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Etoricoxib inhibits peripheral inflammation and alters immune responses in intracerebroventricular colchicine injected rats

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ABSTRACT

The present study was designed to investigate the effectiveness of etoricoxib induced inhibition of neuroinflammation by studying the peripheral inflammatory markers and select immune parameters in intracerebroventricular colchicine injected rats (ICIR). Results showed time dependent upregulation of the inflammatory markers in the serum along with alterations of peripheral immune parameters in ICIR and dose-dependent recovery was observed upon administration of etoricoxib to ICIR; most of these effects were greater with the longer duration of study. The present study indicates that colchicine induced neuroinflammation may cause systemic inflammation and alteration of immune responses which are mediated by increased cox- 2 activity.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease (Huberman et al., 1999; Jellinger and Bancher, 1996) but the mechanism of neurodegeneration is complicated and not yet clearly known. Different types of animal models have been used to investigate the mechanism of neurodegeneration in AD (Eijkenboom et al., 2000; Kumar et al., 2007; Rai et al., 2013; Nazem et al., 2015). Several authors have proposed neuroinflammation to be one of the causative factor for neurodegeneration in different chemically induced animal models of AD, e.g., streptozotocin (STZ), okadaic acid and intracerebroventricular (icv) colchicine injected rats (Nazem et al., 2015; Sil et al., 2015). Sil et al. (2014) reported cyclooxygenase (cox) induced neuroinflammation mediated neurodegeneration in icv colchicine injected rats, as naproxen (a non-specific cox blocker) could inhibit this neuroinflammation mediated degeneration. Kumar et al. (2006) also reported that colchicine induced cognitive impairments, oxidative and nitrosative stress was inhibited by cox- inhibitors. Sil and Ghosh (2016) has further shown that cox-2 expression and activity were increased in the hippocampus of intracerebroventricular colchicine injected rats (ICIR) and specific cox-2 blocker etoricoxib inhibited not only cox-2 expression and activity but also neuroinflammation, neurodegeneration and memory impairments. Thus it appears that cox-2 plays a major role in the process of colchicine induced neurodegeneration.

Neuroinflammation in the brain may have influence on the periphery as blood brain barrier was impaired in neuroinflammation (de Vries et al., 1997). A bi - directional communication between brain and periphery has been proposed by several workers (Nicola et al., 2013). In ICIR the higher level of TNF α, reactive oxygen species (ROS) and nitrite in brain were associated with the increased levels of these inflammatory markers in the periphery (Sil et al., 2014). Even some of the peripheral immune responses were found to be changed in colchicine injected rats indicating the altered inflammatory status in the periphery (Sil et al., 2014). Furthermore cox-2 expression and its activity as measured by PGE_2 in hippocampus were increased in ICIR (Sil and Ghosh, 2016). It was opined that the higher level of inflammatory markers in periphery may also induce the cox activity and thereby may further influence the immune responses (Sil et al., 2014). The peripheral inflammation and altered immune responses in ICIR are probably reflections of the central inflammation in these rats. In support of this contention it was found that administration of non-specific cox blocker not only inhibited the neuroinflammation in ICIR, but also showed concurrent regaining of the colchicine induced alteration of observed peripheral immune responses (Sil et al., 2014). Though the direct effect of cox inhibitor on periphery is a possibility but the brain source of inflammatory markers will continue to pass in periphery and may not be completely controlled by peripheral action of cox blockers.

Etoricoxib has been able to reduce the colchicine induced

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neuroinflammation in the hippocampus (Sil and Ghosh, 2016) but the effectiveness of etoricoxib on colchicine induced central inflammation may be further understood by studying its effect on the peripheral inflammation and immune responses in these rats. The link between etoricoxib mediated inhibition of brain inflammation, and alteration of peripheral inflammation and peripheral immune parameters in ICIR may be investigated by observing the levels of inflammatory markers in serum and the status of immune responses in these rats. The magnitude of inhibition on the peripheral inflammatory markers and immune responses has not been investigated in ICIR after application of etoricoxib. Therefore, this study was designed to investigate the effectiveness of etoricoxib induced inhibition of neuroinflammation by studying the peripheral inflammatory markers and select immune parameters in ICIR.

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; for use in passaging of Ehrlich ascites cells in one study outlined below) 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 in a facility maintained at 25 ± 1 °C with a 12 h 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

2.2.1. Experiment I

Rats were allocated into two groups (with sub-groups therein): Group I (14- day study) or Group II (21- day study). In each case the sub-groups were: control rats; sham-operated rats (receiving only icv injection of artificial CSF) and ICIR (receiving only icv colchicine). On Day 14/Day 21 after the icv injection of colchicine or artificial CSF, all rats (including controls) were euthanized by ether, and 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 white blood cells (WBC), levels of TNF α, ROS, nitrite (as marker for NO), cox-2 and PGE_2 in the serum were also assessed.

2.2.2. Experiment II

Rats were divided into two groups (with sub-groups therein): Group I (14-day study) or Group II (21-day study). In each case the sub-groups were: control rat + daily *per os* etorocoxib (10, 20, 30 mg/kg body weight); sham (icv artificial CSF) + daily *per os* 10, 20, 30 mg etoricoxib/kg; and ICIR (icv colchicine) + daily per os 10, 20, 30 mg (etoricoxib/kg). The various parameters as noted in Experiment I were also measured here, following the same experimental design in all the groups of rats.

2.3. Intracerebroventricular injection of colchicine in rats

Colchicine (7.5 μg in 2.5 μl of artificial CSF) was injected into the lateral ventricle of each side of the rat brain using stereotaxic coordinates: AP: - 0.6 mm from bregma, L. ± 1.5 mm from midline and V: 2.8 mm from the skull surface (Paxinos and Watson, 1986). Rats were anaesthetized with Na-thiopentone (50 mg/kg BW), and the head of the rat was fixed on the stereotaxic apparatus (ST141, INCO Ambala, Delhi, India) with the help of ear bars, incisor bar and nose clip. The skin covering the skull was opened by midline incision and periosteum over the skull surface was retracted. Burr holes were made on the two points over the skull surface (right and left side) according to the AP and L coordinates, with a dental drill. A steel micro-cannula (0.45 mm diameter) connected to a 10 μl Hamilton syringe (Hamilton, Australia) with polyethylene tubing, was inserted into the lateral ventricle using the V coordinate. The cannula was left in place for 2–3 min after the icv injection. Sham-operated rats received the same volume (2.5 μl) of artificial CSF (Kumar et al., 2007) 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, Mumbai, India) was applied on the cut end of skin/muscles to minimize pain during surgery. Neosporin powder was sprayed over the cut surface as an antiseptic measure.

2.4. Treatment of etoricoxib

Etoricoxib (Ranbaxy, India) was dissolved in 10% alcohol and it was administered orally through a gastric cannula attached to a 1-ml syringe. The daily dose of etoricoxib was divided equally into two parts given at 6 h interval. Three doses of etoricoxib (10, 20 and 30 mg/kg body wt.) were given p.o. to different groups of rats for 14/21- days each starting from 4 days prior to colchicine (icv) injection (ICIR) and 4 days prior to vehicle (icv) injection (for sham-operated rats). Control rats were also treated with etoricoxib for the same time period.

2.5. Blood collection

Upon ether dosing, blood for use in the flow cytometric (FACS) analyses was collected (0.5 ml, between 11:00–11:30 AM) from the heart of each rat using syringes containing 100 μl Na-citrate (3.8%, Sigma, St. Louis, MO). Another 1.5 ml blood was also collected without any anti - coagulant and used for serum isolation.

2.6. Cytokine assay

TNF α levels were assayed in the serum using a Rat TNF Flex Set, and a BD Cytometric Bead Array (CBA) Rat Soluble Protein Master Buffer kit (BD Biosciences, San Jose, CA) in BD Verse FACS instrument. FCAP Array software was used for the data analysis. A sample (25 μl) of this material was assessed for protein content using the method of Lowry et al. (1951), with bovine serum albumin (BSA) as standard. An additional sample (400 μl) was then mixed with 500 μl of a solution containing 10 mM Tris-HCl buffer, 1% BSA, and 0.2% Tween-20. An aliquot (50 μl) of this solution was then used to assay TNF α levels, according to manufacturer protocols. Cytokine levels were expressed as pg TNF α/100 mg protein in sample.

2.7. ROS estimation

Reactive oxygen species (ROS) levels in the serum were estimated according to the method of Socci et al. (1999). The serum (10 μl) samples were each incubated with 5 μM Dichloro-dihydro-fluorescein diacetate (DCF-DA) (Loba Cheme) for 30 min at 37 °C waterbath. Thereafter, formation of fluorescent DCF product was measured in FP 6200 spectrofluorometer (JASCO, Baltimore, MD) at excitation and emission wavelengths of 495 and 529 nm, respectively. ROS levels were expressed as % of control levels.

2.8. Nitrite estimation

Nitrite levels were estimated by the method of Green et al. (1982). In brief, 10 μl serum were mixed with an equal volume of Griess reagent [0.1% N-(1-naphthyl)-ethylenediamine 2- HCl, 1% sulfanilamide, 2.5% phosphoric acid] and incubated at 37 °C for 10 min. Thereafter, the absorbance in the sample was assessed at 550 nm in a