Full length article

Minocycline diminishes the rotenone induced neurotoxicity and glial activation via suppression of apoptosis, nitrite levels and oxidative stress

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A B S T R A C T

The study was conducted to evaluate the effect of minocycline against pesticide rotenone induced adverse effects in different rat brain regions. Assessment of oxidative stress, nitrite levels, degenerating neurons and level of cleaved caspase-3 was done in frontal cortex, mid brain, hippocampus and striatum regions of rat brain. In addition the expression profile of neuronal (MAP2), astrocytes (GFAP) and microglia (cd11b) markers was done after treatments. Rotenone induced DNA fragmentation was also assessed in all studied rat brain regions by utilizing comet assay. Rotenone administration caused significantly decreased level of glutathione along with increased level of nitrite and lipid peroxidation. Significant oxidative and nitrosative stress was also observed after rotenone administration which was considerably inhibited in minocycline treated rats in time dependent manner. Fluorojade staining and levels of cleaved caspase 3 showed the degeneration of neurons and apoptosis respectively in studied rat brain regions which were further inhibited with minocycline treatment. Rotenone administration caused significantly increased reactivity of astrocytes, microglia and altered neuronal morphology in rat brain regions which was also partially restored with minocycline treatment. In conclusion, present study showed that minocycline treatment attenuated the rotenone induced oxidative stress, nitrite level, degeneration of neurons, augmented glial reactivity and apoptosis.

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1. Introduction

Minocycline is highly lipophilic, semi-synthetic tetracycline derivative which can easily cross blood brain barrier (Klein and Cunha, 1995). Minocycline shows anti-inflammatory, antimicrobial, (Smilack, 1999) as well as neuroprotective effect in variety of central nervous system (CNS) diseases model in (Kim and Suh, 2009). It has also been reported that minocycline offer neuronal survival by decreased levels of cleaved caspases-3, inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS) and inhibited mitogen-activated protein kinase p38 (MAPKp38) activation (Tikka et al., 2001). Minocycline protects the liver against ischemia/reperfusion injury by reducing oxidative stress and inhibiting the release of pro-inflammatory cytokines (Li et al., 2015). It could also reduce the microglial activation, and provides neuroprotection against excitotoxicity. Minocycline protects the

cultured neurons against N-methyl-D-aspartate receptor (NMDA) excitotoxicity (Tikka and Koistinaho, 2001). It also inhibits the NMDA-induced proliferation of microglial cells and augmented nitric oxide level and proinflammatory cytokine in microglia cultures (Tikka and Koistinaho, 2001).

Minocycline also exert neuroprotective effects over various experimental models such as delay in progression of Hungtington's disease in mouse, protective role in cerebral ischemia (Yrjanheikki et al., 1999), Parkinson's disease (Wu et al., 2002), traumatic brain injury (Sanchez et al., 2001), amyotrophic lateral sclerosis (Zhu et al., 2002), multiple sclerosis (Popovic et al., 2002) and Alzheimer's disease (Choi et al., 2007). Though the interference of minocycline in mitochondria mediated pathway is reported (Wang et al., 2003) but its mechanism of action is yet not well explored. The study by Wang et al. (2004) showed that minocycline could act through its action on mitochondria and able to suppress Bax accumulation, cytochrome c translocation and augmented Bcl-2 levels. Recently the anti-inflammatory effects of minocycline have also been suggested (Hayashida et al., 2016). Valproic acid induced increase in locomotion, oxidative and nitrosative stress, inflammation, calcium levels and blood brain barrier permeability

was also inhibited with minocycline treatment in neonatal rat brains (Kumar and Sharma, 2016). Minocycline induced protective effect against rotenone induced dopaminergic death have also been reported in primary culture (Radad et al., 2010). Minocycline prevented the rotenone induced decreased tyrosine hydroxylase expression and apoptosis of neuronal culture (Casarejos et al., 2006). Recent study by Stokes et al. (2017) has shown that minocycline treatment could block the glial cell activation in respiration centers during chronic hypoxia.

Rotenone is a naturally occurring pesticide which causes neurotoxicity via inhibiting mitochondrial complex-I activity. It interferes with the electron transport chain of mitochondria thus consequently affect the energy metabolism and ATP levels (Betarbet et al., 2002; Liu et al., 2003; Lapointe et al., 2004; Bove et al., 2005). Rotenone also causes neurotoxicity due to generation of reactive oxygen species (ROS), which further leads to various adverse effects in cellular physiology (Trojanowski, 2003; Uversky, 2004; Radad et al., 2006; Hu et al., 2007) therefore selected in this study. In this study the rotenone was injected in intracerebroventricular (ICV) region for its better diffusion in all rat brain regions which are related to various disease pathologies. In this regard earlier we have reported the variable susceptibility of the rotenone in different rat brain regions (Swarnkar et al., 2009) and cerebral neuronal damage by diminished mitochondrial enzyme activity and increased levels of ROS (Swarnkar et al., 2010). The present study was done to investigate the effect of minocycline on rotenone induced neurotoxicity and glial reactivity specifically in brain regions related to Alzheimer's and Parkinson's disease.

2. Material and methods

2.1. Chemical used

Antibodies mouse anti beta-actin antibodies (A3854), bovine serum albumin (BSA), copper sulphate, calcium chloride, dichlorofluorescein diacetate (DCF-DA), dihydroethidium (DHE), disodium hydrogen phosphate, dimethyl sulphoxide (DMSO), ethidium bromide, ethylene diamine tetra acetic acid (EDTA), Folin-Ciocalteu reagent, glucose, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), hydrochloric acid (HCl), low melting agarose, magnesium chloride, melatonin, NADH, paraformaldehyde (PFA), phenylenediamine, potassium chloride, propidium iodide (PI), RNase, and tris buffer were procured from SIGMA, USA. Antibody mouse polyclonal anti-caspase-3 (SC-56055) was procured from Santacruz. Cresyl violet acetate purchased from MP Biomedical. Laboratory chemicals like sodium bicarbonate, sodium carbonate, sodium chloride, sodium dihydrogen phosphate, sodium hydrogen biphosphate, sodium hydroxide, sodium potassium tartarate and sodium pyruvate were procured from SRL, India.

2.2. Animals, treatments and groups

The experiments were carried out with adult male Sprague Dawley rats of 180–200 g (age, 2 to 3 months). The animals were procured from the Laboratory Animal service Division of Central Drug Research Institute – CSIR. Animals were provided after approval from institutional animal ethical committee and experiments were performed according to international guidelines. The animals were kept in transparent polyacrylic cage and maintained under standard housing conditions (room temperature $22 \pm 1 \,^\circ C$) with 12 h light and dark cycle and acclimatized to the laboratory conditions before the experiment. The food was given in the form of dry standard rat chow and water *ad-libitum*.

Animals were divided in 5 groups labeled as control (no treatment), vehicle (DMSO, 3 μl bilateral by intracerebroventricular injection), minocycline per se, rotenone [bilateral intracerebroventricular injections of each 3 μl (12 μg)], rotenone + minocycline (3 mg/kg intraperitoneal, 30 min prior to rotenone administration). Number of animals per group per parameter per time point was ranged between 8 to 10.

2.3. Stereotoxic surgery

Rats were anesthetized with ketamine: xylazine (100 mg/kg: 10 mg/kg) (Sophocleous and Idris, 2014) and placed on a stereotaxic frame (Stoelting, USA) for surgery. Rotenone was solubilized in DMSO and infused (3 μl) into the intracerebroventricular region. Infusion of toxins/drugs into the ventricle reproduces many of the behavioral, electrophysiological, neurochemical and neuropathological changes associated with neurodegenerative disease (Rosi et al., 2004). The stereotaxic coordinates were antero-posterior from the bregma −0.8 mm, media-lateral from the bregma ±1.6 mm, dorso-ventral −3.5 mm (Goswami et al., 2015; Kamat et al., 2012). Proper postoperative care was done until animals recover completely. Animals were sacrificed after 1st, 3rd, 5th and 7th day after treatment as per assay requirement. Rats were anesthetized and perfused with pre chilled saline by intra-cardiac injection. After perfusion the brain was dissected to isolate the frontal cortex (FC), hippocampus (HP) mid brain (MB) and striatum (STR) (Swarnkar et al., 2010). Rat brain homogenate of different regions (w/v 10%,) was prepared in sodium phosphate buffer (30 mM, pH 7.0) by using Ultra-Turrax T25 homogenizer (USA) at a speed of 9500 x rpm. The homogenate was collected and immediately processed for biochemical estimations.

2.4. Glutathione (GSH) estimation

GSH is considered as a marker of oxidative stress. The level of GSH was estimated by following a protocol of Sharma and Gupta (2003). For deproteinization of samples 1:1 ratio of rat brain homogenates and 1% trichloroacetic acid (TCA) was taken and incubated for 20 min at 4 °C. After deprotenisation the homogenate was centrifuged for 5 min at 5000× rpm at 4 °C. For GSH estimation 10 μl of supernatant was taken from each sample, then 140 μl of potassium phosphate buffer (0.1 M, pH 8.4) was added. The reaction was initiated with addition of 50 μl of 5-5′-dithiobis[2-nitrobenzoic acid] (5 mM) to each sample and absorbance was read immediately at wavelength of 412 nm using ELISA plate reader (BIO-TEK Instruments). The assay for each sample was run in triplicate. The GSH concentration in the brain samples was extrapolated from the standard GSH curve obtained by plotting the optical density of the standard GSH concentrations. Results are expressed as GSH μg/mg protein.

2.5. Malondialdehyde (MDA) estimation

Estimation of MDA was done as a marker of lipid peroxidation (Colado et al., 1997). Brain homogenate was deproteinized using 30% TCA, 5N HCl and 2% TBA (prepared in 0.5 N sodium hydroxide). This reaction mixture was incubated on a water bath at 90 °C temperature for 15 min and after cooling the reaction mixture was centrifuged in Biofuge Fresco (Heraeus, Germany) at 5000 x rpm for 10 min. The supernatant of reaction mixture was collected and the absorbance was measured at wavelength 532 nm, using ELISA plate reader (BIO-TEK Instruments). The assay for each sample was run in triplicate. The MDA concentration in the samples was extrapolated from the standard curve of tetraethoxypropane. Results in graphs are expressed as MDA nmol/mg protein.

2.6. Estimation of nitrite level

Nitrite levels were estimated with slight modifications in the protocol reported by Esposito et al. (2008). Briefly, the brain