Increased cyclooxygenase-2 and nuclear factor-κB/p65 expression in mouse hippocampi after systemic administration of tetanus toxin

BING CHUN YAN1,2*, YONG HWAN JEON3*, JOON HA PARK4, IN HYE KIM4, JEONG-HWI CHO4, JI HYEON AHN4, BAI HUI CHEN5, HYUN-JIN TAE6, JAE-CHUL LEE4, JI YUN AHN7,8, DONG WON KIM8,9, JUN HWI CHO6, MOO-HO WON4 and SEONGKWEON HONG10

1Jiangsu Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Treatment of Senile Diseases, Yangzhou, Jiangsu 225009, P.R. China; 2Jiangsu Key Laboratory of Zoonosis, Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, Jiangsu 225009, P.R. China; 3Department of Radiology and 4Neurobiology, School of Medicine, Kangwon National University, Chunchon, Gangwon 200-701; 5Department of Physiology, College of Medicine, Kangwon National University, Chunchon, Gangwon 200-701; 6Department of Biomedical Science and Research Institute of Bioscience and Biotechnology, Hallym University, Chunchon, Gangwon 200-702; 7Department of Emergency Medicine, Sacred Heart Hospital, College of Medicine, Hallym University, Anyang, Gyeonggi 431-796; 8Department of Emergency Medicine, School of Medicine, Kangwon National University, Chunchon, Gangwon 200-701; 9Department of Emergency Medicine, Chunchon Sacred Heart Hospital, College of Medicine, Hallym University, Chunchon, Gangwon 200-702; 10Department of Surgery, School of Medicine, Kangwon National University, Chunchon, Gangwon 200-701, Republic of Korea

Received December 22, 2014; Accepted October 1, 2015

DOI: 10.3892/mmr.2015.4490

Abstract. Brain inflammation has a crucial role in various diseases of the central nervous system. The hippocampus in the mammalian brain exerts an important memory function, which is sensitive to various insults, including inflammation induced by exo/endotoxin stimuli. Tetanus toxin (TeT) is an exotoxin with the capacity for neuronal binding and internalization. The present study investigated changes in inflammatory mediators in the mouse hippocampus proper (CA1-3 regions) and dentate gyrus (DG) after TeT treatment. The experimental mice were intraperitoneally injected with TeT at a low dosage (100 ng/kg), while the control mice were injected with the same volume of saline. At 6, 12 and 24 h after TeT treatment, changes in the hippocampal levels of inflammatory mediators cyclooxygenase-2 (COX-2) and nuclear factor kappa-B (NF-κB/p65) were assessed using immunohistochemical and western blot analysis. In the control group, moderate COX-2 immunoreactivity was observed in the stratum pyramidal (SP) of the CA2-3 region, while almost no expression was identified in the CA1 region and the DG. COX-2 immunoreactivity was increased by TeT in the SP and granule cell layer (GCL) of the DG in a time-dependent manner. At 24 h post-treatment, COX-2 immunoreactivity in the SP of the CA1 region and in the GCL of the DG was high, and COX-2 immunoreactivity in the SP of the CA2/3 region was highest. Furthermore, the present study observed that NF-κB/p65 immunoreactivity was obviously increased in the SP and GCL at 6, 12 and 24 h after TeT treatment. In conclusion, the present study demonstrated that systemic treatment with TeT significantly increased the expression of COX-2 and NF-κB/p65 in the mouse hippocampus, suggesting that increased COX-2 and NF-κB/p65 expression may be associated with inflammation in the brain induced by exotoxins.

Introduction

In the immune system, excessive innate immunity in defense against bacterial or viral infections is a response to a variety of pathological conditions, such as chronic inflammation (1,2). During the inflammatory process, numerous pro-inflammatory mediators are generated and the major mediators of inflammatory events are members of the cyclooxygenase (COX) family (3). Two major COX isofoms, COX-1 and COX-2, catalyze the first step of the synthesis of prostaglandin E2 (PGE₂), which is the transformation of arachidonic acid (4). In particular, COX-2 expression is enhanced by stimuli from...
inflammatory mediators, including lipopolysaccharide (LPS) and several pro-inflammatory cytokines (5-8). In addition, COX-2 is located in the perinuclear membrane and exerts pathological effects though the biosynthesis of prostaglandins several hours after the stimuli (9,10).

Nuclear transcription factor kappa-B (NF-κB), one of the most important transcription factors, has critical roles in inflammation and immunity as well as cell proliferation, differentiation and survival (11). The activation of NF-κB involves the phosphorylation of inhibitor of NF-κB (IκB). Once IκB is phosphorylated, the resulting free NF-κB then translocates to the nucleus, where it binds to κB binding sites in the promoter regions of target genes and induces the transcription of pro-inflammatory mediators, including inducible nitric oxide synthase (iNOS), COX-2 and tumor necrosis factor (TNF)-α (2,10,12).

It is well known that brain inflammation has a crucial role in various diseases of the central nervous system (CNS), including Alzheimer's disease and epilepsy (13,14). The hippocampus in the mammalian brain, which is important in memory function (15), is a vulnerable to certain types of brain damage (16-20). In particular, it is highly sensitive to various insults, including inflammation induced by exo/endotoxin stimuli (5,21-24). Tetanus toxin (TeT), an exotoxin, has a capacity for neuronal binding and internalization (25-27). When systemically administered to animals, TeT reaches the CNS via retrograde transportation through nerve axons (28). A previous study by our group reported that systemic administration of TeT caused responses in the mouse hippocampus, including the secretion of certain inflammatory cytokines and glial activation, while neuronal death was not observed (27,29). However, in studies regarding TeT, few have focused upon the effect of TeT treatment on alterations in inflammatory mediators in the hippocampus. Therefore, to further investigate changes in inflammatory mediators induced by TeT, the present study observed changes in the immunoreactivities and protein levels of COX-2 and NF-κB/p65 in the mouse hippocampus after the systemic administration of TeT.

Materials and methods

Experimental animals. A total of 56 male ICR mice (BW; weight, 25-30 g; age, eight weeks) were purchased from the Jackson Laboratory (Maine, ME, USA). The animals were housed under standard conditions with a 12-h light/dark cycle at 23±3°C, 55±5% relative humidity and free access to food and water. All animal care and experimental procedures were performed according to the National Institutes of Health (NIH) guidelines (NIH Guide for the Care and Use of Laboratory Animals; NIH publication no. 85-23, 1985) and were approved by the Institutional Animal Care and Use Committee (IACUC) at Kangwon National University (approval no. KIACUC-140409-1; Chuncheon, Republic of Korea). All efforts were made to minimize animal suffering, as well as the number of animals used.

Treatment with TeT. The mice were intraperitoneally injected with a low dose of TeT (100 ng/kg; Dawinbio, Seoul, Republic of Korea) and the control animals were injected with the same volume of saline (pH 7.4). The mice (n=14 at each time-point) were sacrificed at 6, 12 and 24 h following treatment with TeT.

Preparation of tissue samples for histology. Animals were anesthetized with sodium pentobarbital (40 mg/kg; JW Pharmaceutical Co., Ltd., Seoul, Republic of Korea) and transcardially perfused with 0.1 M phosphate-buffered saline (PBS; pH 7.4; Sigma-Aldrich, St. Louis, MO, USA) followed by 4% paraformaldehyde (Sigma-Aldrich) in 0.1 M PBS. The brains were removed and post-fixed in 4% paraformaldehyde for 6 h. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight (Sigma-Aldrich). Subsequently, tissues were frozen and serially sectioned using a cryostat (Leica Microsystems GmbH, Wetzlar, Germany) to obtain 30-μm coronal sections, which were then collected in six-well plates containing PBS.

Immunohistochemical analysis. The expression of neuronal nuclei (NeuN), COX-2 and NF-κB/p65 was determined using immunohistochemistry. Coronal sections from control- and TeT-treated animals (n=7 for each time-point) were incubated with with 0.3% hydrogen peroxide (Sigma-Aldrich) in PBS for 30 min at room temperature. Subsequent to washing three times with PBS (each for 10 min), the sections were incubated with 10% normal goat serum (Vector Laboratories, Inc., Burlingame, CA, USA) in 0.05 M PBS for 30 min at room temperature. The samples were subsequently incubated with polyclonal rabbit anti-NeuN (ABN78; 1:1,000; Chemicon; EMD Millipore, Billerica, MA, USA), polyclonal rabbit anti-COX-2 (160126; 1:500; Chemicon) or polyclonal rabbit anti-NF-κB/p65 (sc-372; 1:2,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) antibody overnight at 4°C. Subsequent to washing three times with PBS (each for 10 min), the samples were exposed to biotinylated goat anti-rabbit immunoglobulin (Ig)G (BA-1000) and streptavidin-biotinylated hors eradish peroxidase complex (SA-5004) (1:200; Vector Laboratories, Inc.) for 2 h at room temperature. Antibodies were then visualized using 3,3′-diaminobenzidine tetrachloride (Sigma-Aldrich) in 0.1 M Tris-HCl buffer (pH 7.2) and samples were mounted on gelatin-coated slides. Following dehydration by immersion in serial dilutions of ethanol, the sections were mounted in Canada balsam (Kanto Chemical, Tokyo, Japan). In order to test the specificity of the immunostaining, a negative control sample was prepared using pre-immune serum instead of primary antibody (data not show).

Eight sections per animal were selected to quantitatively assess immunoreactivity for COX-2 and NF-κB/p65. Digital images of the hippocampus proper and dentate gyrus were captured using an AxioM1 light microscope (Carl Zeiss, Oberkochen, Germany) equipped with a digital camera (AxioCam MRC 5; Carl Zeiss) connected to a PC monitor. According to the method used in previous studies by our group (27,30), immunostaining intensities were semi-quantified using digital image analysis software (MetaMorph 4.01; Universal Imaging Corp., Bedford Hills, NY, USA). The level of the immunoreactivity was scored as (-), (±), (+), (+++) or (++++) representing no staining (gray scale value ≤49), weakly positive (gray scale value, 50-149), moderate (gray scale value, 100-149), high (gray scale value, 50-99) or very high (gray scale value ≥99), respectively.
Western blot analysis. The protein expression of COX-2 and NF-κB/p65 in the hippocampus of the control- and TeT-treated animals (n=7 at each time point) was determined by western blot analysis. Subsequent to sacrifice by cervical dislocation, mice were decapitated and the brains were removed. The brains were then serially and transversely cut into 400-µm section using a vibratome (Leica Microsystems GmbH), and the hippocampal region was dissected using a surgical blade. The tissues were homogenized in 50 mM PBS (pH 7.4) containing 0.1 mM ethylene glycol tetraacetic acid (pH 8.0), 0.2% Nonidet P-40, 10 mM ethylenediamine tetraacetic acid (pH 8.0), 15 mM sodium pyrophosphate, 100 mM β-glycerophosphate, 50 mM NaF, 150 mM NaCl, 2 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol (DTT) (All from Sigma-Aldrich). Subsequent to centrifugation at 16,000 x g for 20 min, the protein concentration of the supernatants was determined using a Micro Bicinchoninic Acid protein assay kit with bovine serum albumin as a standard (Pierce Biotechnology, Inc.). Aliquots containing 50 µg total protein were boiled in loading buffer containing 150 mM Tris (pH 6.8), 3 mM DTT, 6% SDS (Sigma-Aldrich), 0.3% bromophenol blue (Sigma-Aldrich) and 30% glycerol (Junsei Chemical Co., Ltd., Tokyo, Japan). Aliquots were then subjected to 5% SDS-PAGE and electrotransferred to nitrocellulose membranes (Pall Corp, Port Washington, NY, USA). To reduce background staining, the membranes were incubated with 5% non-fat dry milk (Sigma-Aldrich) in PBS containing 0.1% Tween 20 (Sigma-Aldrich) for 45 min and subsequently incubated with rabbit polyclonal anti-COX-2 (160126; 1:1,000; Chemicon), rabbit polyclonal NF-κB/p65 (sc-372; 1:1,000; Santa Cruz Biotechnology, Inc.) and rabbit polyclonal anti-β-actin (ab8227; 1:2,000; Abcam, Cambridge, UK) secondary antibody for 1 h at room temperature. Antibodies were visualized using an enhanced chemiluminescence kit (Pierce Biotechnology, Inc.). The blots were exposed to X-ray film (X-max; Kodak, Rochester, NY, USA) and scanned using a Hewlett Packard ScanJet 3200C at 300dpi (HP, Inc., Palo Alto, CA, USA). Subsequently, densitometric analysis was conducted using Scion Image software (Scion Corp., Frederick, MD, USA) in order to quantify the bands, with normalization to β-actin.

Statistical analysis. Values are expressed as the mean ± standard error of the mean. SPSS software, version 17.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Differences between groups were assessed using one-way analysis of variance. P<0.05 was considered to indicate a statistically significant difference between values.

Results
TeT does not cause neuronal damage in hippocampi of mice. In the present study, the neuronal damage/death in the hippocampi
Table I. Semi-quantitative analysis of cyclooxygenase-2 immunoreactivity in hippocampal regions after treatment of mice with 100 ng/kg TeT.

| Region | Layer | Time after TeT treatment |
|--------|-------|--------------------------|
|        |       | Control | 6 h | 12 h | 24 h |
| CA1    | SO    | -       | ±   | ±    | +    |
|        | SP    | ±       | +   | +    | ++   |
|        | SR    | -       | ±   | ±    | +    |
| CA2-3  | SO    | -       | ±   | ±    | +    |
|        | SP    | +       | ++  | ++   | +++  |
|        | SR    | -       | ±   | ±    | +    |
| DG     | ML    | -       | ±   | ±    | ±    |
|        | GCL   | ±       | +   | +    | ++   |
|        | PL    | -       | ±   | ±    | +    |

Levels of immunoreactivity were defined as five grades: (-), negative; (±), weakly positive; (+), moderate; (++) high; and (+++) very high. CA, cornu ammonis; DG, dentate gyrus; GCL, granule cell layer; ML, molecular layer; PL, polymorphic layer; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; TeT, tetanus toxin.

Figure 2. Immunohistochemical detection of COX-2 in the hippocampi of (A-C) the control mice and (D-L) 100 ng/kg TeT-treated mice. At 24 h post-treatment, COX-2 immunoreactivity was distinctively increased in the SP (asterisks) and GCL (asterisk and arrows). TeT, tetanus toxin; COX, cyclooxygenase; SP, stratum pyramidale; GCL, granule cell layer; SO, stratum oriens; SR, stratum radiatum; ML, molecular layer; PL, polymorphic layer. Scale bar, 50 µm.
Table II. Semi-quantitative analysis of nuclear factor-κB/p65 immunoreactivity in hippocampal regions after treatment with 100 ng/kg TeT.

| Area | Layer | Control | 6 | 12 | 24 |
|------|-------|---------|----|----|----|
| CA1  | SO    | ±       | +  | +  | +  |
|      | SP    | +       | ++ | ++ | ++ |
|      | SR    | ±       | +  | +  | ++ |
| CA2-3| SO    | ±       | +  | +  | ++ |
|      | SP    | +       | ++ | ++ | ++ |
|      | SR    | ±       | +  | +  | +  |
| DG   | ML    | -       | +  | +  | +  |
|      | GCL   | ±       | ++ | ++ | ++ |
|      | PL    | ±       | +  | +  | +  |

Levels of immunoreactivity were defined as four grades: (-), negative; (±), weakly positive; (+), moderate; and (++), high. CA, cornu ammonis; DG, dentate gyrus; GCL, granule cell layer; ML, molecular layer; PL, polymorphic layer; SO, stratum oriens; SP, stratum pyramidale; SR, stratum radiatum; TeT, tetanus toxin.

Figure 3. Immunohistochemical detection of NF-κB/p65 in the hippocampi of (A-C) control mice and (D-L) 100 ng/kg TeT-treated mice. NF-κB/p65 immunoreactivity was apparently increased in the SP (asterisks) and GCL (asterisks) after TeT treatment. TeT, tetanus toxin; NF, nuclear factor; SP, stratum pyramidale; GCL, granule cell layer; SO, stratum oriens; SR, stratum radiatum; ML, molecular layer; PL, polymorphic layer. Scale bar, 50 µm.
of mice treated with TeT was observed by immunostaining for NeuN (Fig. 1). NeuN-immunoreactive cells were observed in the hippocampus proper (CA1-3 regions) and dentate gyrus of the control group (Fig. 1A-D). In the TeT-treated groups, the distribution pattern of NeuN-immunoreactive cells was not different from that in the control group at any time-point after TeT treatment (Fig. 1E-P).

**TeT increases COX-2 expression in mouse hippocampi.** In the control group, COX-2 immunoreactivity observed in the stratum pyramidal of the CA1 region was low and that in the stratum pyramidal of the CA2/3 region was moderate; furthermore, low COX-2 immunoreactivity was observed in the granule cell layer of the dentate gyrus (Table I; Fig. 2A-C).

At 6 h post-treatment, COX-2 immunoreactivity was slightly increased in the stratum pyramidal and granule cell layer in all the hippocampal sub-regions compared with that in the control group (Table I; Fig. 2D-F). COX-2 immunoreactivity at 12 h post-treatment was similar to that at 6 h post-treatment (Table I, Fig. 2G-I). Of note, at 24 h post-treatment, COX-2 immunoreactivity in the stratum pyramidal and granule cell layer was significantly increased compared with that at 12 h post-treatment; in particular, the immunoreactivity in the stratum pyramidal of the CA2/3 region was high (Table I; Fig. 2J-L).

**TeT increases NF-κB/p65 expression in mouse hippocampi.** In the control group, moderate NF-κB/p65 immunoreactivity was detected in the stratum pyramidal of the CA1-3 regions, while low NF-κB/p65 immunoreactivity was identified in the granule cell layer of the dentate gyrus (Table II; Fig. 3A-C).

At 6 h post-treatment, NF-κB/p65 immunoreactivity was markedly increased in all layers of all hippocampal sub-regions compared with those in the control group (Table II; Fig. 3D-F). At 12 h post-treatment, the pattern of NF-κB/p65 immunoreactivity was similar to that at 6 h post-treatment (Table II; Fig. 3G-I). At 24 h post-treatment, NF-κB/p65 immunoreactivity in all layers was not significantly changed compared with that at 12 h post-treatment; however, the immunoreactivity was higher than that in the control group (Table II; Fig. 3J-L).

**Effects of TeT on COX-2 and NF-κB p65 protein levels.** Western blot analysis was performed to confirm the changes in the protein levels of COX-2 and NF-κB/p65 in the mouse hippocampi after TeT treatment (Fig. 4). COX-2 protein levels steadily increased in a time-dependent manner until 24 h post-treatment, while NF-κB/p65 levels were significantly increased at 6 h post-treatment and then remained constant until 24 h post-treatment (Fig. 4).

**Discussion**

TeT has been commonly used in experimental studies on neurological disorders or animal models of diseases (31,32). It was reported that intrahippocampal TeT injection induced neuronal damage or death in certain brain regions, particularly in the hippocampus (21,33). However, results of previous studies regarding the induction of neuronal damage or death were inconsistent due to differences in animals, dosages of TeT, routes of administration and time of sacrifice (27,29,34).

In the present study, following intraperitoneal injection of TeT, no neuronal damage or loss in any of the sub-regions of the hippocampus was identified using immunohistochemical analysis of NeuN expression.

In the present study, COX-2 immunoreactivity and expression levels were significantly increased compared with those in the control group at 24 h after injection of 100 ng/kg TeT; in particular, enhanced COX-2 immunoreactivity was identified in the stratum pyramidal of the hippocampus proper (CA1-3 regions) and in the granule cell layer of the dentate gyrus. In analogy with this finding, a previous study showed that systemic administration of LPS increased COX-2 immunoreactivity in the mouse hippocampus (5). The brain and the immune system are extensively interconnected and regulate each other (35,36). The communication within the hippocampus contributes to region-specific vulnerability to certain insults (35). In the hippocampus, certain types of stimulation, such as bacterial toxin stimuli, evoke a rapid immune response accompanied with specific cellular and molecular changes (5,37). For example, intraperitoneal treatment with LPS caused a reduction in the mRNA levels of interleukin-1β and 6 in the cerebral cortex and hippocampus of mice (38). In addition, upon activation with LPS, microglia in the olfactory bulb were shown to secrete a variety of cytokines in a rat model of neuroinflammation (39). A previous study also showed that microglia in rat hippocampi were activated following injection of TeT into the ventral hippocampus (40). Furthermore, recent studies by our group revealed marked changes in inflammatory cytokines accompanied with glial activation in all hippocampal sub-regions after intraperitoneal administration of 100 ng/kg TeT (27,29).

NF-κB, a heterodimer of its p65 and p50 sub-units, is located in the cytoplasm as an inactive complex bound to its inhibitor, which is phosphorylated and subsequently degraded and dissociated to produce activated NF-κB (10,12). The present

---

Figure 4. Western blot analysis of COX-2 and NF-κB/p65 in the hippocampi of control- and 100 ng/kg TeT-treated mice (n=7 in each group). (A) Representative western blot. ROD of (B) COX-2 and (C) NF-κB/p65 normalized to β-actin. Values are expressed as the mean ± standard error of the mean. *P<0.05 vs. control group; †P<0.05 vs. preceding time-point. NF, nuclear factor; COX, cyclooxygenase; TeT, tetanus toxin; ROD, relative optical density.
study observed changes of NF-κB/p65 immunoreactivity and expression levels in mouse hippocampi after systemic administration of TeT. NF-κB/p65 immunoreactivity was increased in the cytoplasm of pyramidal neurons and granule cells after TeT treatment. It was reported that NF-κB was activated by numerous different types of stimuli and NF-κB regulated the expression of COX-2 in the CNS (41). Therefore, these results lead to the hypothesis that the increased COX-2 expression may be closely associated with the increase of NF-κB/p65 immunoreactivity in neurons after TeT treatment.

In conclusion, the present study suggested that the systemic administration of 100 ng/kg TeT did not cause neuronal damage; however, it markedly increased the expression of COX-2 and NF-κB/p65 in mouse hippocampi after TeT treatment.

Acknowledgements

The authors would like to thank Mr. Seung Uk Lee (Department of Neurobiology, School of Medicine, Kangwon National University, Chuncheon, Republic of Korea) for his technical assistance in the experiments of this study. The present study was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (grant no. 2010-0010580) and by a 2014 Research Grant from Kangwon National University (Chuncheon, Korea).

References

1. Chae HS, Kang OH, Lee YS, Choi JG, Oh YC, Jang HJ, Kim MS, Kim JH, Jeong SI and Kwon DY: Inhibition of LPS-induced iNOS, COX-2 and inflammatory mediator expression by parthenol through the MAPKs inactivation in RAW 264.7 cells. Am J Chin Med 37: 181-194, 2009.
2. Kim JB, Han AR, Park EY, Kim JY, Cho W, Lee J, Seo EK and Lee KT: Inhibition of LPS-induced iNOS, COX-2 and cytokines expression by poncirin through the NF-kappaB inactivation in RAW 264.7 macrophage cells. Biol Pharm Bull 30: 2345-2351, 2007.
3. Matheus AS, Coelho AM, Sampietre S, Patzina R, Jukemura J, Cunha JE and Machado MC: Effect of inhibition of prostaglandin E2 production on pancreatic infection in experimental acute pancreatitis. HPB (Oxford) 9: 392-397, 2007.
4. Ji K and Tsirka SE: Inflammation modulates expression of iNOS, COX-2 and inflammatory mediator expression by poncirin through the NF-kappaB inactivation in RAW 264.7 macrophage cells. Biol Pharm Bull 30: 2345-2351, 2007.
5. Darlington CL: Cytosolic glucocorticoid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. Exp Brain Res 162: 309-314, 2005.
6. Benn SC, Ay I, Bastia E, Chia RJ, Celia SA, Pepinsky RB, Fishman PS, Brown RH Jr and Francis JW: Tetracosactin fragment C fusion facilitates protein delivery to CNS neurons from cerebrospinal fluid in mice. J Neurochem 95: 765-770, 2005.
7. Baietto et al.: Inhibition of LPS-induced iNOS, COX-2 and cytokines expression by poncirin through the NF-kappaB inactivation in RAW 264.7 macrophage cells. Biol Pharm Bull 30: 2345-2351, 2007.
8. Feng J, Wu Q, Zhang D and Chen BY: Hippocampal impairments are associated with intermittent hypoxia of obstructive sleep apnea. Chin Med J (Engl) 125: 106-119, 2012.
9. Zuloaga KL, O'Connor DT, Handa RJ and Gonzales RJ: Estrogen receptor beta dependent attenuation of cytokine-induced cyclooxygenase-2 by androgens in human brain vascular smooth muscle cells and rat mesenteric arteries. Steroids 77: 835-844, 2012.
10. Lee CH, Park JH, Kim IH, Choopani S, Choopani S, Choopani S, Chopani S and Chopani S: Role of baicalin in regulating Toll-like receptor 2/4 after ischemic neuronal injury. Chin Med J (Engl) 125: 1586-1593, 2012.
11. Kudah A and Chopra K: Effect of sesamol on diabetes-associated cognitive decline in rats. Exp Brain Res 185: 411-420, 2008.
12. Zhang Y, Yang X, Wang Y, Yang L, Liu P, Bi J, Zheng Y, Smith PF and Darlington CL: Cytosolic glucocorticoid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. Exp Brain Res 162: 309-314, 2005.
13. McAleer A, Coelho AM, Sampietre S, Patzina R, Jukemura J, Cunha JE and Machado MC: Effect of inhibition of prostaglandin E2 production on pancreatic infection in experimental acute pancreatitis. HPB (Oxford) 9: 392-397, 2007.
14. Ji K and Tsirka SE: Inflammation modulates expression of iNOS, COX-2 and inflammatory mediator expression by poncirin through the NF-kappaB inactivation in RAW 264.7 macrophage cells. Biol Pharm Bull 30: 2345-2351, 2007.
15. Darlington CL: Cytosolic glucocorticoid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. Exp Brain Res 162: 309-314, 2005.
16. Feng J, Wu Q, Zhang D and Chen BY: Hippocampal impairments are associated with intermittent hypoxia of obstructive sleep apnea. Chin Med J (Engl) 125: 696-701, 2012.
17. Li HY, Yuan ZY, Wang YG, Wan HJ, Hu J, Chai YS, Lei F, Xing DM and DU LJ: Role of baicalin in regulating Toll-like receptor 2/4 after ischemic neuronal injury. Chin Med J (Engl) 125: 1586-1593, 2012.
18. Bagetta G, Corasaniti MT, Nisticò G and Bowery NB: Behavioural and neuropathological effects produced by tetanus toxin injected into the hippocampus of rats. Neuropharmacology 29: 765-770, 1990.
19. Kudah A and Chopra K: Effect of sesamol on diabetes-associated cognitive decline in rats. Exp Brain Res 185: 411-420, 2008.
20. Zhang Y, Yang X, Wang Y, Yang L, Liu P, Bi J, Zheng Y, Smith PF and Darlington CL: Cytosolic glucocorticoid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. Exp Brain Res 162: 309-314, 2005.
21. McAleer A, Coelho AM, Sampietre S, Patzina R, Jukemura J, Cunha JE and Machado MC: Effect of inhibition of prostaglandin E2 production on pancreatic infection in experimental acute pancreatitis. HPB (Oxford) 9: 392-397, 2007.
22. Ji K and Tsirka SE: Inflammation modulates expression of iNOS, COX-2 and cytokines expression by poncirin through the NF-kappaB inactivation in RAW 264.7 macrophage cells. Biol Pharm Bull 30: 2345-2351, 2007.
23. Darlington CL: Cytosolic glucocorticoid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. Exp Brain Res 162: 309-314, 2005.
31. Halliday AJ, Campbell TE, Nelson TS, McLean KJ, Wallace GG and Cook MJ: Levetiracetam-loaded biodegradable polymer implants in the tetanus toxin model of temporal lobe epilepsy in rats. J J Clin Neurosci 20: 148-152, 2013.
32. Juruska P, Shtaya AB, Bodansky DM, Chang WC, Gray WP and Jefferys JG: Dentate gyrus progenitor cell proliferation after the onset of spontaneous seizures in the tetanus toxin model of temporal lobe epilepsy. Neurobiol Dis 54: 492-498, 2013.
33. Bagetta G, Nistico G and Bowery NG: Characteristics of tetanus toxin and its exploitation in neurodegenerative studies. Trends Pharmacol Sci 12: 285-289, 1991.
34. Lee CL, Hannay J, Hrachovy R, Rashid S, Antalffy B and Swann JW: Spatial learning deficits without hippocampal neuronal loss in a model of early-onset epilepsy. Neuroscience 107: 71-84, 2001.
35. Urra X, Obach V and Chamorro A: Stroke induced immunodepression syndrome: From bench to bedside. Curr Mol Med 9: 195-202, 2009.
36. Williamson LL and Bilbo SD: Chemokines and the hippocampus: A new perspective on hippocampal plasticity and vulnerability. Brain Behav Immun 30: 186-194, 2013.
37. Quan N, Whiteside M and Herkenham M: Time course and localization patterns of interleukin-1beta messenger RNA expression in brain and pituitary after peripheral administration of lipopolysaccharide. Neuroscience 83: 281-293, 1998.
38. Henry CJ, Huang Y, Wynne A, Hanke M, Himler J, Bailey MT, Sheridan JF and Godbout JP: Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior and anhedonia. J Neuroinflammation 5: 15, 2008.
39. Doursout MF, Schurdell MS, Young LM, Osuagwu U, Hook DM, Poindexter BJ, Schiess MC, Bick DL and Bick RJ: Inflammatory cells and cytokines in the olfactory bulb of a rat model of neuroinflammation; insights into neurodegeneration? J Interferon Cytokine Res 33: 376-383, 2013.
40. Shaw JA, Perry VH and Mellanby J: Tetanus toxin-induced seizures cause microglial activation in rat hippocampus. Neurosci Lett 120: 66-69, 1990.
41. O'Neill LA and Kalschmidt C: NF-kappa B: A crucial transcription factor for glial and neuronal cell function. Trends Neurosci 20: 252-258, 1997.