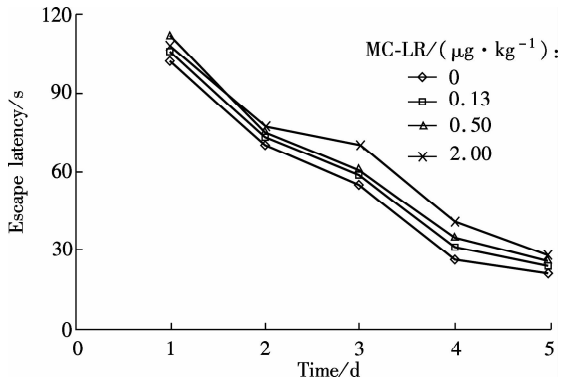

Fig. 1 Escape latency of rats in the Morris water maze test after exposure to MC-LR

2.2 Effects of MC-LR on spontaneous behavior in rats

The results show that, compared with those of the control, the edge region duration in rats exposed to 2.00 μg/kg MC-LR was increased significantly, while the central region duration evidently decreased ($P < 0.05$). The total distance of the rats treated with 2.00 μg/kg MC-LR was significantly longer than that of rats treated with the vehicle ($P < 0.05$). With respect to the edge region duration, no significant difference was observed among the groups (see Fig. 3).

2.3 Effects of MC-LR on NAA in the hippocampus of rats

The 1H-MRS for the hippocampus exhibiting the peaks of NAA was identified at 2.0×10^{-6}. Student's t-tests showed that the ratio of NAA/Cr in rats exposed to 2.00 μg/kg MC-LR was significantly decreased ($P < 0.05$), compared with that of rats exposed to the vehicle (see Fig. 4). However, no significant changes on hippocampal volumes were found between saline and MC-LR exposed groups.

The neurotoxicity of MC-LR remains poorly understood, although previous studies have demonstrated that long-term exposure to low-doses of MC-LR can pose

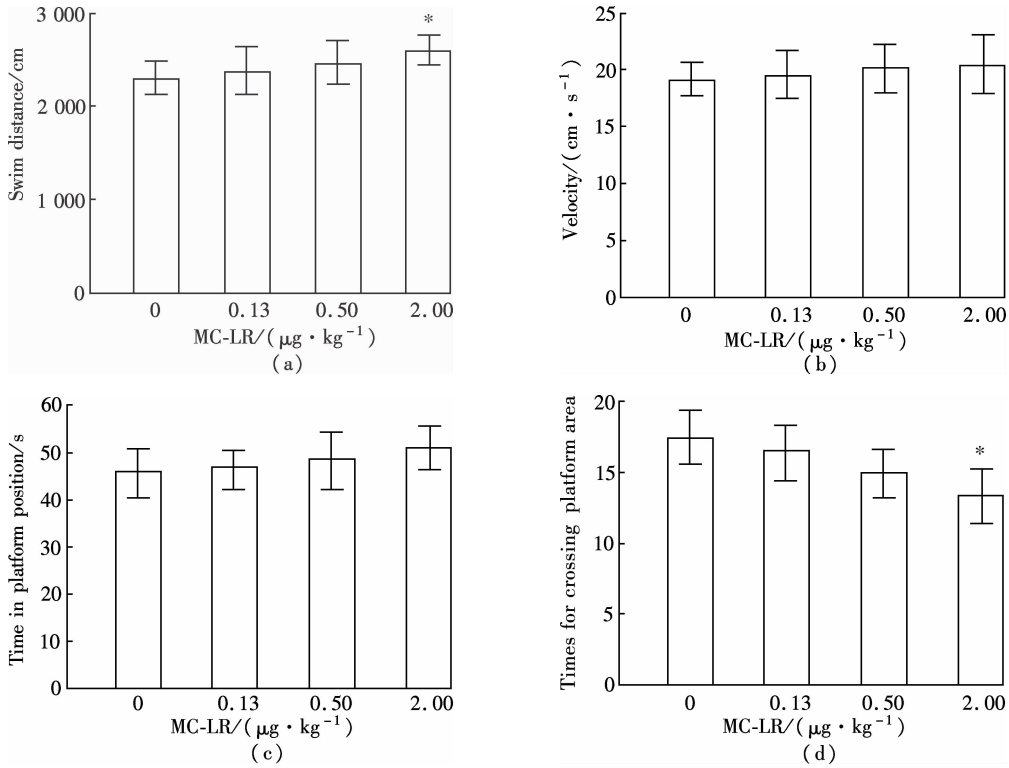

Fig. 2 Spatial probe test on rats after exposure to MC-LR. (a) Swim distance; (b) Velocity; (c) Time in platform position; (d) Times for crossing the platform area

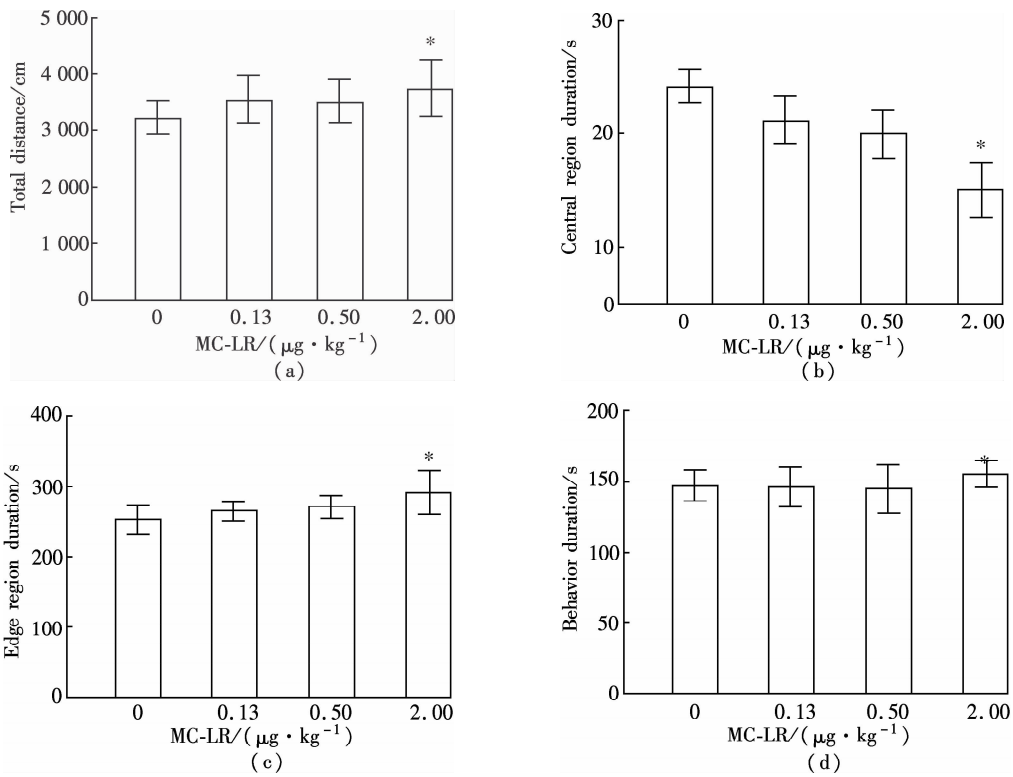

Fig. 3 Spontaneous behavior in rats after exposure to MC-LR. (a) Total distance; (b) Central region duration; (c) Edge region duration; (d) Behavior duration

significant health risks in humans[16]. In the present study, we observed adverse effects of MC-LR on the neuronal function and behavior in rats.

Magnetic resonance spectroscopy (MRS) revealed lower levels of NAA in the hippocampus after exposure to MC-LR, although no significant changes in brain volume were observed. NAA is synthesized in the mitochondria of neurons and represents one of the most concentrated

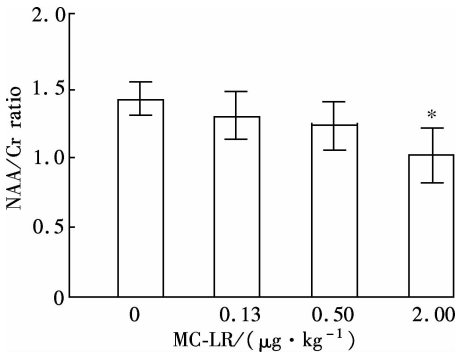

Fig. 4 Effects of MC-LR on levels of NAA in hippocampus of rats

molecules in the brain, maintaining neuronal integrity and the osmotic balance of brain fluid[9]. To the best of our knowledge, MRS has not yet been used to investigate the effects of MC-LR exposure on the rat brain. Conversely, the dangers of exposure to lead, manganese and other neurotoxins in humans are well documented[17-18], and these toxins have significantly reduced NAA expression in the temporal lobe, including the hippocampus[19]. Reduced NAA levels were likely responsible for neuronal dysfunction. In conclusion, our findings show that MC-LR might be involved in neuronal damage in the hippocampus.

Hippocampal neurons are highly vulnerable to harm[20]. Behavioral training is dependent on hippocampal function[21]; therefore, the loss of hippocampal neurons can impair learning and memory processes[22]. Intra-hippocampal injections of microcystin significantly altered the long-term memory (LTM) of rats[23]. In this paper, we demonstrate that exposure to MC-LR induced neuronal damage and significant changes in learning ability and memory abilities. The ability of rats to adapt to new circumstances can be monitored in the open field[24]. We showed that the exposure to MC-LR can affect the exploratory and locomotor behavior of rats, indicating that MC-LR can influence the spontaneous performance in rats. It is well established that the dopaminergic circuits between the substantia nigra and the cortex are involved in the control of motor activity[25]. The changes we observed can be explained by the effect of MC-LR on dopamine receptors, which can influence locomotor behavior performance.

3 Conclusion

We assess the effects of MC-LR on cognitive ability and biochemical metabolism changes in the hippocampus region, which are associated with learning and memory. Our findings suggest that MC-LR can induce neuronal damage and alter neuronal function in the hippocampus. There are several limitations to the study. Other metabolites, including Cho, Cr and Lac, which may be involved in the hippocampal processes, are not investigated. Additionally, future studies should explore the relationship between alterations of NAA in hippocampus and impairment of capacity of learning and memory.

References

[1] Mezhoud K, Praseuth D, Puiseux-Dao S, et al. Global quantitative analysis of protein expression and phosphorylation status in the liver of the medaka fish (Oryzias latipes) exposed to microcystin-LR: Ⅰ. Balneation study [J]. *Aquatic Toxicology*, 2008, **86**(2): 166-175.

[2] Dietrich D, Hoeger S. Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): a reasonable or misguided approach? [J]. *Toxicology and Applied Pharmacology*, 2005, **203**(3): 273-289.

[3] Msagati T A, Siame B A, Shushu D D. Evaluation of methods for the isolation, detection and quantification of cyanobacterial hepatotoxins [J]. *Aquatic Toxicology*, 2006, **78**(4): 382-397.

[4] Zegura B, Zajc I, Lah T T, et al. Patterns of microcystin-LR induced alteration of the expression of genes involved in response to DNA damage and apoptosis [J]. *Toxicon*, 2008, **51**(4): 615-623.

[5] Fischer W J, Altheimer S, Cattori V, et al. Organic anion transporting polypeptides expressed in liver and brain mediate uptake of microcystin [J]. *Toxicology and Applied Pharmacology*, 2005, **203**(3): 257-263.

[6] Jiang Y, Zheng W, Long L, et al. Brain magnetic resonance imaging and manganese concentrations in red blood cells of smelting workers: search for biomarkers of manganese exposure [J]. *Neurotoxicology*, 2007, **28**(1): 126-135.

[7] Wang Q, Xie P, Chen J, et al. Distribution of microcystins in various organs (heart, liver, intestine, gonad, brain, kidney and lung) of Wistar rat via intravenous injection [J]. *Toxicon*, 2008, **52**(6): 721-727.

[8] Baganz D, Staaks G, Pflugmacher S, et al. Comparative study of microcystin-LR-induced behavioral changes of two fish species, Danio rerio and Leucaspius delineatus [J]. *Environ Toxicol*, 2004, **19**(6): 564-570.

[9] Bates T E, Strangward M, Keelan J, et al. Inhibition of N-acetylaspartate production: implications for 1H MRS studies in vivo [J]. *Neuroreport*, 1996, **7**(8): 1397-1400.

[10] Xi G, Hui J, Zhang Z, et al. Learning and memory alterations are associated with hippocampal N-acetylaspartate in a rat model of depression as measured by 1H-MRS [J]. *PloS One*, 2011, **6**(12): 28686-1-28686-9.

[11] Ju J, Ruan Q, Li X, et al. Neurotoxicological evaluation of microcystin-LR exposure at environmental relevant concentrations on nematode Caenorhabditis elegans [J]. *Environmental Science and Pollution Research*, 2013, **20**(3): 1823-1830.

[12] Li X B, Zhang X, Ju J, et al. Alterations in neurobehaviors and inflammation in hippocampus of rats induced by oral administration of microcystin-LR [J]. *Environ Sci Pollut Res*, 2014, **21**(21): 12419-12425.

[13] Loureiro M, Cholvin T, Lopez J, et al. The ventral midline thalamus (reuniens and rhomboid nuclei) contributes

to the persistence of spatial memory in rats [J]. *The Journal of Neuroscience*, 2012, **32**(29): 9947 - 9959.
[14] Frankland P W, Bontempi B. The organization of recent and remote memories [J]. *Nature Reviews Neuroscience*, 2005, **6**(2): 119 - 130.
[15] Bernasconi N, Bernasconi A, Caramanos Z, et al. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region [J]. *Brain*, 2003, **126**(2): 462 - 469.
[16] Sierosławska A, Rymuszka A, Bownik A, et al. The influence of microcystin-LR on fish phagocytic cells [J]. *Human & Experimental Toxicology*, 2007, **26**(7): 603 - 607.
[17] Meng X M, Zhu D M, Ruan D Y, et al. Effects of chronic lead exposure on 1H MRS of hippocampus and frontal lobes in children [J]. *Neurology*, 2005, **64**(9): 1644 - 1647.
[18] Xu X, Xiang Y, Yang M. Subacute toxicity of exogenous manganese on rat hippocampal neurons [J]. *Neural Regeneration Research*, 2008, **3**(5): 550 - 553.
[19] Ross A J, Sachdev P S. Magnetic resonance spectroscopy in cognitive research [J]. *Brain Research Reviews*, 2004, **44**(2): 83 - 102.
[20] Sandi C. Stress, cognitive impairment and cell adhesion molecules [J]. *Nature Reviews Neuroscience*, 2004, **5**(12): 917 - 930.
[21] Vorhees C V, Williams M T. Morris water maze: procedures for assessing spatial and related forms of learning and memory [J]. *Nature Protocols*, 2006, **1**(2): 848 - 858.
[22] Dash P, Mach S, Moore A. Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury [J]. *Journal of Neuroscience Research*, 2001, **63**(4): 313 - 319.
[23] Maidana M, Carlis V, Galhardi F G, et al. Effects of microcystins over short-and long-term memory and oxidative stress generation in hippocampus of rats [J]. *Chemico-Biological Interactions*, 2006, **159**(3): 223 - 234.
[24] Kondratova A A, Dubrovsky Y V, Antoch M P, et al. Circadian clock proteins control adaptation to novel environment and memory formation [J]. *Aging*, 2010, **2**(5): 285 - 297.
[25] Van den Heuvel D M, Pasterkamp R J. Getting connected in the dopamine system [J]. *Progress in Neurobiology*, 2008, **85**(1): 75 - 93.

微囊藻毒素-LR 对大鼠海马组织中 N-乙酰门冬氨酸及神经行为的作用

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摘要:探讨了微囊藻毒素-LR(MC-LR)对大鼠神经行为及海马组织中神经递质 N-乙酰门冬氨酸(NAA)水平的影响. 用不同剂量的 MC-LR 对雄性 sprague-dawley(SD)大鼠灌胃染毒 90 d 后,进行旷场实验和 Morris 水迷宫试验来评估大鼠的运动行为、空间学习能力及记忆功能. 行为学试验结果表明:MC-LR 染毒剂量为 2.00 μg/kg 时可显著损伤大鼠的学习记忆能力及自发活动能力;核磁共振成像(MRI)试验结果显示,染毒大鼠海马中的 NAA/Cr 比值显著降低($P < 0.05$). 研究提示,低剂量 MC-LR 能够导致神经行为及海马组织神经元的损伤.

关键词:微囊藻毒素-LR;N-乙酰门冬氨酸;神经行为;核磁共振成像;神经元
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