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Sodium Selenite inhibits mitophagy, downregulation and mislocalization of blood-testis barrier proteins of bovine Sertoli cell exposed to microcystin-leucine arginine (MC-LR) via TLR4/NF-kB and mitochondrial signaling pathways blockage

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ABSTRACT

This study was conducted to investigate the ameliorative effect of selenium on microcystin-LR induced toxicity in bovine Sertoli cells. Bovine Sertoli cells were pretreated with selenium (Na_2SeO_3) for 24 h after which selenium pretreated and non-pretreated Sertoli cells were cultured in medium containing 10% heat activated fetal bovine serum FBS + 80 μg/L MC-LR to assess its ameliorative effect on MC-LR toxicity. The results show that selenium pretreatment inhibited the MC-LR induced mitophagy, downregulation and mislocalization of blood-testis barrier constituent proteins in bovine Sertoli cells via NF-kB and cytochrome c release blockage. The observed downregulation of electron transport chain (ETC) related genes (mt-ND2, COX-1, COX-2) and upregulation of inflammatory cytokines (IL-6, TNF-α, IL-1β, IFN-γ, IL-4, IL-10, 1 L-13, TGFβ1) in non-pretreated cells exposed to MC-LR were ameliorated in selenium pretreated cells. There was no significant difference ($P > 0.05$) in the protein levels of blood-testis barrier constituent proteins (ZO-1, occludin, connexin-43, CTNNB1, N-cadherin) and mitochondria related genes (mt-ND2, COX-1, COX-2, ACAT1, mtTFA) of selenium pretreated Sertoli cell compared to the control. Taken together, we conclude that selenium inhibits MC-LR caused Mitophagy, downregulation and mislocalization of blood-testis barrier proteins of bovine Sertoli cell via mitochondrial and TLR4/NF-kB signaling pathways blockage.

1. Introduction

The increasing rate of male reproductive disorders and infertility has been attributed to exposure to environmental toxicants (Georges et al., 2011; Chen et al., 2011; Pant et al., 2013). Research findings of both laboratory and field studies revealed that apart from the liver, high concentrations of MC-LR also accumulate in the gonad Chen and Xie (2005); Zhang et al. (2007); Zhang et al. (2009); Chen et al. (2009); Papadimitriou et al. (2012); Wang et al. (2008)), indicating that the mammalian reproductive system is the second most important target organ of MC-LR (Wang et al., 2013). Recent studies have shown that MC-LR accumulation in the testis elicit toxic effects on the reproductive system (Yang et al., 2013; Lone et al., 2015). MC-LR represents a serious hazard to human reproductive health because animal studies provided strong evidences of interplay between exposure to MC-LR and reproductive toxicity (Sukenik et al., 2006; Chen et al., 2011; Li et al., 2008). Lesions, including a wide range of histological changes to both spermatogonia and Sertoli cells, were reported seen in immature male Japanese white rabbits treated with 12.5 μg MC-LR (Liu et al., 2010). According to the findings of Bu et al. (2006), it resulted in embryotoxicity in pregnant Kunming mice, indicating that MC-LR is a potential causative agent of reproductive toxicity in both males and females. In male reproductive system, Sertoli cells have been named the potential target of MC-LR for reproductive toxicity (Xiong et al., 2009; Li and Han, 2012). MC-LR induced oxidative stress in rat Sertoli cells (Dan et al., 2011). The in vitro toxicity of MC-LR to Sertoli cells was demonstrated by decreased cell viability (Li et al., 2008; Li and Han, 2012; Zhang et al., 2011). MC-LR has been found to be transported into sertoli cells resulting in cell injury (Chen et al., 2013). MC-LR could induce autophagy and apoptosis in a dose-dependent manner (Chen

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et al., 2013). Meanwhile, toll like receptors (TLRs) activated nuclear factor-kappa B (NF-kB) and mitochondrial signaling pathways have been implicated in mechanism of MC-LR induced inflammation and toxicity in mouse leydig and rat Sertoli cells (Adegoke et al., 2018a; Chen et al., 2017; Huang et al., 2016). Previous studies (Li and Han, 2012; Chen et al., 2013, 2016, 2017; Zhou et al., 2015) addressed the damages and toxicity caused by MC-LR while the detoxification and ameliorative measures remain unaddressed.

Our findings in previous research on influence of selenium on bovine Sertoli cells (Adegoke et al., 2018b) revealed that among different concentration of selenium tested, 0.5 mg/L in culture medium best upregulates expression of immune genes and blood-testis-barrier constituent proteins of bovine sertoli cell in vitro suggesting that it may have ameliorative measure against toxicity. Apart from our findings, other researchers have documented functions of selenium that qualify it as ameliorative measures against toxicity (Hawkes et al., 2001; Yu et al., 2015; Lee and Wan, 2002).

Selenium is an essential component of several enzymes important to optimal immune function (Hawkes et al., 2001). Selenoenzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) have been effective antioxidants that help protect immune cells from oxidative damage from reactive oxygen species released to kill engulfed bacteria (Ebert-Dumig et al., 1999). In addition, selenium supplementation has been confirmed to enhance lymphocyte activity and inhibit the oxidative stress of cells, which resulted to an increased number of lymphocytes (Lee and Wan, 2002). Moreover, selenoprotein W (SelW) was reported protected immune organs from oxidative stress by inhibiting inflammation and apoptosis (Yu et al., 2015). Selenium antioxidant and anti-inflammatory properties have been speculated to be responsible for its efficiency in the treatment of diseases whose major cause of their development is oxidative stress (Mario et al., 2011). The sertoli cells perform an indispensable roles in male reproduction and fertility. They provide nourishment and immunoprotective factors that are essential for the development and protection of sperm cells (Eddy et al., 2012; Mital et al., 2010). The tight junction between adjacent Sertoli cell form the blood-testis barrier which prevents the access of harmful substances from blood to the developing germ cells and at the same time preventing the entry of sperm related proteins from entering the blood circulation (Cheng and Mruk, 2012). Till date, the threat of MC-LR toxicity remains a concern to livestock and human reproductive health. This study therefore aims at investigating the ameliorative effect of selenium on MC-LR induced toxicity in bovine sertoli cells in vitro to make recommendation on mitigating MC-LR reproductive toxicity.

2. Materials and method

2.1. Isolation of bovine primary Sertoli cell

All animal works were approved by the Animal Ethics Committee of Northeast Agricultural University, Harbin, China and performed with strict adherence to the guide for the Care and Use of Animal for Research Purpose. The testes of twelve Holland Holstein newborn calves were obtained from Harbin Modern Biological Technical Co. Ltd, China. The differential adherent selection method as reported by Zheng et al. (2013), Adegoke et al. (2018b) and Yang et al. (2014) was used to isolate Sertoli cells. Briefly, the testes were obtained from newborn calves and quickly washed with sterile phosphate-buffered saline (PBS). Following the removal of tunica albuginea, the testes were cut into 1 mm^3 pieces. Then, the seminiferous tubules were transferred into new 60-mm Petri-dishes containing 5 mL of 1.0 mg/mL collagenase IV/DNase solution (Sigma, USA), and incubated at 37 °C in a humid environment with agitation for 20 min. After washing with PBS, the tubules were re-suspended in PBS and further digested with 2.5 mg/mL trypsin for 15–20 min at 37 °C. After digestion, the mixture was passed through a 120 μm stainless mesh and washed with PBS. After decantation of the enzyme solution by centrifugation at 200 ×*g* for 10 min, the cell pellet obtained was re-suspended in DMEM/F12 containing 10% fetal bovine serum (FBS). Floating and contaminating germ cells were removed by changing the medium several times 4–6 h after culture. After 36 h, Sertoli cells were treated with 20 mM Tris, pH 7.4 for 2 min to get a purity of > 98% (Mruk and Cheng, 2011; Lee et al., 2004). Fresh medium was added for culture at 37 °C in a humid environment containing 5% CO_2

2.2. Reagents and chemicals

MC-LR was purchased from Enzo Life Sciences (USA). Sodium selenite (Na2SeO3) as a selenium source was obtained from Sigma-Aldrich (USA). Rabbit anti-bovine occludin, connexin-43, zonula-occluden (ZO-1), CTNNB1, N-cadherin, caspase-3, cytochrome-c, TLR4, and NF-kB were purchased from Protein tech (Wuhan China). Mitophagy detection kit were purchased from Dojindo Molecular Technologies, Inc (Japan). Trizol was purchased from Invitrogen Corporation, USA. cDNA synthesis kit was purchased from ABM, Canada.

2.3. Cell culture

Bovine Sertoli cells were plated into 6 wells culture plates at the density of 6.0×10^4 cells/well in a culture medium containing 10% heat-activated fetal bovine serum (FBS, Hyclone USA), 1% penicillin-streptomycin (Corning, USA) for 24 h while those used for determination of the ameliorative effects of selenium on MC-LR toxicity were pretreated with 0.5 mg/L selenium (Na_2SeO_3) for 24 h. Following 24 h, selenium pretreated and non-pretreated bovine Sertoli cells were cultured in medium containing 10% heat activated FBS + 80 μg/L MC-LR at 37 °C and 5% CO_2 for 24 h. The control group was cultured in medium containing 10% FBS. Each treatment was replicated three times. Cells were collected from each treatment at 24 h for RNA isolation, reverse transcription synthesis of cDNA and western blots

2.4. RNA isolation and cDNA synthesis

Total RNA was extracted from the control, selenium pretreated (MC-LR + Se) and non-pretreated (MC-LR) bovine Sertoli cells using the Trizol® reagent (Invitrogen Corporation, USA) according to the manufacturer's instructions. The RNA preparations were treated with AccurRT Reaction Mix 4 × (ABM) to remove contaminating genomic DNA and incubated at 42 °C for 2 min. Approximately 2 μg of total RNA was reversed transcribed to complementary DNA (cDNA) using 5x All In One RT MasterMix in a volume of 20 μl and incubated at 25 °C for 10 min, then at 42 °C for 50 min. The reaction was inactivated at 85 °C for 5 min and Chilled on ice.

2.5. Real-time quantitative PCR analysis

Real-time quantitative PCR (RT-qPCR) was performed using the comparative method ($2^{-\Delta\Delta Ct}$) to quantify the expression of target genes. One μl of the cDNA product was used as the template for RT-qPCR which was done on QuantStudioTM Real-Time PCR System (Applied Biosystems, USA) using the FastStart universal SYBR Master (ROX) (Roche, USA) according to the manufacturer's instructions. The reaction mixture (10 μl) contained 5 μl of the FastStart universal SYBR Master (ROX), 0.3 μl of sense primer and 0.3 μl of anti-sense primer. All primers used are listed in Table 1. The reaction was performed at 94 °C for 5 min, followed by 40 cycles at 95 °C for 40 s, 56 °C for 40 s, and 72 °C for 40 s. Fluorescence was measured following each cycle and analyzed by the QuantStudioTM Real-Time PCR software v1.3 (Applied Biosystems, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene.