In vitro toxicity of colistin on primary chick cortex neurons and its potential mechanism

Chongshan Dai\textsuperscript{a}, Dexian Zhang\textsuperscript{a,b}, Ruixia Gao\textsuperscript{c}, Xiuying Zhang\textsuperscript{a}, Jian Li\textsuperscript{d,∗∗}, Jichang Li\textsuperscript{a,∗}

\textsuperscript{a} College of Veterinary Medicine, Northeast Agricultural University, 59 Mucai Street, Xiangfang District, Harbin 150030, PR China
\textsuperscript{b} College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110161, PR China
\textsuperscript{c} College of Life Science, Northeast Agricultural University, 59 Mucai Street, Xiangfang District, Harbin 150030, PR China
\textsuperscript{d} Facility for Anti-infective Drug Development and Innovation, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Victoria 3052, Australia

\textbf{A B S T R A C T}

Colistin is increasingly used as the last-line therapy for infections caused by Gram-negative 'superbugs'. Although colistin neurotoxicity was reported in the literature, there has no data on its mechanism. In the present study, we examined the effect of colistin on primary chick neuron cells, which were treated with 0.83, 4.15 and 8.3 µg/mL colistin for 6, 12 and 24 h. Cell viability was evaluated with 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assays after exposure to colistin. Formation of reactive oxygen species (ROS), nuclear morphology, caspase-3 activity and internucleosomal DNA fragmentation were examined. The results showed that, compared with the control, no significant change was observed in cell viability, ROS formation and caspase-3 activity in cells treated for 6, 12 and 24 h with 0.83 µg/mL colistin. However, in the 4.15 and 8.3 µg/mL colistin-treated groups, the viability of chick primary neurons significantly decreased at 12 and 24 h, respectively; caspase-3 activities were significantly increased to 5.1 and 7.4 fold at 6 h, more earlier than the changes of ROS, which was significant increased to 124.5% and 143.5% (P<0.01) of control at 12 h, respectively. The apoptosis of neuron cells was revealed by both nuclear morphological observations and internucleosomal DNA fragmentation in 4.15 and 8.3 µg/mL colistin-treated groups at 6, 12 and 24 h. Our data demonstrated that colistin can induce apoptosis in primary chick cortex neurons through caspase-3 activation, which may be contributed with ROS-dependent and independent mechanism.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Over the last decade, there has been a remarkable increase in resistance to almost all currently available antibiotics in Gram-negative 'superbugs' (e.g. Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae), especially the emergence of New Delhi metallo-β-lactamase-producing Enterobacteriaceae in clinical patients (Docobo-Pérez et al., 2012; Dai et al., 2012, 2013; Li et al., 2006; Talbot et al., 2006;
Rasheed et al., 2013). Unfortunately, no new antibiotics will be available for these ‘superbugs’ at least a decade (Payne et al., 2007). Colistin, which had been replaced by “less” toxic aminoglycosides and fluoroquinolones in the 1970s due to its potential nephro- and neurotoxicity, has re-entered and even as the last-line therapeutic option in clinical use in recent years with the development of ‘superbugs’ (Li et al., 2005; Falagas et al., 2005; Evans et al., 1999; Dai et al., 2013).

Despite the special physicochemical properties of colistin (the larger molecular and lower lipid solubility) resulting to the low blood–brain barrier (BBB) penetration, it has the ability to enter brain mediated neurotoxicity when the BBB was injured (Jin et al., 2009, 2011, 2012). The interaction of colistin with neurons that have high lipid content has been associated with neurotoxicity, including peripheral, oral, and facial parenthesis, vertigo, visual disturbances, hallucinations, mental confusion, ataxia, and seizures. In a recent study (Wallace et al., 2008), signs of overt neurotoxicity, notably muscular weakness and ataxia were observed in rats after a single dose of 150 mg/kg colistin methanesulfonate, a non-active prodrug of colistin. In our previous studies (Dai et al., 2012, 2013), the significant neurotoxicity including peripheral and central neurotoxicity were certified by the clinical signs and pathology changes when the female mice were intravenously injected colistin sulfate 15 mg/kg for 7 days. These animal experiments and the previous literatures have certified that colistin-induced neurotoxicity is positively relative with the time and dose of its administration in patients and animals (Dai et al., 2012, 2013; Landman et al., 2000; Falagas and Kasiakou, 2006). However, up to now, there is no proper information on the mechanism of its potential neurotoxicity. This paper is to investigate the toxicity in vitro chick primary neurons and further study the potential mechanism of colistin induced neurotoxicity.

2. Materials and methods

2.1. Materials

Colistin (sulfate, 99% purity) was purchased from Max Pharmaceutical Company (Wuhan, China). Poly-l-lysine, 2,7-dichlorofluorescein diacetate (DCFH-DA), trypsin, anhydrous dimethyl sulfoxide (DMSO), 3-[4,5-dimethylthiazol-2-y]-2,5-diphenyl tetrazolium bromide (MTT) and colorimetric caspase-3 substrate (7-amino-4-methylcoumarin-N-acetyl-l-aspartyl-l-glutamyl-l-valyl-l-aspartic acid amide, Ac-DEVD-AMC) were purchased from Sigma–Aldrich (New York, USA). l-Glutamine and cytosine β-d-arabinofuranoside (ARA-C) were from Invitrogen (New York, USA). For cell culture experiments, Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), penicillin (5000 U/mL) and streptomycin (5000 μg/mL) were obtained from Celbio (Milan, Italy). Flasks and 96-well plates were from Costar, Corning Inc. (Corning, New York, USA). DNA Fragmentation Detection ELISAFlus® kit was from Roche Applied Sciences, Switzerland.

2.2. Cell culture of chick primary neuron

Primary cultures of chick cortical neurons were prepared as described previously (Hartbauer et al., 2001; Yoo et al., 2002) with modifications. Briefly, the cerebral cortex of 6–8 d old chick embryo (Laboratory Animal Center of Northeast Agricultural University, Harbin, China) was dissected under sterile conditions and rapidly pooled into D-Hank’s balanced with sodium salts without calcium and magnesium. The meningeal tissue was quickly peeled off. The cortical tissue was minced before further enzymatic dissociation with 0.25% trypsin at 37 °C for 10 min, then transferred into 10 mL DMEM supplemented with 20% heat-inactivated fetal calf serum to terminate trypsinization. The tissue samples were mechanically dissociated by repeated triturating with a pipette. Dissociated cells were passed through sterile nylon gauze (80-μm pore size) and seeded in 25-cm² plastic flasks and 35-mm dishes coated with poly-l-lysine at a density of 4 × 10⁵ cells/cm². Cells were suspended in DMEM containing 20% FBS, 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin, and cultured at 37 °C in a humidified incubator with 5% CO₂ for 48 h. The neurons were then purified by 5 μg/mL ARA-C. The purified cells reached a confluence after culturing for another 4 days. The primary cells were digested with 0.125% trypsin in Hank’s Balanced Salt Solution (HBSS; CaCl₂ 1.26 mM, KCl 5.37 mM, KH₂PO₄ 0.44 mM, MgCl₂ 0.49 mM, MgSO₄ 0.41 mM, NaCl 140 mM, NaHCO₃ 4.17 mM, Na₂HPO₄ 0.34 mM and glucose 5.55 mM), and re-seeded at a density of 4 × 10³ cells/cm² onto poly-l-lysine-coated coverslips and 96-well plates. The culture medium was refreshed every 2 days.

After incubation in DMEM with 10% FBS for 5 days, cells were collected for experimentation. These culture cells were used in the experiment (see Fig. 1) and they were divided into 4 different groups: Group 1 to 3, the cells were exposed to 1, 5 and 10 μg/mL colistin sulfate, respectively equally to 0.83, 4.15 and 8.3 μg/mL colistin base by the biologically relevance, and a control group (0.2% DMSO with no colistin). Cells in each group were subsequently cultured at 37 °C in a humidified incubator with 5% CO₂ and examined at various time points. All experiments were conducted in three replicates.

2.3. Measurement of cell viability by MTT assay

Chick neuron cells were plated at a density of 1 × 10⁶ cells/well in a 96-well plate, cultured for 5 days and then treated with colistin at 0.83, 4.15 and 8.3 μg/mL for 24 h. An aliquot (50 μL) of 5 mg/mL MTT was pipetted into each well and the plate was incubated for 4 h at 37 °C in the presence of 5% CO₂. The medium was discarded and 150 μL DMEM was added to each well followed by incubation and mechanical shaking for 10 min at room temperature and optical density (OD) at 540 nm was measured using an ELISA plate reader (Bio-RAD, NY, USA). All results were normalized to the OD values of an identically conditioned well but without cell culture. For trypan blue staining, 10 μL of trypan blue solution (4 mg/mL in PBS) was mixed with 10 μL of cell suspension from each sample and incubated at room temperature for 2 min. Unstained live cells were counted using a hemacytometer (JWFU, Shanghai, China). For cell counting, at least four optical fields with 200 cells were observed.
2.4. *Measurement of reactive oxygen species (ROS)* formation

Primary cells were cultured on a 96-well plate as described above. After exposure to colistin for 6, 12 and 24 h, respectively at 0.83, 4.15 and 8.3 μg/mL, cells were washed twice in phosphate-buffered saline (PBS) and DCFH-DA was diluted in fresh DMEM medium at a concentration of 10 μM DCFH-DA for 20 min at room temperature according to the manufacturer’s instructions. DCFH-DA was deacetylated intracellularly by nonspecific esterase, which was further oxidized by ROS to the fluorescent compound 2,7-dichlorofluorescein (DCF). The fluorescence was monitored at an excitation wavelength of 485 nm and emission wavelength of 535 nm using a Wallac Victor II Multilabel Counter (Perkin Elmer, Vernon Hills, USA).

2.5. *Examination of nuclear morphology*

Cells were exposed to colistin at different concentrations in high potassium HBSS (25 mM KCl) at 37 °C for 24 h, with 5% CO₂ and constant humidity. Condensed and fragmented nuclei were evaluated by intercalation of the fluorescent probe Hoechst 33258 (final concentration 0.010 mg/mL) into nuclear DNA. Cell-permeable Hoechst 33258 was employed to label both intact and apoptotic nuclei. Visualization was conducted at an excitation wavelength of 480 nm and emission wavelength of 520 nm by fluorescence microscopy (Olympus IMT-2) and images were taken with an Olympus Camedia C-5060 digital camera. All experiments were conducted in 3 replicates. Cells were viewed to visualize the nuclear morphology, and the number of apoptotic nuclei, which look smaller than normal and in which the chromatin appears condensed, was counted and compared with the total number of cells in each field. For cell counting, at least four optical fields with 200 cells were observed.

2.6. *Measurement of caspase-3 activity*

Caspase-3 activity was measured with an ApoAlert Caspase Assay Kit (Clontech Laboratories, USA). Briefly, cells were cultured in 25 cm² tissue culture flasks and then exposed to colistin at the three different concentrations for 2, 6 and 12 h. Cells were washed with PBS and collected into tubes. The tubes were centrifuged and the supernatants were removed. Cell pellet was suspended in lysis buffer (10 mM Tris, 1% Triton X-100 in PBS, pH 7.5), placed on ice for 20 min and centrifuged (30 min, 12,000 × g) at 4 °C. Aliquots of cell lysate (25 μg protein) were dissolved in 175 μL protease assay buffer (20 mM HEPES at pH 7.5, 2 mM dithiothreitol and 10% glycerol), then 25 μL Ac-DEVD-AMC (15 μM) in the assay buffer was added to start the reaction. After 1-h incubation at 37 °C in dark, substrate cleavage was measured fluorometrically in black 96-well plates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. Fluorescence values of cell-free wells containing 15 μM Ac-DEVD-AMC in the assay buffer were subtracted from the fluorescence values of cell samples. Cell lysate containing cytosolic fractions was collected and total protein concentration was determined by BCA™ protein assay kit (Fierce, Bonn, Germany). The microplate reader output corresponding to caspase-3 activity was corrected for the total protein. Caspase-3 activity was reported as a comparative activity in different groups compared with the control group (arbitrarily set at 1).

2.7. *Internucleosomal DNA fragmentation*

Quantification of DNA fragmentation was measured with the agarose gel electrophoresis method and cell death detection ELISAPlus kit. Cells were exposed to the three different concentrations of colistin in high potassium HBSS and harvested in 400 μL PBS before DNA isolation. An apoptotic DNA ladder kit (Roche Diagnostics Scandinavia AS) was employed. After lysis of the cells in binding buffer, the lysate was transferred to a filter tube with glass fiber fleece and centrifuged at 5000 × g for 1 min at 4 °C. During this procedure, DNA bound specifically to the surface of glass fibers. Residual impurities were removed by a washing step and subsequently DNA was eluted in elution buffer. After incubation for 30 min at 37 °C in the presence of 2 μg/mL RNase, isolated DNA was mixed with loading buffer (0.1% sodium dodecyl sulfate, 3% glycerol, 0.4% bromphenol blue). Fragments were separated by electrophoresis (at 75 V for 90 min) on a 2% agarose gel containing 0.008 μg/mL ethidium bromide. DNA bands were visualized with a UV light source and the gel was photographed.
Fig. 2 – Measurement of neuronal viability by MTT after exposure to colistin for 6, 12 and 24 h, respectively. Data are presented as mean (% of control) ± SEM of three independent measurements. *P < 0.05, **P < 0.01 versus DMSO-exposed control group.

For ELISA, cells were lysed with DNA lysis buffer (200 μL) and the cell lysate (20 μL) was mixed with an Ab solution provided by the supplier (80 μL) in 96-well plates at room temperature for 2 h. After washing with the incubation buffer, the substrate buffer (100 μL) was added to each well and incubated for 10 min at 37 °C. OD was then measured using a microplate reader at a wavelength of 405 nm.

2.8. Statistical analyses

All data were expressed as mean ± SEM. Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by followed by LSD and Dunnett’s T3 test, which were provided by SPSS 11.5 statistical software (Chicago, USA). The P values less than 0.05 were considered to be significant.

3. Results

3.1. Effect of colistin on neuronal cell viability

The effects of colistin on neuronal cell viability were showed in Fig. 2. Respectively exposure of chick neuronal cells used different dose of colistin for 6, 12 and 24 h, there was not significantly decrease in cell viability at the 0.83 μg/mL colistin group. However, at 6 h after chick neuronal cells exposed colistin 8.30 μg/mL, the cell viability began to significantly decrease to 93.53% (P < 0.05), compared with the control group. At 12 h and 24 h after cells exposed colistin 4.15 and 8.30 μg/mL, the cell viability both significantly decreased. Especially at 24 h, the 4.15 and 8.30 μg/mL colistin treatment significantly (both P < 0.01) decreased the percentage of viable cells to approximately 85% and 65%, respectively of the control (Fig. 2).

3.2. Effect of colistin on ROS formation of chick primary neuron cells

ROS formation of chick primary neuron cells after colistin treatment was both time and concentration dependent, the results showed in Fig. 3. ROS formation did not increase after exposure to colistin at all three concentrations at 6 h.

Fig. 3 – Effect of colistin on ROS formation in neuron cells. Data are presented as mean (% of control) ± SEM of three independent measurements. *P < 0.05, **P < 0.01 versus DMSO-exposed control group.

Treatment with colistin at 0.83 μg/mL significantly (P < 0.01) increased ROS formation only at 24 h, while exposure to 4.15 and 8.30 μg/mL colistin significantly increased ROS formation to 124.5% and 140% (both P < 0.01), 143.5% and 180% (both P < 0.01), respectively at both 12 h and 24 h compared with the control group (Fig. 3).

3.3. Effect of colistin on cell morphology

Colistin induced apoptotic characteristics in nuclear morphology associated with chromatin condensation (Fig. 5). After 6, 12 and 24-h exposure to colistin at 8.30 μg/mL, 20.4%, 30.2 and 43.5% (all P < 0.01) apoptotic cells were observed, respectively. After 12 and 24-h exposure to colistin 4.15 μg/mL, 16.5% and 28.2% (P < 0.05 or 0.01) apoptotic cells were observed, respectively. The percentage of apoptotic cells after 0.83 μg/mL colistin treatment for 6, 12 and 24 h was not different from the control group treated with vehicle alone (DMSO) (Fig. 4).

3.4. Effect of colistin on the caspase-3 activity of chick neuron cells

The caspase-3 activities were in a both dose and time-dependent manner of the colistin-treated chick neuron cells,

Fig. 4 – Percentage of apoptotic cells (condensed or fragmented nuclear) according to nuclear morphological observations after 6, 12 and 24-h exposure to DMSO (control) or colistin. Values are presented as mean ± SEM (n = 3). *P < 0.05, **P < 0.01 versus DMSO-exposed control group.
the results showed in Fig. 6. The caspase-3 activity in the control group was set as “1”, and the strongest hydrolysis effect, respectively increased to (7.4 ± 2.8), (14.3 ± 3.4) and (21.5 ± 4.5) fold (both P<0.01) in this study was observed in the cells treated with colistin at 8.3 μg/mL for 6, 12 and 24 h, while increased to (5.1 ± 1.85), (7.2 ± 2.5) and (9.8 ± 3.14) fold with colistin at 4.15 μg/mL. Colistin treatment with 0.83 μg/mL up to 24 h did not cause any significant increase (P>0.05) in caspase-3 activity compared with the correspondingly control group.

3.5. The examination of DNA fragmentation

Internucleosomal DNA cleavage, a late hallmark of apoptosis, was examined with cells exposed to colistin at different concentrations for 6, 12 and 24 h (Fig. 7). As showed in Fig. 7-A, for 6 h, the DNA fragmentation increased to 115.3% (P<0.05) and 122.4% (P<0.01) (Fig. 7-A) in the 4.15 and 8.3 μg/mL colistin groups, for 12 and 24 h, the DNA fragmentation both significantly increased (all P<0.01), compared with the control. At 24 h, we also tested the DNA fragmentation formation by the
of major adverse effect. Unfortunately, there is a dearth of information on the mechanism of colistin neurotoxicity in the literatures. In the present study, we employed chick neuron primary cells to examine the effects of colistin treatment on the viability, ROS-formation, nuclear morphology and apoptosis, and further investigate its potential mechanism.

In clinical, visible neurotoxicity occurred at the lower rates (about 0–7%) when patients received the currently recommended dosage regimens than the nephrotoxicity (over 40%) (Falagas and Kasiakou, 2006; Lim et al., 2010; Yousef et al., 2011). However, of note that most of these patients were old or some being treated with sedatives and myorelaxants, assessment of the neurotoxicity is commonly difficult, the truly neurotoxicity rates may be more than the present (Nasnas et al., 2009; Wabhy et al., 2010). Some authors suggested colistin-induced neurotoxicity mediated by center nerve system (CNS) would need to permeate the BBB. In health animal, the BBB penetration rates of colistin was very low, the brain-to-plasma concentration (B/P) ratio of colistin was about 2–4%. The B/P ratios will significantly increase when the BBB are dysfunction (Jin et al., 2011, 2012). Jin et al. (2011) confirmed that the B/P ratio of colistin when mice were subcutaneous administration of colistin sulfate 40 mg/kg and intraperitoneal administration of LPS (from Salmonella) 3 mg/kg, the B/P ratio increased to 10–16% and the brain concentrations of colistin increased to 1–10 μg/g during 4 h. In addition, the accumulation of colistin occurs with repeated doses in clinical or animal (Kucers et al., 1997; Falagas and Kasiakou, 2006; Jin et al., 2012) and the central and peripheral neurotoxicity occurred when mice were continuously intravenously administrated colistin sulfate 15 mg/kg for 7 days. The excessively produced oxidants from colistin in kidneys is also a risk at its neurotoxicity, the oxidant overload could migrate to the lung through systemic circulation and further resulted the system toxicity including nerve injury (Ozyilmaz et al., 2011).

In our current study, we demonstrated the cell toxicity of colistin in chick cortex cells by the decreased cell viability in dose and time-dependent manners (Fig. 2). At the 0.83 μg/mL colistin group, the data of MTT had reduced but no significant changes at 6, 12 and 24 h, they suggested that it might have little toxicity at the concentration of 0.83 μg/mL. However, the significant changes were showed at the 4.15 (both p < 0.05) and 8.30 (both p < 0.01) μg/mL colistin groups at 12 and 24 h. These dose ranges of caused neurons cell injury may be supported by the previous studies (Jin et al., 2011, 2012). Landman et al. (2000) found that the dose of 40 mg/kg body weight of colistin sulfate injected subcutaneously induced rapid (within 1–3 h) mortality in young ostriches avian and the clinical signs of apathy, lethargy and hypotonia indicative of neurotoxicity of the compound were observed, and the maximum serum concentrations were 36.1 μg/mL colistin, whereas the contribution of colistin in young ostriches’ brains were unknown.

The process of apoptosis is controlled by a diverse range of cell signals, which may originate either extracellularly (extrinsic inducers) or intracellularly (intrinsic inducers). Extracellular signals may include toxins, hormones, growth factors, nitric oxide or cytokines, that must either cross the plasma membrane or transduce to effect a response (Thompson, 1995; Rubin, 1997). These signals may positively (i.e., trigger) or negatively (i.e., repress, inhibit, or dampen)
affect apoptosis. The binding of nuclear receptors by glucocorticoids, heat, radiation, nutrient deprivation, viral infection, hypoxia and increased intracellular calcium concentration, for example, by damage to the membrane, can all trigger the release of intracellular apoptotic signals by a damaged cell (Mattson and Chan, 2003). The structure of five 5 positively charged amino groups in colistin is considered as the major structure of killing the bacteria and inducing toxicity in the mammalian cells though changing the membrane permeability by effecting the concentration of Na⁺, K⁺ and Ca²⁺ (Falagas and Kasiakou, 2006). The ability of colistin to modify or disrupt the lipid membrane due to its fatty acid components may help to explain the toxic vulnerability of the highly lipid neurons (Dai et al., 2012). In this study, the caspase-3 and DNA fragmentation significantly increased after neuron cells exposed colistin for 6 h at the 4.15 and 8.3 μg/mL colistin groups (Figs. 6 and 7). These results suggested that colistin may activate caspases in chick neuron cells and then induce the cell apoptosis. Several previous studies have demonstrated that caspase-3 is able to activate Poly (ADP-ribose) polymerase (PARP)-dependent apoptotic pathway in cells (Nunez et al., 1998), and cause chromosomal DNA break and finally the occurrence of apoptosis (Salvesen and Dixit, 1999; Eldadah BA and Faden, 2000; Marks and Berg, 1999). In addition, the decreased viability, ROS generation, increased caspase-3 activity and internucleosomal DNA fragmentation after exposure to colistin (Figs. 2, 3, 6 and 7, respectively) strongly support the colistin-induced neuronal apoptosis observed in chick primary neurons (Figs. 4 and 5) in our study.

Colistin treatment, as an exogenous stimulus, also induced the generation of ROS (Le et al., 2005). The functions of ROS include stimulating mitotic cell division and inducing cellular senescence. ROS are also important regulators of apoptosis (Moon et al., 2010). In the literature, it has been demonstrated that excessive generation of ROS can cause DNA damage in neurons (Koh and Cheung, 2006) and other cell damages, and initiate various other effects (Davies and Hochstein, 1982).

ROS can lead to degeneration of cytomembranes, which cause descending fluidity and elevating permeability of the cell membrane and even collapse of membrane structure (Moon et al., 2010). Oxidative stress mediated by ROS played an important role in colistin induced nephrotoxicity due to its special structure, i.e. d-amino acid and fatty acid component (Ozyilmaz et al., 2011). In the present study, a dramatic ROS burst was observed in cells treated with colistin (4.15 and 8.3 μg/mL), especially at 12 and 24 h (Fig. 3); this suggests that ROS generation is involved in the colistin-induced apoptosis in chick primary neurons (Fig. 4). The dramatic ROS burst may explain the internucleosomal DNA fragmentation observed in Fig. 7. In addition, compared with the results of caspase-3 activities and ROS changes in the present study, the significant changes of caspase-3 activities occurred at 6 h, more earlier than ROS at 12 h, which suggested that ROS-independent mechanism also play an important role in colistin induced apoptosis in neuron cells (Perdomo et al., 2013). The increase in the activity of caspase-3 was associated with up-regulation of the pro-apoptotic factor Bax and also with the release of cytochrome c from mitochondria (Wang, 2001; Perdomo et al., 2013; Dai et al., 2013). These changes of mitochondria injury may not firstly come from the ROS, but rather the other activity or inhibitor of the signal pathway, such as the inhibitor of anti-apoptotic signaling PI3K-Akt-Bcl signal pathway, the activity of apoptotic signaling Fal and p53 signal pathway (Donovan et al., 2001). The previous studies suggested that colistin may competitively inhibit the nerve growth factor (NGF), a factor in neuron cell survival (Tajti et al., 1988). The polymyxin B, the brother member of colistin, had been certified to own the ability of inhibiting protein kinase C (PKC), a membrane-associated physiological mediators (Jiang et al., 2006). The role of ROS-independent mechanism in colistin-induced neuron apoptosis should be studied in the future.

5. Conclusions

In conclusion, our study first examined the toxic effects of colistin on chick neuron cells and revealed that colistin induced apoptosis. Very likely there are complex interactions among ROS production, and caspase-3 activity after exposure to colistin and the subsequent effects contribute to the toxic damages on chick primary neurons. Of note that, the caspase-3 activity may play a key role in colistin induced neuron cell apoptosis. Further investigation on the mechanism of apoptotic effect of colistin on neuron cells is warranted and will provide new insights into colistin neurotoxicity.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in.

Acknowledgements

This study was supported by the National Nature Science Foundation of China (No. 31272613 and 31201954) and the Scientific Research Fund of Heilongjiang Provincial Education Department (12521043). J.L. is supported by research grants from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (R01AI070896 and R01AI079330) and Australian National Health and Medical Research Council (NHMRC).

REFERENCES


