New insight in colistin induced neurotoxicity with the mitochondrial dysfunction in mice central nervous tissues

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A B S T R A C T

In the present study, the mechanism of colistin-induced neurotoxicity was investigated with a focus on behavioral characters, mitochondrial ultrastructures and functions of the central nerve tissues in mice followed by administrating intravenously 15 (divided into two dose and 12 h apart), 7.5 and 5 mg/kg bw colistin sulfate for 1, 3 or 7 days successively. To assess the reversibility of colistin-induced neurotoxicity, the neurotoxicity was also examined on day 15 (8 post colistin sulfate administration for 7 days). The results showed that, the spontaneous activities of mice were significantly decreased on days 3 and 7 in the 15 mg/kg group compared with the correspondingly control group. The abnormal ultrastructure changes of mitochondria were presented in their nervous tissues and changed in a dose- and time-dependent manner, e.g. severe vacuolation and fission on days 3 and 7 in the 15 mg/kg group and more slight on day 7 in the 7.5 mg/kg group. In addition, mitochondrial permeability transition (MPT), membrane potential (∆ψm) and activities of mitochondrial succinate dehydrogenase changed, showing that colistin affected the mitochondrial functions. The recoverability of colistin-induced neurotoxicity was showed and only slight injury occurred in the nerve tissues of mice on day 15 in the 15 mg/kg group and it had no abnormal changes in the behavioral and neuropathology characters in mice on day 15 in the 7.5 and 5 mg/kg groups. The results suggested that mitochondrial dysfunction might partly account for the mechanism of neurotoxicity induced by colistin sulfate.

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1. Introduction

In recent ten years, colistin (also known as polymyxin E) is clinically being used increasingly as a last-line therapeutic option for infections of multidrug resistant gram-negative bacteria, e.g. Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae (Hermse, et al., 2003; Falagas and Kasiakou, 2006; Landman et al., 2008; Lim et al., 2010); It is possible of inducing neurotoxicity in human beings and sensitive animals, affecting its optimal therapeutic efficacy in clinic (Landman et al., 2000; Falagas and Kasiakou, 2006; Lim et al., 2010). The neurotoxic signs and symptoms include apathy, ataxia, myopathy, muscular weakness, polymyoneuropathy, facial and peripheral paresthesia and even neuromuscular blockade (Landman et al., 2000, 2008; Lin et al., 2005; Falagas and Kasiakou, 2006; Wabhiy et al., 2010; Dai et al., 2012a). Though several literatures reported that the proposed mechanism of colistin neurotoxicity was a noncompetititve myoneuronal synapptic blockade of acetylcholine release, hypocalcemia-induced prolongation of depolarization, or the interaction of colistin with neurons owning high lipid content induced neurotoxicity (Duncan, 1973; Falagas and Kasiakou, 2006), the proper mechanism is unknown. In our pervious study (Dai et al., 2012a,b), the ultrastructural studies of sciatic nerves showed that axonal degeneration and demyelization, and the overtly abnormal electrophysiological characterizations of sciatic nerves occurred when the female mice were treated with colistin sulfate (15 mg/kg per day) intravenously for 3 and 7 days successively. Mitochondria are important regulators of neuronal function in addition to their role in energy production, and their dysfunction is a key determinant of neurodegeneration (Mancuso et al., 2006; Masoud et al., 2009), such as some neurodegenerative diseases including Huntington’s disease, Parkinson’s disease, Alzheimer’s disease, or neurotoxicity induced by some chemotherapy agents including the paclitaxel and cisplatin (Lin and Beal, 2006; Mancuso et al., 2006; Melli et al., 2008; Jewel et al., 2011). Up to now, little is known about the correlations between the mitochondrial dysfunction and neurotoxicity induced by colistin. So we built the animal models of the colistin-induced neurotoxicity by the behavioral tests in mice, followed by examination of the ultrastructural and functional changes of the mitochondria in central nervous tissue (CNS) in a dose- and time-dependent manner, to illustrate whether mitochondrial dysfunction account for the development of the colistin neurotoxicity.
2. Materials and methods

2.1. Chemicals, solvents and reagents

Colistin sulfate was purchased from Sigma-Aldrich (Lot: 09SK1048, St. Louis, MO, USA; 20195 U/mg). Rhodamine 123, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). BCATM protein assay kit was purchased from Wuhan Boster Bio-engineering Limited Co. (Wuhan, Hubei, China). All other chemicals were of highest quality commercially available. The NZ-6 mice spontaneous activity apparatus was produced by Chengdu Taimeng Science and Technology, Co., Ltd. (Chengdu, Sichuan, China). JSM25610LV transmission electron microscope was produced by JEOL Ltd. (JEOL Ltd., Japan).

2.2. Animals

Female Kunming mice (supplied by the Harbin Veterinary Research Institute, China) at 8 weeks of age and weighing 18–20 g were used in these experiments, were kept in separate cages, had free access to feed and water and were acclimated to housing and environmental conditions (temperature, humidity, light, and ventilation) for at least 1 week prior to beginning of treatments. Animals were housed in a limited access animal facility where animal room temperature and relative humidity were set at 22 ± 2 °C and 55 ± 10%, respectively. Artificial lighting provided a 24 h cycle of 12 h light/12 h dark (light 7 a.m.–7 p.m.). All procedures involving animals were approved by the Animal Care and Use Committee of Heilongjiang Province, PR China.

2.3. Experimental design

Three experiments were set up to investigate the effect of colistin sulfate on the behavior changes of mice (experiment 1), the structures (experiment 2) and functions (experiment 3) of mitochondria in central nerve tissues including the brains and spinal cords. Doses of 15, 7.5 and 5 mg/kg colistin sulfate were administered intravenously as 10 ml/kg solutions for a successive treatment period of 1, 3 and 7 days, respectively in each group, and the dose regimens were showed in Table 1. For the 15 mg/kg colistin sulfate group, the dose of 15 mg/kg was divided into two dosages and 12 h apart, i.e. 7.5 mg/kg/12 h, which was consistent with the previous study (Dai et al., 2012a). And for the 7.5 and 5 mg/kg groups, the dose was once a day. Those in the control group were given 0.9% normal saline of the same volume.

In the experiment 1, the effects of colistin sulfate on the behavioral changes including the spontaneous activity and gait scores were studied. Forty mice were randomly divided into four groups with 10 mice in each group. Before the mice administration, the spontaneous activity and gait scores were tested as the base line. Then the mice were administrated for 7 days continuously. The spontaneous activity and gait scores were tested on days 1, 3 and 7 after the first administration. To observe the reversibility of the neurotoxicity induced by colistin sulfate, on day 15, i.e. on the 8th day after 7 days of successive administration, these behavioral indexes were also been tested.

In the experiment 2, fifty two mice were randomly divided into four groups, which were randomly divided into 15 mg/kg (n = 16), 7.5 mg/kg (n = 16) and 5 mg/kg (n = 16) colistin sulfate–given groups and the control group (n = 4). For the colistin sulfate groups, the mice were administrated colistin sulfate successively for 1, 3 and 7 days including 4, 4 and 8 mice, respectively, and on days 1, 3, 7 and 15 after the first administration, they (four at each time point) were respectively anesthetized by intraperitoneal injection of an overdose of sodium pentobarbital (80 mg/kg bw), perfused transcardially with 100 ml 0.9% saline and 1000 ml 0.1 M phosphate buffer (PB; pH 7.4) containing 4% (w/v) paraformaldehyde. The cerebral cortex and spinal cord were obtained and used to observe the changes of mitochondrial ultrastructures.

In the experiment 3, one hundred and sixty mice were randomly divided into four groups, i.e. 15, 7.5, 5 mg/kg colistin sulfate–given groups and the control group (n = 40 in each group). Ten mice in each group were randomly selected and sacrificed on the corresponding time points of days 1, 3, 7 and 15, respectively (n = 10 in each time point). The cerebral cortex and spinal cord were quickly dissected and prepared for mitochondrial functional assay.

2.4. Determination of neurological behavior

2.4.1. Gait scores

In the experiment 1, on days 1, 3, 7 and 15 after the first intravenous administrations of colistin sulfate, the gait abnormalities were measured as the previous study (Dai et al., 2012a,b). The tested mice were placed in a clear plexiglas box and were observed for 3 min each time, the assigned scores were as follows: 1 = normal gait; 2 = slightly abnormal gait (slight ataxia, hopping gait and foot splay); 3 = moderately abnormal gait (obvious ataxia and foot splay with limb abduction during ambulation); 4 = severely abnormal gait (inability to support body weight and foot splay).

2.4.2. Spontaneous activities

The tested mice were placed into the mice spontaneous activity apparatus, 1 mouse in each cabin, the times of spontaneous activities and the times of arising within 10 min were recorded after adaptation for 5 min (Li, 1992).

2.5. Observation of mitochondrial ultrastructures

In the experiment 2, on days 1, 3, 7 and 15, the cerebral cortex and spinal cord were obtained from 4 mice for the ultrastructural studies in each group; the method of ultrastructure observation was as our previous method (Dai et al., 2012a,b). Small pieces of tissue were dissected from the cerebral cortex and spinal cord and cut into approximately 1 mm cubes, then were fixed by immersion with 2.5% glutaraldehyde in 0.12 mM phosphate buffer (pH 7.2), and kept overnight at 4 °C in the fixative. Post-fixation was performed with 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for

### Table 1

The dose regimens in all the experiments.

<table>
<thead>
<tr>
<th>Doses (mg/kg)</th>
<th>Times of administration</th>
<th>Times of investigation</th>
<th>The volume of injection</th>
<th>Route of administration</th>
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<tbody>
<tr>
<td>15</td>
<td>Successive 1 to 7 days in</td>
<td>On days 1, 3, 7, and 15</td>
<td>10 ml/kg</td>
<td>Administered intravenously in the tail vein</td>
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<tr>
<td>7.5</td>
<td>experiment 1 and 3, 7 and</td>
<td>after the first administration</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>days, respectively in experiments 2 and 3</td>
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a 15 mg/kg per day was divided into two doses, 12 h apart (Dai et al., 2012a,b).

b Day 15, i.e. 8 days after the mice were administrated colistin sulfate for 7 d.

c Days 1, 3, 7, and 15 were based on the days of the first dose as day 0.

d It is a convenient intravenous procedure in mice.
2 h at 4°C. The specimens were then dehydrated in graded acetone solutions and embedded in araldite-epon. Ultrathin sections (50 nm) were prepared and counterstained with uranyl acetate and lead citrate, examined with the JSM25610LV transmission electron microscope.

2.6. Measurement of mitochondrial function

2.6.1. Isolation and purification of the mitochondria

In the experiment 3, mitochondria of the mice central nerve tissues were isolated by the method described by Clark and Nicklas (1970). Briefly, the dissected cerebrum and spinal cord samples were homogenized (10% w/v) in ice-cold buffer (0.25 M sucrose, 10 mM Tris–HCl, 0.5 mM K+-EDTA, pH 7.4). Then the homogenate was centrifuged at 2000 × g for 4 min at 4°C, the supernatant was further centrifuged at 12,500 × g for 8 min at 4°C. The crude mitochondrial pellet was resuspended in a 3% Ficoll medium (3% Ficoll, 0.12 M mannitol, 0.03 M sucrose, 25 mM K+-EDTA, 5 mM Tris–HCl, pH 7.4), this suspension was carefully layered onto a 6% Ficoll medium (6% Ficoll, 0.24 M mannitol, 0.06 M sucrose, 50 mM K+-EDTA, 10 mM Tris–HCl, pH 7.4) and centrifuged at 11,500 × g for 30 min at 4°C, the supernatant was decanted and the mitochondrial pellet was resuspended in isolation medium and recentrifuged at 12,500 × g for 10 min at 4°C to get the purified mitochondria. The final pellet was reconstituted in the isolation medium. The protein concentration of the mitochondrial suspension was quantified by the BCA™ protein assay kit.

2.6.2. Measurement of mitochondrial permeability transition (MPT)

MPT is associated with mitochondrial swelling, which was assayed by the absorbance at 540 nm (A540 nm) (Uyemura et al., 1997). The isolated mitochondria were modulated to 0.5 mg/mL and incubated in the assay buffer (125 mM sucrose, 65 mM KCl, 5 mM succinate, 5 mM rotenone, 10 mM Tris–HCl, pH 7.4). MPT was initiated by adding 50 μM calcium chloride, and monitored by measuring the A540 nm at 37°C using an ultraviolet spectrophotometer in 5 min.

2.6.3. Detection of mitochondrial membrane potential (ΔΨm)

Changes in mitochondrial membrane potential were monitored in the presence of the fluorescent dye Rhodamine 123 (Rh123) according to Emaus et al. (1986a,b) with modifications. Briefly, fluorescence with excitation at 503 nm and emission at 527 nm was detected in a reaction buffer (250 mM sucrose, 2 mM HEPES, 0.5 mM KH₂PO₄, 4.2 mM sodium succinate, pH 7.4, and 0.3 mM Rh123) using F-4500FL Spectrophotometer (Hitachi High-Technologies Co., Japan). The mitochondria were diluted to 0.5 mg/mL in the buffer and incubated for 3 min. The fluorescence was recorded again, and the alteration of the ΔΨm was detected by the decrease of the fluorescence.

2.6.4. Activities of the mitochondrial respiratory chain

MTT reduction was used to assess the activities of the mitochondrial respiratory chain by the method of Liu et al. (1997). The dye was converted to water-insoluble purple formazan on the reductive cleavage of its tetrazolium ring by the succinate dehydrogenase system of the active mitochondria. The reaction mixture containing mitochondrial preparation (80–100 mg protein) and 0.02 mL MTT (0.1 mg/mL) was incubated at 37°C for 30 min and then centrifuged at 1000 × g for 5 min at room temperature to obtain the formazan pellet. The pellet was dissolved in 1 mL acidic isopropanol and the mixture was recentrifuged at 1000 × g for 5 min at room temperature. Then the absorbance of the supernatant was measured at 595 nm. Results were expressed as A595 nm/mg protein.

2.7. Statistical analyses

Results are represented as mean ± standard deviations (SD). The data were analyzed with one-way analysis of variance (ANOVA), followed by LSD’s post hoc tests to compare the control and treatment groups. The differences were considered significantly at p < 0.05 level.

3. Results

3.1. Gait scores

On days 0, 1, 3, 7 and 15, the gait scores were measured and the data were shown in Fig. 1. Some mice showed slightly abnormal gait on day 3 in the 15 mg/kg group (mean clinical score = 1.23 ± 0.44).
Clinical signs progressed to disturbances with slight ataxia, hopping gait and foot splay, and signs were severe on day 7 thereafter (mean clinical score = 2.2 ± 0.63) in the 15 mg/kg group, and the gait scores of two mice were 3. In the 7.5 mg/kg group, mice began to take on slightly abnormal gait on day 7 thereafter (mean clinical score = 1.3 ± 0.48). On day 15, forty percent of the mice showed abnormal gait in the 15 mg/kg group. In contrast, little clinical signs of the neurotoxicity were observed in mice in the 5 mg/kg and control groups.

3.2. Changes of spontaneous activities

On days 0, 1, 3, 7 and 15, the changes of spontaneous activities were shown in Fig. 2. In the 15 mg/kg group, markedly change of the times of spontaneous activities and times of arising within 10 min were showed on days 3 and 7 (p < 0.05 and p < 0.01, respectively), and the changes recovered on day 15 compared with the control. However, there were no significant changes in 7.5 and 5 mg/kg groups during the experiment period.

3.3. Ultrastructure changes of mitochondria

3.3.1. Ultrastructure changes of mitochondria in the cerebral cortex

In the cerebral cortex, progressive morphological changes of mitochondria were observed and showed in Fig. 3. In the 15 mg/kg group, decreases of mitochondrial cristae were shown on day 3 (Fig. 3C), and more severe pathology changes including the swollen and vacuolated mitochondria, and ruptured, decreased, even disappeared mitochondrial cristae were shown on day 7 (Fig. 3D); Some
3.3.2. Ultrastructural changes of mitochondria in the spinal cord

In the spinal cord, the progressive morphological changes of mitochondria were observed and shown in Fig. 4. In the 15 mg/kg group, a little mitochondria began to show slightly abnormal changes (Fig. 4C) on day 3, and almost all in neurons were vacuolated on day 7 (Fig. 4D), which led to necrosis of neuron including karyopyknosis and increase of its perinuclear space (Fig. 4E); up to day 15, the mitochondrial structures in spinal cord showed slight abnormalities, such as mild swelling and slight decrease of mitochondrial cristae, while the swollen endoplasmic reticulum was much more obvious than others (Fig. 4F). In the 7.5 and 5 mg/kg groups, the slightly abnormal changes in the neuron cells and mitochondria occurred only on day 7 (Fig. 4H).

3.4. Effects of colistin sulfate on mitochondrial functions

3.4.1. Effects of colistin sulfate on Ca²⁺-induced MPT

Compared with the control group, with time went on, on day 3, the A540 nm was significantly decreased by 14.7% and 22.2% (p < 0.05 and p < 0.01, respectively) in cerebrum and spinal cord in the 15 mg/kg group, which were decreased by 30.2% and 48.7%
Fig. 5. Effects of colistin sulfate on Ca\(^{2+}\)-induced mitochondrial permeability transition (MPT). (A) cerebrum; (B) spinal cord. The results were represented as the mean ± SD (n = 10). Statistical significance was determined by using one-way analysis of variance (ANOVA). *p < 0.05 and **p < 0.01 compared with the control group. Ca\(^{2+}\)-induced MPT were shown as the progressive drop of A540 nm.

Fig. 6. Effects of colistin sulfate on Δ\(\psi\)\(_{m}\) (A) cerebrum; (B) spinal cord. The results were represented as the mean ± SD (n = 10). Statistical significance was determined by using one-way analysis of variance (ANOVA). *p < 0.05, **p < 0.01 compared with the control group. Significant differences of Δ\(\psi\)\(_{m}\) were only presented in the spinal cord of the high dose group treated mice on days 7 and 15 (p < 0.01 and p < 0.05, respectively).

Fig. 7. The activity of mitochondrial succinate dehydrogenase by mitochondria from mice central nerve tissues following administration of colistin sulfate; (A) cerebrum; (B) spinal cord. The results were represented as the mean ± SD (n = 10). Statistical significance was determined by using one-way analysis of variance (ANOVA). *p < 0.01 compared with the control group. The activities of mitochondrial succinate dehydrogenase were expressed as A595 nm/mg protein.

(both p < 0.01) on day 7. In the 7.5 mg/kg group, the A540 nm was significantly decreased by 18.8% (p < 0.05) in spinal cord, while it had no significant changes (p > 0.05) in the cerebrum. On day 15, the A540 nm was still decreased by 19.3% and 19.9% (both p < 0.05) in cerebrum and spinal cord in the 15 mg/kg group, and no significant changes were shown in the other groups (Fig. 5).

3.4.2. Effect of colistin sulfate on the Δ\(\psi\)\(_{m}\)
Δ\(\psi\)\(_{m}\) was examined by monitoring the fluorescence quenching of Rh123 dynamically (Fig. 6). There was no significant difference in the cerebrum, but significant differences of Δ\(\psi\)\(_{m}\) were shown in the spinal cord on days 7 and 15 (p < 0.01 and p < 0.05, respectively) in the 15 mg/kg group.

3.4.3. Activities of the mitochondrial respiratory chain
The results showed in Fig. 7. In the cerebrum, there was no significant decrease in the activity of mitochondrial succinate dehydrogenase on days 1 and 3; however, on day 7 in the 15 mg/kg group, the enzyme activities were significantly decreased by 14.8% (p < 0.01) compared with the correspondingly control group. In the spinal cord, the activities of mitochondrial succinate dehydrogenase were decreased by 9.6% (p < 0.01) and 15.7% (p < 0.01), respectively on days 3 and 7 in the 15 mg/kg group. On day 15, it still decreased by 9.84% (p < 0.01) and 8.82% (p < 0.01) in the cerebrum and spinal cord in the 15 mg/kg group compared with the control group.

4. Discussion
Neuro- and nephrotoxicities are potential adverse effects that are of greatest concern to physicians when colistin as a valuable therapeutic option used against Gram-negative pathogens. Fewer rates of neurotoxicity (about 0–7%) occurred in clinical when patients received the currently recommended dosage regimens than the nephrotoxicity (Falagas and Kasiakou, 2006; Lim et al., 2010; Yousef et al., 2011). However, most of these patients were old and some were treated with sedatives and myorelaxants, assessment of the central and especially peripheral toxicity is difficult, the neurotoxicity rates may be more than the present (Nasnas et al., 2009; Wabby et al., 2010). In our previous study, we had confirmed that the peripheral neurotoxicity occurred when mice were intravenously administrated colistin sulfate at 15 mg/kg per day (divided into two dosages) for 3 days (Dai et al., 2012a). And we also found that the mitochondrial dysfunction may play an
important role in colistin-induced neurotoxicity in vivo (Dai et al., 2012b).

In this paper, these signs including slight ataxia, hopping gait and foot splay were shown in the mice administrated intravenously with 15 mg/kg colistin sulfate, which were similar with the previous study (Landman et al., 2000; Dai et al., 2012a). The spontaneous activities in mice, and the functions and structure of mitochondria also changed in the dose- and time-dependent manners (Landman et al., 2000; Falagas and Kasiakou, 2006; Landman et al., 2008; Lim et al., 2010), which may be relative with the concentration of colistin in the nerve tissues or serum level. When the chick and mice were treated subcutaneously with 40 mg/kg colistin sulfate, significant neurotoxicity was only found in chick at the maximum serum concentration of 55.6 μg/mL but no any signs of neurotoxicity in mice at the maximum serum concentration of 36.1 μg/mL (Landman et al., 2000; Jin et al., 2009). When colistin is used in patients, the drug accumulation will occur, and a serum level of 15 μg/mL was reported following dosing of 2.5 mg/kg (total dosage per day) for 7 days, but if the neurotoxicity occurred was not known (Landman et al., 2008).

In the previous studies, the cell apoptosis or necrosis had been certified in colistin-induced nephrotoxicity by Wallace et al. (2008), the same results were also been certified by Yousef et al. (2011, 2012). Some authors suggested that colistin should induce neurotoxicity mediated by the α-amino acid and fatty acid components of colisin, especially the lipid A of its model structure, which is interacted with the high lipid neuron, as same as the colistin induced nephrotoxicity (Liu et al., 1997; Falagas and Kasiakou, 2006; Nasnas et al., 2009; Ozyilmaz et al., 2011). In the present study, the mitochondria ultrastructure changed evidently, significant increase of Ca\(^{2+}\)-induced MPT and obvious decrease in the activity of mitochondrial enzymes in the central nerve tissues in mice treated with 15 mg/kg colistin sulfate for 7 days. It suggested that the mitochondrial dysfunction played an important role, which was same as the precious study in vivo (Dai et al., 2012b).

The mitochondria are vital cellular machineries for maintaining basic cellular functions such as cellular energy metabolism, which generate ATP accompanied with the production of reactive oxygen species (ROS) (Lin and Beal, 2006; Masoud et al., 2009; Smith et al., 1999). Consequently, oxidative stress-induced cell apoptotic or necrosis in neurons has been shown to be related with the elevation of ROS levels-induced mitochondrial damage (Schulz et al., 2000; He et al., 2007; Tastekin et al., 2006). In this paper, the degeneration or necrosis in neurons of cerebral cortex and spinal cord were presented in the 15 mg/kg group (Figs. 3 and 4). Colistin may produce amount of ROS in vivo on primary chick cortex neurons because of the oxidative stress (Zhang, 2011). Excessive ROS could impair nervous system by triggering the cascade of peroxida- tive events, leading to damage of cell membranes and intracellular cytoplasmic organelles, such as increased MPT, decreased Δψ\(_{m}\) or reduced MTT of mitochondria in this study (Figs. 5–7), which suggested that mitochondrial dysfunction might be partly responsible for the development of the neurotoxicity induced by colistin sulfate. The obvious depemrination, decrease of spontaneous activities or myasthenia of limbs occurred in mice treated with colistin sulfate intravenously, which were same as Lin et al. (2005), it may be related with the metabolism of energy resulting in reduction of ATP level in nervous system.

It is known that mitochondrial Ca\(^{2+}\) homeostasis is at the center of wide interest in the scientific community today because of its role both in the modulation of numerous physiological responses and its involvement in cell death (Giacomello et al., 2007). Alterations in Ca\(^{2+}\)-induced MPT and Δψ\(_{m}\) are now thought to be a central regulatory mechanism for cell death induction (Lopachin and Lehning, 1997). Once mitochondria membrane permeabilization occurs, cells would die either by apoptosis or necrosis. When the excessive mitochondrial Ca\(^{2+}\) loads occurred, it might be incurred the Ca\(^{2+}\)-induced respiratory impairment by inhibition of electron transport chain and oxidative phosphorylation, which may activate the key enzymes responsible for increased ROS generation (Giacomello et al., 2007; Kaur et al., 2007). The structure of five positively charged amino groups in colistin sulfate, which were considered as the major structure inducing toxicity in the mammalian cell and the mechanism of its killing the bacteria, was thought to change the membrane permeability by effecting the concentration of Na\(^{+}\), K\(^{+}\) and Ca\(^{2+}\) (Landman et al., 2008). Ozyilmaz et al. (2011) reported that endothelial NOS (eNOS), Ca\(^{2+}\) and calmodulin (CaM)-dependent activation, was deceased in kidney and lung tissue after rats were administered 10 mg/kg intraperitoneal (i.p.) colistin for 6 days. In our previous study (Dai et al., 2012b), the changes were certified in vivo on the primary chick cortex neurons.

Especially, on day 15, the capability of self-recovery was shown from the behavioral tests and mitochondrial functions, which were consistent with some literatures, i.e., the colistin-induced neurotoxicity had recoverability (Hermson et al., 2003; Falagas and Kasiakou, 2006; Wabhy et al., 2010; Dai et al., 2012a,b). But up to now, the recoverability mechanism was unknown. It may be relative with the self-regulation of Bac-2 and nerve growth factor (NGF), which had been confirmed in regularizing neurotoxicity caused by diabetis or cisplatin (Hellweg and Hartung, 1990; Gill and Windebank, 1998; Schmidt et al., 2000; Sayre et al., 2008).

In this paper, the result showed obvious pathological changes, increased Ca\(^{2+}\)-induced MPT, decrease of Δψ\(_{m}\) and mitochondrial succinate dehydrogenase occurred in the mice injected with colistin sulfate, indicating that mitochondrial dysfunction might be partly responsible for the development of the neurotoxicity induced by colistin sulfate.

Conflict of interest

The authors declare that there are no conflicts of interest.

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