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Minocycline attenuates colistin-induced neurotoxicity via suppression of apoptosis, mitochondrial dysfunction and oxidative stress

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Background: Neurotoxicity is an adverse effect patients experience during colistin therapy. The development of effective neuroprotective agents that can be co-administered during polymyxin therapy remains a priority area in antimicrobial chemotherapy. The present study investigates the neuroprotective effect of the synergistic tetracycline antibiotic minocycline against colistin-induced neurotoxicity.

Methods: The impact of minocycline pretreatment on colistin-induced apoptosis, caspase activation, oxidative stress and mitochondrial dysfunction were investigated using cultured mouse neuroblastoma-2a (N2a) and primary cortical neuronal cells.

Results: Colistin-induced neurotoxicity in mouse N2a and primary cortical cells gives rise to the generation of reactive oxygen species (ROS) and subsequent cell death via apoptosis. Pretreatment of the neuronal cells with minocycline at 5, 10 and 20 μM for 2 h prior to colistin (200 μM) exposure (24 h), had an neuroprotective effect by significantly decreasing intracellular ROS production and by upregulating the activities of the anti-ROS enzymes superoxide dismutase and catalase. Minocycline pretreatment also protected the cells from colistin-induced mitochondrial dysfunction, caspase activation and subsequent apoptosis. Immunohistochemical imaging studies revealed colistin accumulates within the dendrite projections and cell body of primary cortical neuronal cells.

Conclusions: To our knowledge, this is first study demonstrating the protective effect of minocycline on colistin-induced neurotoxicity by scavenging of ROS and suppression of apoptosis. Our study highlights that co-administration of minocycline kills two birds with one stone: in addition to its synergistic antimicrobial activity, minocycline could potentially ameliorate unwanted neurotoxicity in patients undergoing polymyxin therapy.

Introduction

The two clinically used polymyxins, polymyxin B and colistin (Figure 1), are lipopeptide antibiotics that are used as last-line therapy against problematic Gram-negative pathogens.[1–6] Available population pharmacokinetic and pharmacodynamic data from our group indicated that the currently recommended dosage regimens of polymyxins achieve suboptimal plasma concentrations; and that higher dosing is needed to achieve effective killing and prevent resistance.[7] Neurotoxicity is an unwanted side effect that limits effective polymyxin therapy.[8–11] Patients receiving intravenous colistin methanesulfonate (CMS) (the inactive prodrug of colistin) have been reported to present with neurological symptoms such as confusion, dizziness, facial/peripheral paraesthesia, vertigo, seizures, respiratory muscle weakness, apnoea and ataxia.[8,10–13]

The development of effective neuroprotective agents that can be co-administered during polymyxin therapy remains a priority area for antimicrobial chemotherapy with these very important last-line antibiotics. Minocycline is a broad-spectrum tetracycline antibiotic that has been reported to display antioxidant and neuroprotective activities.[14–18] Moreover, given that minocycline and colistin produce a pharmacodynamically synergistic therapeutic effect,[19–22] their co-administration could have the advantages of reduced dosage and toxicity at an equal or improved level of efficacy. The present study investigates the neuroprotective action of minocycline against colistin-induced neurotoxicity using mouse

neuroblastoma-2a (N2a) and primary cortical neuronal cell culture models. We also explored the ability of minocycline to suppress colistin-induced oxidative stress, mitochondrial dysfunction and apoptosis in N2a neuronal cells. The presented findings are discussed in the context of the clinical potential of minocycline as a synergistic antibiotic that can be preferentially co-administered during polymyxin therapy due to its neuroprotective properties.

Materials and methods

Materials

Colistin sulfate was obtained from Zhejiang Shenghua Biology Co., Ltd (Zhengjiang, China). Minocycline (hydrochloride, purity ≥98%) was purchased from Aladdin Reagent Co., Ltd (Shanghai, China). MTT and DMSO were purchased from AMRESCO Inc. (Solon, OH, USA). 2′,7′-Dichlorofluorescensin diacetate (DCFH-DA) was purchased from Beyotime (Haimen, China). DMEM and FBS were obtained from Life Technologies Corporation (Grand Island, NY, USA). All other reagents were of the highest analytical grade available.

Cell culture

The mouse N2a cells (ATCC CCL-131™) were cultured in DMEM medium supplemented with 10% (v/v) FBS, 110 mg/L sodium pyruvate, 100 units/mL penicillin and 100 mg/L streptomycin (Beyotime, Haimen, China) at 37°C in 5% CO_2. The media were changed once per day. Mouse primary cortical neurons were prepared from C57/BL6 embryonic day 14 mice under sterile conditions.[23] Briefly, embryonic day 14 C57/BL6 mice cortices were removed, dissected free of meninges and dissociated in 0.025% (w/v) trypsin in Krebs buffer. The dissociated cells were triturated using a filter-plugged fine pipette tip, pelleted, resuspended in plating medium (minimum Eagle's medium containing 10% fetal calf serum and 5% horse serum) and counted. Cortical neuronal cells were seeded at 150 000 cells/well onto a poly-D-lysine-coated 48-well plate in plating medium for 2 h, then replaced with freshly prepared neurobasal medium containing B27 supplements, gentamicin and 0.5 mM glutamine [all tissue culture reagents were purchased from Invitrogen (Australia) unless otherwise stated]. The neuronal cells were allowed to mature for 7 days in culture before commencing treatment using freshly prepared primary culture media [neurobasal medium plus B27 supplements (minus antioxidants)]. All cultures were maintained in an incubator set at 37°C with 5% CO_2. This method resulted in cultures highly enriched in neurons (>95% purity) with minimal astrocyte and microglial contamination.

Measurement of cell viability

The concentrations of the drugs used and the duration of exposure were empirically derived from preliminary exposure–response experiments in the neuronal cell culture model. Cell viability was measured using the MTT assay. Briefly, N2a cells (1×10^4) were seeded into 96-well tissue culture plates. After culture for 12 h, cells were treated with colistin (200 μM). After 24 h, the medium was discarded and replaced with 100 μL serum-free DMEM media containing 10 μL MTT (5 mg/mL) and the cells were incubated for 4 h at 37°C. Finally, the medium was discarded and 100 μL DMSO was added. After incubation for 20 min at room temperature, the absorbance was read at 570 nm in a microplate reader (Molecular Devices, Sunnyvale, CA, USA). To assess the neuroprotective effects, cells were pretreated with minocycline (5, 10 or 20 μM) for 2 h. After 2 h, the medium containing minocycline was discarded and the cells were washed with cold PBS and incubated with colistin (200 μM) for an additional 24 h and then cell viability was assessed. Mouse primary cortical neurons were pretreated with minocycline (5, 10 and 20 μM) or vehicle (DMSO) for 2 h, then media were removed and fresh NB media containing colistin (200 μM) or vehicle (PBS) were added to the wells and incubated for 24 h. At the conclusion of the experiment, MTT reagent was added to the treated cultures for 4 h at 37°C, after which the culture media were removed and the formazan by-product fully dissolved using DMSO. A 100 μL aliquot of the MTT/DMSO was transferred to a 96-well clear-walled plate and the absorbance measured at 570 nm in a plate reader. The data were normalized and calculated as a percentage of untreated vehicle control values.

Immunostaining of colistin accumulation in mouse primary cortical neurons

Anti-polymyxin B mouse IgM antibody (Thermo Fisher Scientific, Rockford, IL, USA) was diluted to 1:500 and incubated with the colistin (400 μM)-treated cells overnight at 4°C.[24] The cells were then washed and incubated with MOM biotinylated anti-mouse secondary link (Vector Labs, CA, USA) for 10 min, followed by incubation with an AlexaFluor647 streptavidin conjugate at a 1:500 dilution (Life Technologies, VIC, Australia).

Measurement of apoptosis

The apoptosis assay was performed using an annexin V-FITC apoptosis detection kit according to the manufacturer's protocol (Vazyme Biotech Co., Ltd, Nanjing, China). For the flow cytometric analysis, cells were harvested with 0.25% trypsin without EDTA, washed twice with cold PBS and resuspended in 500 μL binding buffer supplied by the manufacturer. The cells were then incubated with 5 μL annexin V-FITC (40 μg/mL) and 5 μL propidium iodide (PI) (40 μg/mL) in the dark for 10 min. Analysis was performed using a BD FACSAria™ flow cytometer (Becton Dickinson, San Jose, CA, USA) set at an excitation wavelength of 488 nm and an emission wavelength of 605 nm.

Measurement of caspase-3/7 and -9 activities

N2a cells (1.5×10^4 cells/well) were cultured in 96-well plates and treated with minocycline (5, 10 or 20 μM) for 2 h at 37°C. After removing the medium containing minocycline, the cells were then incubated in media containing colistin (200 μM) for 24 h at 37°C. The caspase-3/7 and -9 activities were determined using the Caspase-Glo®-3/7 and -9 assay kits according to the manufacturer's instructions (Promega Corp., Madison, WI, USA). Luminescence was measured using a microplate luminometer (Molecular Devices, Sunnyvale, CA, USA).

Measurement of intracellular reactive oxygen species (ROS)

Intracellular ROS production was measured using the ROS-specific fluorescent dye DCFH-DA according to the manufacturer's protocol (Beyotime, Haimen, China). N2a cells were plated into 12-well plates at a density of 2×10^5 cells/well and pretreated with minocycline at final concentrations of 5, 10 or 20 μM for 2 h at 37°C and washed with cold PBS. Cells were then treated with colistin (200 μM) for 24 h. The control cells were treated with minocycline at 20 μM *per se* or the vehicle (0.1% DMSO in PBS). After treatment, DCFH-DA (10 μM) was added into the medium for a further 30 min at 37°C. After three washes with cold PBS, the DCFH-DA fluorescence was imaged using a fluorescent microscope (Leica DMLS) (excitation wavelength 488 nm, emission wavelength 530 nm) and the fluorescence was measured using a multimode plate reader (Varioskan Flash Top, Thermo Fisher Scientific, Germany).

Measurement of intracellular superoxide dismutase (SOD) and catalase (CAT) activities

The SOD and CAT activities levels were detected using specific assay kits according to the manufacturer's instructions (Nanjing Jiancheng Co., Ltd, Nanjing, China). In brief, N2a cells were plated onto 6-well plates at a

density of 5×10^5 cells/well and pretreated with minocycline (5, 10 or 20 μM) at 37°C for 2 h. After removing the medium containing minocycline, the cells were incubated in colistin (200 μM) for 24 h. The negative control cells were treated with minocycline (20 μM) or the vehicle (0.1% DMSO in DMEM). Cells were washed with cold PBS and lysed using the cell lysis buffer provided by the manufacturer. The cell lysates were centrifuged at 14 000 **g** for 10 min at 4°C. Supernatants were collected and assayed for SOD and CAT activities. Protein concentrations were quantified using the BCA protein assay kit (Beyotime, Haimen, China).

Measurement of the change in mitochondrial membrane potential ($\Delta\psi_m$)

The $\Delta\psi_m$ was detected using the fluorescent indicator JC-1 (Beyotime, Haimen, China). N2a cells were plated onto 12-well plates at a density of 2×10^5 cells/well and pretreated with minocycline (5, 10 or 20 μM) at 37°C for 2 h, followed by treatment with colistin (200 μM) for 24 h. After treatment, N2a cells were incubated in DMEM containing 10 μM JC-1 at 37°C for 15 min, washed with PBS and observed under a fluorescence microscope (Leica Microsystems, Wetzlar, Germany). A shift of fluorescence from red to green represents a loss of ψ_m. JC-1 red fluorescent emission (normal ψ_m) was measured at 583 nm with an excitation wavelength of 525 nm, and JC-1 green fluorescence emission (loss of ψ_m) was measured with an excitation wavelength of 525 nm and emission wavelength of 530 nm. For quantitative analysis, at least 100 regions of interest were selected in each treatment and the ratios between fluorescence intensity in the green and red channels were calculated. An increase in the ratio was interpreted as the loss of ψ_m.

Statistical analysis

Data from the control and treatment groups were analysed with one-way analysis of variance, followed by the LSD *post hoc* test using SPSS v. 13.0 (SPSS Inc., Chicago, IL, USA). A P value <0.05 was considered as significant.

Ethics

Mouse primary cortical neurons were prepared from C57/BL6 embryonic day 14 mice according to procedures as previously described and approved by the Melbourne University Animal Ethics Committee.[23]

Results

Minocycline attenuates colistin-induced neurotoxicity in mouse N2a and primary cortical neuronal cells

Treatment of mouse N2a and primary cortical neuronal cells with colistin (200 μM) for 24 h produced a >50% decrease in cell viability ($P<0.01$) (Figure 1a and b). Pretreatment of the neuronal cells with minocycline at 5, 10 and 20 μM for 2 h prior to colistin exposure increased the cell viability (Figure 1a and b), with the neuroprotective effect being most significant at 20 μM minocycline. Minocycline treatment *per se* had no impact on cell viability. Furthermore, the binding of colistin to primary cortical cells was visualized by confocal microscopy using an anti-polymyxin monoclonal antibody (Figure 2). The imaging results revealed a punctuate localization pattern where colistin binds to both the neurites and the cell soma.

Minocycline attenuates colistin-induced apoptosis and caspase activation in N2a cells

Exposure of N2a cells to 200 μM colistin for 24 h induced apoptotic rates up to 46.4% ($P<0.01$) (Figure 3a and b). Pretreatment of the N2a cells with minocycline at 5, 10 and 20 μM for 2 h prior to colistin exposure decreased the apoptotic rates to ∼40%, ∼30% and ∼25%, respectively (Figure 3b). The apoptotic rates in the minocycline (20 μM)-only treatment were essentially comparable to untreated control cells. Colistin exposure at 200 μM for 24 h significantly (all $P<0.01$) increased the activities of caspases-3 and -9 to ∼2.5-fold, compared with the untreated control cells. Minocycline pretreatment (20 μM) significantly (all $P<0.01$) down-regulated the activation of caspases-3 and 9, compared with the colistin-only treated cells (Figure 3c and d).

Minocycline attenuates colistin-induced loss of mitochondrial membrane potential

The neuroprotective effect of minocycline against colistin-induced mitochondrial dysfunction was assessed using the JC-1 mitochondrial membrane potential (MTP) assay. Colistin treatment (200 μM) for 24 h induced mitochondrial dysfunction in N2a cells, seen as an increase in green fluorescent JC-1 (JC-1 monomeric form); the green/red fluorescence radio increased ∼3-fold ($P<0.01$) compared with that in the control (Figure 4). This reflects the loss of mitochondrial membrane potential (ψ_m). Pretreatment of the N2a cells with minocycline at 5, 10 and 20 μM for 2 h prior to colistin exposure protected against colistin-induced loss of ψ_m, as evidenced by the decreases in the green/red fluorescence ratio (decreased to ∼2-fold at 20 μM minocycline, $P<0.01$) compared with the colistin-only treatment.

Minocycline attenuates colistin-induced generation of cellular ROS and induces SOD and CAT activities

The treatment of Na2 cells with colistin (200 μM) for 24 h increased the intracellular ROS levels to ∼220% relative to the untreated control cells (Figure 5a and b). Pretreatment of the N2a cells with minocycline at 5, 10 and 20 μM for 2 h prior to colistin exposure significantly decreased the intracellular ROS levels in a dose-dependent fashion (Figure 5b). Notably, a ∼100% decrease in the ROS levels was seen with the 20 μM minocycline pretreatment, relative to the colistin-only treated cells. Moreover, minocycline pretreatment at 20 μM significantly increased ($P<0.01$, compared to colistin-only treatment) the SOD and CAT activities (Figure 5c and d). Minocycline treatment alone had no effect on cellular ROS levels, SOD and CAT activities.

Discussion

MDR Gram-negative bacteria have become a crisis in hospitals worldwide, due to their proclivity to spread rapidly and the diminishing therapeutics available to effectively treat infections caused by these pathogens.[25,26] Although polymyxins remain effective against these problematic Gram-negative bacteria, pharmacodynamic and pharmacokinetic data on polymyxins largely from our group suggest that caution is required with monotherapy. There have been increasing reports of infections caused by Gram-negative *Acinetobacter baumannii* resistant to all available antibiotics, including polymyxins, and the recent emergence of plasmid-mediated colistin resistance due to its unchecked agricultural use is most alarming.[27,28] The emergence and spread of polymyxin-resistant isolates highlights the urgent need to

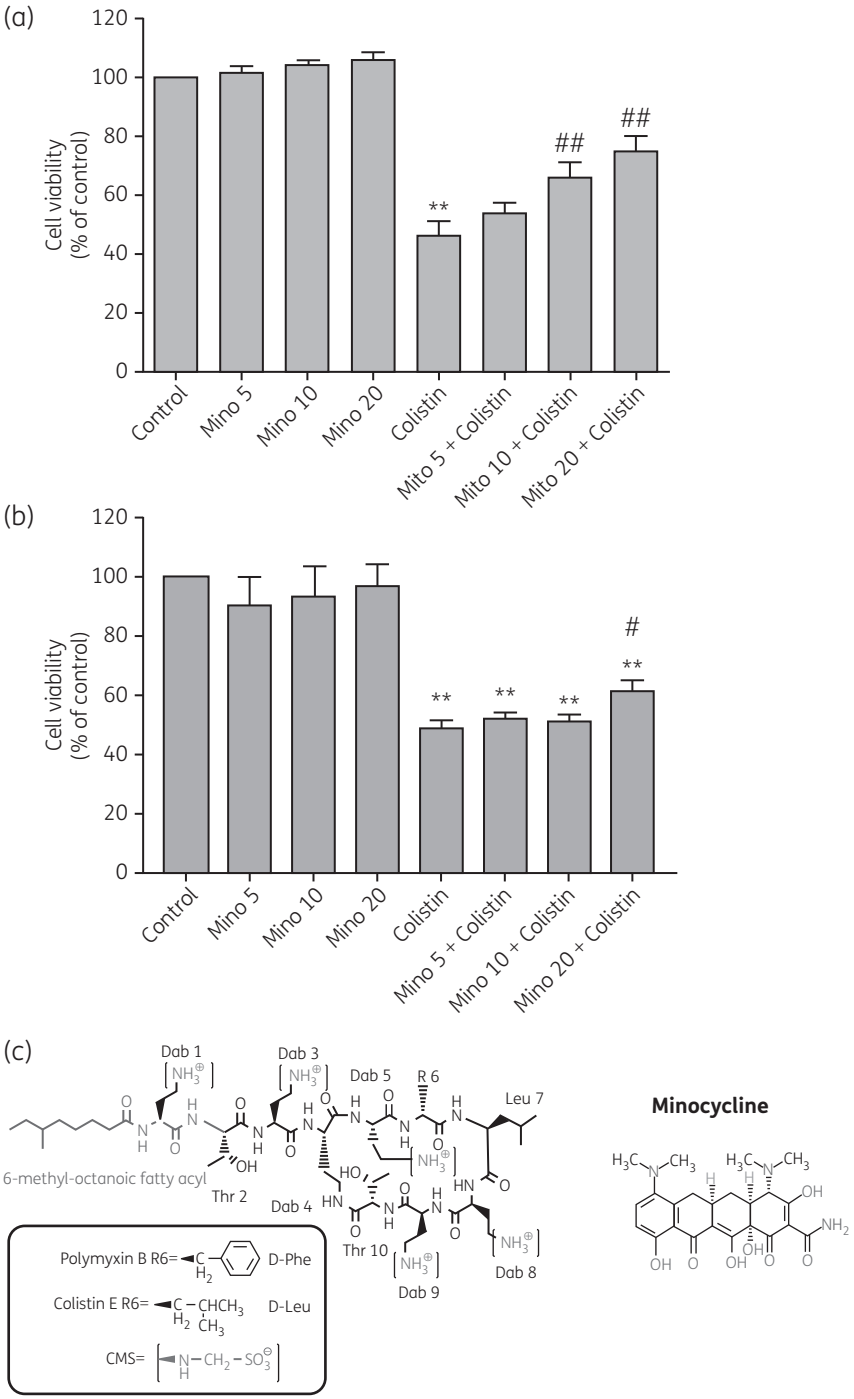

Figure 1. Protective effect of minocycline against colistin-induced neurotoxicity in mouse neuronal N2a and primary cortical cells. (a) Impact of minocycline (Mino) pretreatment (5, 10 and 20 μM for 2 h) on colistin (200 μM)-induced cytotoxicity in N2a cells (24 h incubation). (b) The neuroprotective effect of minocycline pretreatment (5, 10 and 20 μM for 2 h) in mouse primary cortical neurons against colistin (200 μM)-induced cell death. The cell viability data were normalized and calculated as a percentage of untreated vehicle control values. All cell viability data shown represent the mean ± SD from five independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the untreated control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with colistin treatment. (c) The chemical structures of the clinically used polymyxins and minocycline. Leu, leucine; Phe, phenylalanine; Dab, α,γ-diaminobutyric acid.

investigate novel approaches for maintaining and improving the clinical efficacy of these important last-line antibiotics. Another, untoward aspect of polymyxin therapy is the nephrotoxicity and neurotoxicity associated with the clinical use of these antibiotics.[2,5,8,10,11,13,29,30] The discovery of neuroprotective agents for co-administration during polymyxin therapy is paramount to prolong the clinical utility of these important last-line antibiotics. In the present study, we provide demonstrable proof

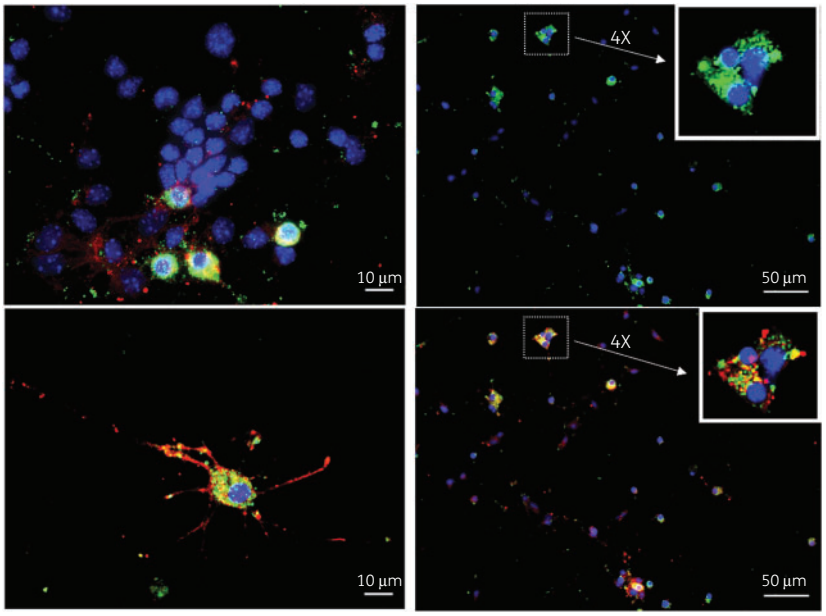

Figure 2. Confocal fluorescence microscopy images of colistin (400 μM)-treated mouse primary cortical neurons stained with anti-polymyxin monoclonal antibody (green channel), phalloidin (red channel) and DAPI nuclear stain (blue channel). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

that minocycline markedly attenuates colistin-induced neurotoxicity in mouse N2a and primary cortical neuronal cells (Figure 1). This represents a 'value-add' in addition to the reported synergy between colistin and minocycline against problematic MDR Gram-negatives such as *A. baumannii*.[19–22]

In our previous mechanistic neurotoxicity study, we reported that colistin-induced apoptosis in neuronal N2a cells is activated via both the death receptor (extrinsic) and the mitochondrial (intrinsic) pathways.[31] In the present report, we have shown that colistin-induced apoptosis could be inhibited by pretreatment of the N2a cells with 20 μM minocycline for 2 h (Figure 3a and b). Caspase-3 is a key apoptotic mediator that can be activated by both the intrinsic (mitochondrial) and extrinsic (death receptor) pathways.[32] Caspase-9 is an important mediator in the mitochondrial apoptosis pathway.[32] Minocycline pretreatment significantly down-regulated the activities of caspases-3 and -9 in colistin-treated N2a cells (Figure 3c and d). Indeed, several other studies have reported that the anti-apoptotic activities of minocycline are inextricably linked to its role in suppressing caspase-dependent and caspase -independent cell death pathways.[33–43]

We previously reported that colistin-induced neurotoxicity involves mitochondrial dysfunction in the mouse cerebral cortex and sciatic nerve tissues in mice intravenously injected with 15 mg/kg/day colistin sulfate for 7 days.[44] In the present study, we found that minocycline could attenuate the colistin-induced loss of mitochondrial membrane potential in a dose-dependent manner in N2a cells (Figure 4). In line with our findings, the anti-apoptotic activity of minocycline in various cells and tissues has been reported to involve its ability to interact directly with mitochondria to up-regulate Bcl-2 levels, and in turn, to suppress cytochrome C and Smac/DIABLO release.[45–50]

The nervous system is highly vulnerable to oxidative damage due to its elevated oxygen demand and high polyunsaturated fatty acid content.[51] In the present study, we found that colistin exposure significantly increased intracellular ROS levels in N2a cells with a concomitant decrease in the activity of the antioxidant enzymes SOD and CAT (Figure 5). Taken together, these findings would suggest that colistin neurotoxicity not only induces ROS production directly, but also decreases the neurons' capacity to breakdown oxygen radicals, further exacerbating ROS-mediated oxidative stress. One of the most remarkable pharmacological properties of minocycline is its antioxidant activity.[16,52] In the present study, we found that minocycline could not only inhibit colistin-induced ROS generation, but also enhance the total antioxidant capacity in N2a cells by up-regulating the activities of SOD and CAT (Figure 5). The potent antioxidant activity of minocycline is related to its ability to chelate mitochondrial iron, which catalyses toxic hydroxyl radical formation.[48] The direct free radical scavenging activity of minocycline is partly due to the four OH-groups in its structure (Figure 1c), which allow the compound to scavenge ROS via sacrificial oxidation of these groups.[16,33,53] Furthermore, minocycline's ability to chelate redox-active metal ions such as Fe^{2+} may also contribute to its antioxidant effect.[48] Coincidently, the reported ability of minocycline to directly chelate Ca^{2+},[47] could mean it also has mechanistic synergy with colistin, as the antibacterial activity of polymyxins involves the displacement of divalent cations (i.e. Ca^{2+} and Mg^{2+}) that stabilize the lipopolysaccharide in the outer membrane of Gram-negative bacteria.[1] Overall, the direct radical scavenging activity of minocycline and its ability to increase the resistance of the neuronal cells by activating their intrinsic antioxidant defence mechanisms, are major factors that are responsible for the observed dose-dependent reduction of colistin-induced neurotoxicity.

Presently, there is a dearth of information on the CNS pharmacokinetics of intravenously administered colistin, and polymyxins in general; essentially the use of polymyxins to treat CNS infections

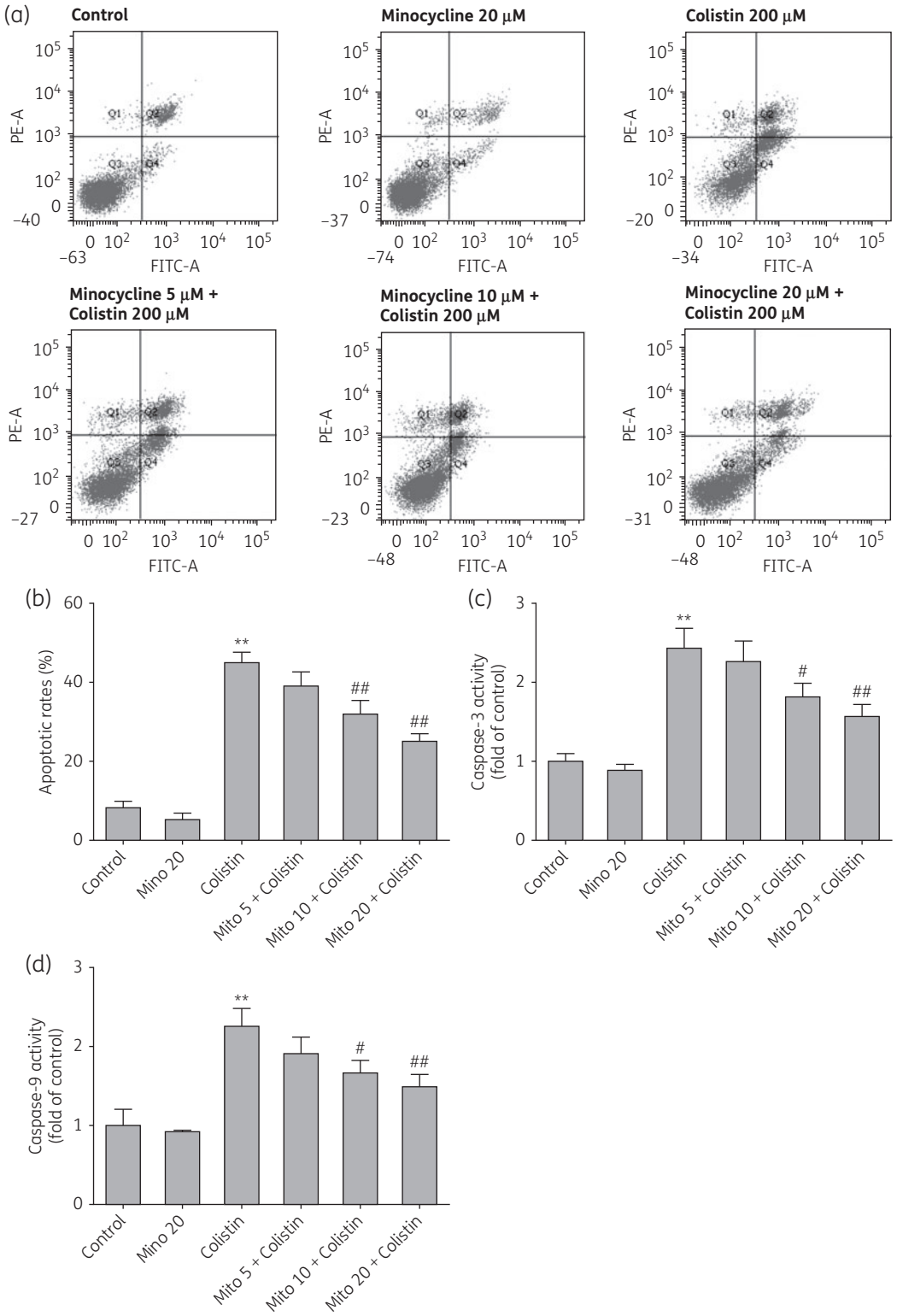

Figure 3. Protective effect of minocycline against colistin-induced apoptosis in N2a cells. (a) Apoptosis of Na2 cells was analysed by flow cytometry following annexin V-FITV/PI staining. Q1, necrosis cells; Q2, later apoptotic cells; Q3, live cells; Q4, early apoptotic cells. Cells were pretreated with minocycline for 2 h at 37 °C. After removing the medium containing minocycline, the cells were then incubated in media containing colistin for 24 h at 37 °C. (b) Apoptotic rate in N2a cells in response to colistin and minocycline pretreatment. (c, d) Caspase-9 and caspase-3 activities in N2a cells in response to colistin and minocycline pretreatment were examined using ELISA. All the data shown represent the mean ± SD from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the untreated control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with colistin treatment.

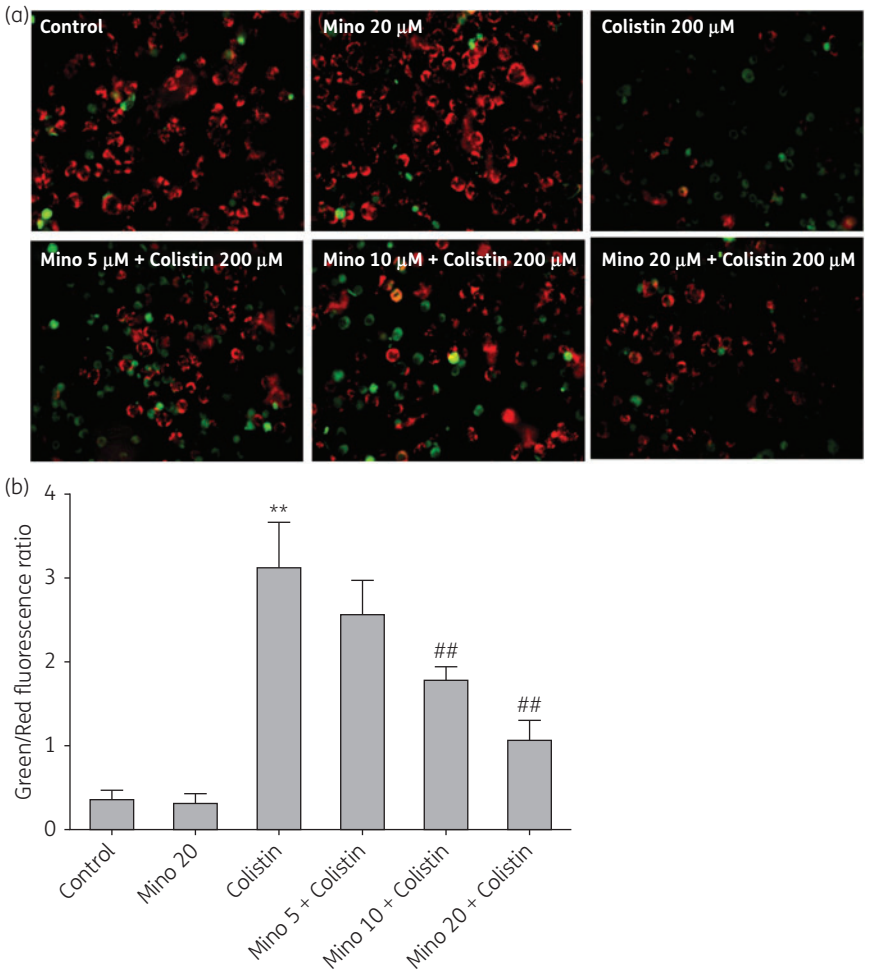

Figure 4. Minocycline attenuates colistin-induced mitochondrial dysfunction. (a) N2a cells were plated onto 12-well plates at a density of 2×10^5 cells/well and pretreated with minocycline at a final concentration of 20 μM at 37 °C for 2 h ($n = 3$). The cells were treated with colistin (200 μM) for an additional 24 h. The change in mitochondrial membrane potential (MTP) was evaluated using the cationic fluorescent indicator JC-1. The JC-1 aggregate form, indicating normal MTP function, appears red. The JC-1 monomeric form, indicating disrupted MTP, appears green; Magnification ×40. (b) Quantitative analysis of the confocal data presented as the ratio between fluorescence intensity in the green (low membrane potential) and red (high membrane potential) channels. An increase in the ratio was interpreted as the loss of ψ_m. Values are presented as the mean ± SD from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the untreated control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with colistin treatment. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

is empirical. To date there have only been a few reports of the concentrations of colistin achievable in the CNS following intravenous or intrathecal/intraventricular administration.[54–57] The few available reports note that following intravenous administration of the prodrug CMS, the CSF concentrations achieved were variable (5%–67% of the serum concentrations).[54–56] In comparison, intrathecal/intraventricular colistin administration achieves much higher CSF/AUC serum ratios and has been associated with better outcomes for severe CNS infections compared to intravenous colistin *per se*.[55–63] However, the use of intrathecal/intraventricular colistin has been associated with neurological side effects including seizures, chemical meningitis and cauda equina syndrome.[59,64,65]

Minocycline has been shown to have neuroprotective effects in a number of models of neurological injury such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, traumatic brain injury, spinal cord injury, intracerebral haemorrhage, global and focal cerebral ischaemia, and amyotrophic lateral sclerosis.[66–68] The neuroprotective action of minocycline is purported to involve multiple mechanisms including anti-inflammatory and anti-apoptotic mechanisms.[66–68]

In humans, following a 200 mg intravenous dose, peak plasma concentrations of ∼4.0 mg/L are achieved; steady-state concentrations following 100 mg orally twice daily for 3 days average 1.4–1.8 mg/L.[68,69] As well as its neuroprotective effects, minocycline has serendipitously been shown to display high blood–brain barrier penetration with CSF concentrations of 11%–56% of the plasma concentrations being achieved (which equates to CSF levels of ∼0.5 mg/L after chronic dosing).[68,69] Notably, it has been demonstrated that peak serum concentrations >3.5 mg/L and trough concentrations >2 mg/L are neuroprotective against temporary focal cerebral ischemia in rats.[68,70]

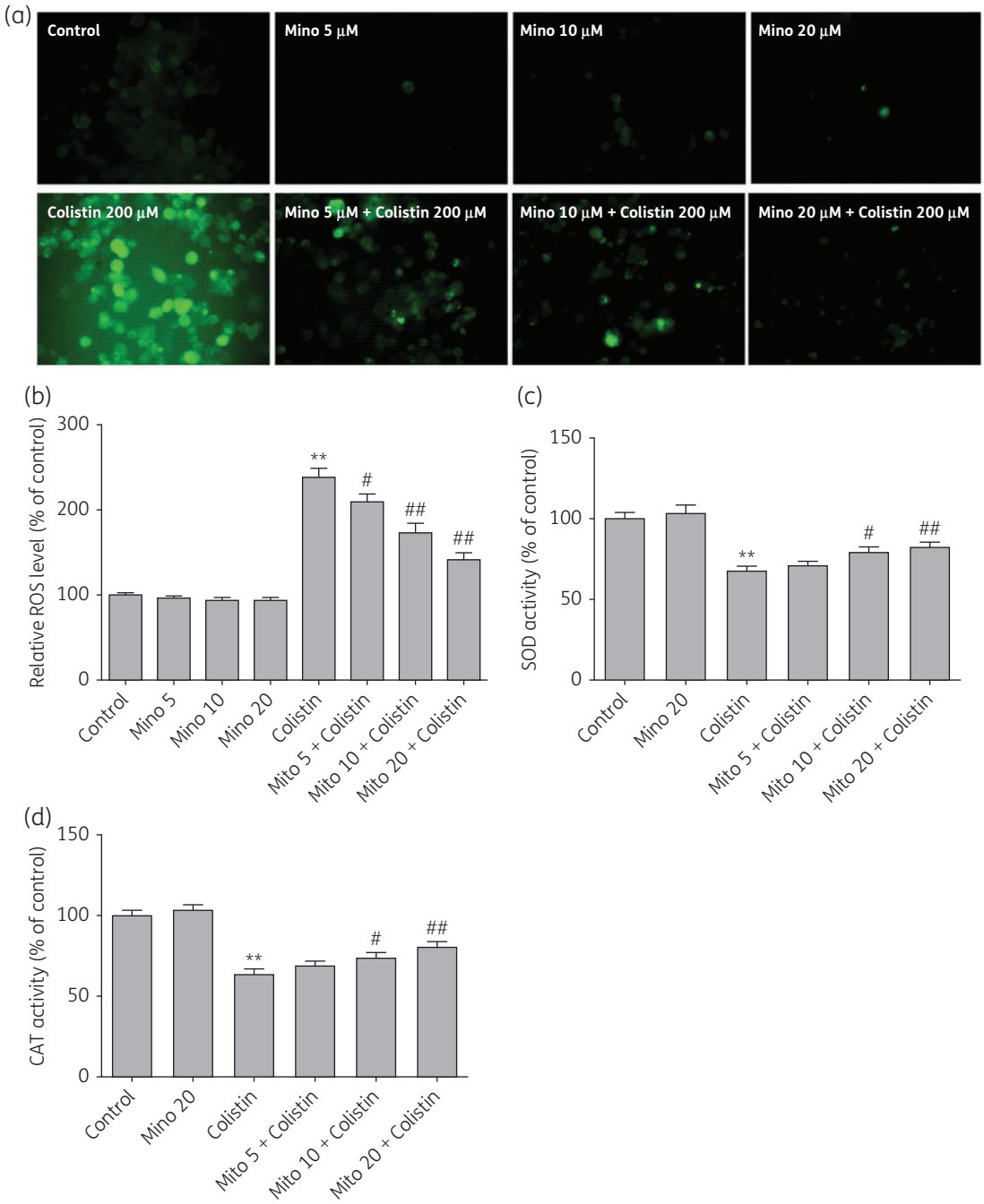

Figure 5. Minocycline protects N2a cells against colistin-induced oxidative stress. (a) Cellular ROS levels were detected using confocal microscopy imaging following staining of N2a cells with the ROS-sensitive dye 2,7-dichlorofluorescein diacetate; Magnification ×40. (b) ROS generated relative to control were quantified. (c, d) The impact of minocycline pretreatment (5, 10 and 20 μM for 2 h) on cellular SOD and CAT activities in N2a cells treated with colistin (200 μM for 24 h). The data shown represent the mean ± SD from three independent experiments. $^{**}P < 0.01$, compared with the untreated control; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with colistin treatment. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

The overall favourable pharmacokinetic profile of intravenous minocycline,[71] along with its synergy with polymyxins,[19–22] neuroprotective/nephroprotective properties[72–75] and stability to many tetracycline resistance mechanisms, indicates a potential role for minocycline/polymyxin combination therapy for treatment of serious MDR Gram-negative CNS infections. Here, we provide demonstrable proof that minocycline ameliorates colistin-induced neurotoxicity by inhibiting oxidative stress, apoptosis and mitochondrial dysfunction. Minocycline combination therapy may represent a promising novel approach for the prevention of neurotoxicity and preventing the emergence of resistance in patients receiving polymyxin therapy.

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Transparency declarations

None to declare.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.

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