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Nitric oxide synthase inhibitor, aminoguanidine reduces intracerebroventricular colchicine induced neurodegeneration, memory impairments and changes of systemic immune responses in rats

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ABSTRACT

Intracerebroventricular (i.c.v.) injection of colchicine induces neurodegeneration, memory impairments and changes of some systemic immune responses in rats. Though the role of cox 2 in these colchicine induced changes have been evaluated, the influence of nitric oxide synthase (NOS) remains to be studied. The present study was designed to assess the role of NOS on the i.c.v. colchicine induced neurodegeneration, memory impairments and changes of some systemic immune responses by inhibiting its activity with aminoguanidine. In the present study the impairments of working and reference memories, neurodegeneration (chromatolysis and plaque formation) and changes of neuroinflammatory markers in the hippocampus (increased TNF α, IL 1β, ROS and nitrite) along with changes of serum inflammatory markers (TNF α, IL 1β, ROS and nitrite) and alteration of systemic immune responses (higher phagocytic activity of blood WBC and splenic PMN, higher cytotoxicity and lower leukocyte adhesion inhibition index of splenic MNC) were measured in the intracerebroventricular colchicine injected rats (ICIR). Administration of aminoguanidine (p.o. 30/50 mg/kg body weight) to ICIR resulted in recovery of neuroinflammation and partial prevention of neurodegeneration which could be corroborated with the partial recovery of memory impairments in this model. The recovery of serum inflammatory markers and the systemic immune responses in ICIR was also observed after administration of aminoguanidine. Therefore, the present study shows that aminoguanidine can protect the colchicine induced neurodegeneration, memory impairments, and changes of systemic immune systemic responses in ICIR by inhibiting the iNOS.

1. Introduction

Colchicine, an alkaloid obtained from the seeds of *colchiciumautumanale* (Shibli et al., 2010), causes neurotoxic effects by binding with tau proteins that results in the depolymerisation of microtubules and disruption of axoplasmic flow (Emerich and Walsh 1990; Wilson, 1986). Intracerebroventricular colchicine injected rats (ICIR) initially shows the neurodegeneration by this direct action of colchicine on neurons but a progressive neurodegeneration and memory impairments have been reported in ICIR (Sil et al., 2014) which may be due to the neuroinflammation triggered by the initial actions of colchicine. The degenerating neurons may activate the glial cells by different inflammatory signals which generates several neuroinflammatory markers (IL 1β, IL 6, TNF α, ROS and RNS) and these markers in turn may further cause increased cox-2 expression and PGE_2 production that in turn activate the microglia resulting in chronic neuroinflammation and neurodegeneration (Sil et al., 2014; Sil and Ghosh, 2016a; Gehrmann et al., 1995). The involvement of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been indicated in the process of neuroinflammation in ICIR (Kumar et al., 2006, 2007; Sil et al., 2014, 2016a; Sil and Ghosh, 2016a,b). During neurodegeneration, induced by neurotoxic chemicals, ROS in the brain may be accumulated due to the impairment of the cellular mechanisms that normally protect it against oxidative stress (Finkel and Holbrook, 2000). Neurons are especially susceptible to ROS as the brain consumes an inordinate fraction (20%) of the total oxygen consumption for its relatively small weight (2%) (Guix et al., 2012). This high metabolic rate produces an increased mitochondrial respiratory chain activity resulting in the production of a large amount of oxidants. Superoxide anions are mainly produced by the mitochondrial electron transport chain, though other sources also contribute to the generation of this radical species (Starkov, 2008). These superoxide anions react extremely fast with nitric oxide, which is generated by the mitochondrial nitric oxide synthase (mNOS) or the neuronal NOS (nNOS), to give peroxynitrite. At lower concentrations the peroxynitrite can alter the function of proteins by irreversibly

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reacting with the phenol ring of tyrosines to yield nitrotyrosine (Radi et al., 2002), leading to the inhibition of tyrosine phosphorylation and blockade of the signal transduction pathways of growth factors (Jonnala and Buccafusco, 2001), necessary for the survival of the neurons. It has been reported that nNOS is activated with persistent stimulation of excitatory amino acid receptors mediating glutamate toxicity, as well as with induction of induicible NOS (iNOS) by diverse stimuli like endotoxin or cytokines and overproduced nitric oxide (NO) (Chabrier et al., 1999). In these conditions, the role of NO changes from a physiological neuromodulator to a neurotoxic factor leading to neurodegenerative diseases (Akama et al., 1998).

Aminoguanidine (AG) has been identified by several authors as specific inducible NOS (iNOS) inhibitor (Di Naso et al., 2010). AG has been shown to inhibit inflammation and to reduce neurodegeneration in experimental autoimmune encephalomyelitis in mice (Cross et al., 1994). It has been reported that AG prevents cognitive deficits and nitrosative stress, in addition to reducing the astroglial activation in the hippocampus in STZ induced model of dementia (Rodrigues et al., 2009). Moreover, it has been shown that daily treatment with AG prevents development of Alzheimer traits (Aβ deposition and cognitive decline) in mice with hypertension obtained by transverse aortic coarctation (TAC) (Carnevale et al., 2012). It has recently been shown that AG prevents cognitive impairment caused by Aβ (25–35) protein in mice (Diaz et al., 2011, 2012, 2014). These studies have shown the potential effects of AMG on different experimental animal models by inhibiting iNOS.

The specific role of ROS and RNS on the colchicine induced neuroinflammation mediated neurodegeneration and cognitive impairments have not been identified. Neuroinflammation in ICIR was also linked with peripheral inflammation and alteration of immune responses probably through leaky blood brain barrier (BBB) (Sil et al., 2014; Sil et al., 2016c). If iNOS is involved with the neurodegeneration ICIR then inhibition of iNOS with specific inhibitor may result in the inhibition of neurodegeneration, cognitive impairments and altered peripheral immune responses. However, there is no report in literature in this regard.

The present study was designed to assess the role of iNOS on the i.c.v. colchicine induced neurodegeneration, memory impairments and changes of some systemic immune responses by inhibiting its activity with aminoguanidine.

2. Materials and methods

2.1. Animals

Charles-Foster rats (male, 200–250 g, 6–8-wk-of-age) and Swiss albino mice (male, 20–30 g, 6–8-wk-of-age) were obtained from local animal supplier (M/s Chakraborty Enterprise, Kolkata, India) for use in this study. All animals were housed individually in polypropylene cages at 25 ± 1 °C with a 12-hr light dark cycle. All animals had ad libitum access to standard rodent chow food pellets and filtered water. The Dept. of Physiology, University of Calcutta Animal Ethics Committee approved all protocols used in these studies.

2.2. Experimental design

Rats were divided into two experiments in the following way.

2.2.1. Experiment I

Rats were divided into three groups: control (C), sham (i.c.v. artificial CSF, S) and colchicine injected rats (i.c.v. colchicine injection, ICIR) in 27-day study. 6 rats from each group were initially habituated in an 8-arm radial maze for 5 days and then trained for 15 days (10 am to 4 pm) to learn and memorize the particular arms to obtain food. Each trial consisted of maximum 5 minutes duration or ended earlier when the rats visited (and consumed) all 4 baited arms. When training of the rats was complete, they were subjected to 27-day experiment. In the first 5 days of the 27-day experiment the memory/behavior parameters were measured in each rat of 3 groups from 10 am to 4 pm to obtain the baseline data and on the 6th day colchicine (in ICIR group) or artificial CSF (in sham operated group) was injected in the lateral ventricles of cerebrum by stereotaxic surgery. Following recovery from surgery (after 8 days of surgery or 14 days of 27-day experiment), the memory/behavioral parameters were again tested in ICIR and sham operated groups along with the control group on 1-15th (9th day after surgery), 18th (12th day after surgery), 21st (15th day after surgery), 24th (18th day after surgery) and 27th (21st day after surgery) days of the 27-day experiment. On day 21after the i.c.v. injection of colchicine or artificial CSF, all rats (including controls) were euthanized by ether, the brain were collected for histopathological study (for plaques and Nissl granules) in each group of rats and then immune parameters were measured in the hippocampus and periphery. The spleens of subsets of three rats within each group (i.e., with 9 rats/group, this yielded three sets of observations) were pooled to provide the requisite number of spleen cells to measure leukocyte adhesive inhibition indices (LAI), or phagocytosis by polymorphonuclear (PMN) cells, or cytotoxic activity of mononuclear cells (MNC). The phagocytic activity of blood WBC, levels of TNF-α, IL 1β, ROS and nitrite in the hippocampus and serum, serum corticosterone level were assessed in 6 rats from each group.

2.2.2. Experiment II

Rats were divided into three divisions: control (C), sham (i.c.v. artificial CSF, S) and colchicine injected ICIR (i.c.v. colchicine injection, ICIR). Each one was divided into 2 groups which contain 9 animals each: Group I (30 mg/kg body wt. of aminoguanidine), Group II (50 mg/kg body wt. of aminoguanidine). Therefore, 6 groups were used in this study. Different parameters as mentioned in Experiment I were measured following the same design in all the groups of rats from Experiment II.

2.3. Intracerebroventricular injection of colchicine in rats

In the lateral ventricle of rat, 7.5 μg of colchicine (SRL, India) dissolved in 2.5 μl artificial CSF (Kumar et al., 2007) was injected slowly for 5 min. The lateral ventricle of both side of the brain was approached stereotaxically (Paxinos and Watson, 1986) (AP −0.6 mm from bregma, L +1.5 mm and V +2.8 mm below cortical surface) through a steel cannula (0.45 mm diameter) connected to a Hamilton syringe in anesthetized rats (Na-thiopentone, 50 mg/kg body wt. i.p.). Thus, 15 μg of colchicine in 5 μl of artificial CSF was injected in each animal of ICIR group. The cannula was left in place for 2–3 min after i.c.v. injection. Rats of sham operated group received same volume (2.5 μl) of artificial CSF in each lateral ventricle by the same procedure. The trephine hole was covered with sterile bone wax after withdrawal of injecting needle. The muscles and skin were then sutured separately. Lignocaine HCl (local anesthetic) (Neon Laboratories Limited, Mumbai, India) was applied on the cut end of skin and muscles to minimize pain during surgery. Neosporin powder was sprayed over the cut surface as antiseptic measure.

2.4. Treatment of aminoguanidine

Aminoguanidine (Sigma Aldrich, USA) was dissolved in distilled water and it was administered orally through a gastric cannula attached to a 1-ml syringe. The daily dose of aminoguanidine was divided equally into two parts given at 6 hours intervals each. Two doses of aminoguanidine (30 and 50 mg/kg body wt.) were given p.o. to different groups of rats for 21-days each starting from 4 days prior to colchicine (i.c.v.) injection (cAD) and 4 days prior to vehicle (i.c.v.) injection (for sham-operated rats). Control rats were also treated with aminoguanidine for the same time period.