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Curcumin attenuates inflammatory response and cognitive deficits in experimental model of chronic epilepsy

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ABSTRACT

Evidence suggests that glial cells play a critical role in inflammation in chronic epilepsy, contributing to perpetuation of seizures and cognitive dysfunctions. The present study was designed to evaluate the beneficial effect of curcumin, a polyphenol with pleiotropic properties, on cognitive deficits and inflammation in chronic epilepsy. Kindled model of epilepsy was induced by administering subconvulsive dose of pentylenetetrazole (PTZ) at 40 mg/kg, i.p. every alternative day for 30 days to Wistar rats. The animals were assessed for cognitive deficits by Morris water maze and inflammatory response in terms of microglial and astrocyte activation. PTZ treated animals had increased escape latency suggesting impaired cognitive functions. Further, an increased expression of astrocyte (GFAP) and microglial (Iba-1) activation markers were observed in terms of mRNA and protein levels in the PTZ treated animals. Concomitantly, mRNA and protein levels of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokine (MCP-1) were increased in hippocampus and cortex. Immunoreactivity to anti-GFAP and anti-Iba-1 antibodies was also enhanced in hippocampus and cortex suggesting gliosis in PTZ treated animals. However, curcumin administration at a dose of 100 mg/kg to PTZ animals prevented cognitive deficits. A significant decrease in pro-inflammatory cytokines and chemokine expression was observed in hippocampus and cortex of PTZ treated rats supplemented with curcumin. In addition, curcumin also attenuated increased expression of GFAP and Iba-1 in animals with PTZ induced chronic epilepsy. Moreover, immunohistochemical analysis also showed significant reduction in number of activated glial cells on curcumin administration to PTZ treated animals. Taken together, these findings suggest that curcumin is effective in attenuating glial activation and ameliorates cognitive deficits in chronic epilepsy.

1. Introduction

Epilepsy is a public health problem that has a social and economic impact affecting an estimated 50 million people worldwide (Wang et al., 2008). Epileptic seizures are clinical manifestations that reflect a temporal dysfunction of a set of neurons in the brain (Engel and Henshall, 2009). Temporal lobe epilepsy (TLE) is one of the most commonly studied types of epilepsy because of its high prevalence and severity (Dogini et al., 2013). Indeed, it has been documented that epileptic seizures trigger neurodegenerative changes that eventually lead to memory impairment (Helmstaedter, 2002). Seizures in TLE are generally known to affect hippocampus leading to hippocampal sclerosis, the most common pathological finding (Blumcke et al., 2002). Chronic seizures in TLE have been often associated with significant memory impairment (Cho et al., 2015; Hermann and Seidenberg, 2007).

Neuroinflammation is a known factor that plays a prominent role in pathophysiology of number of neurological conditions (Czlonkowska and Kurkowska-Jastrzebska, 2011). In brain, the innate immunity is provided by microglial cells and astrocytes that act as a first line of defence against any insult (Solito and Sastre, 2012). The glial cells maintain homeostasis, provide trophic support and protect neurons (Sofroniew and Vinters, 2010). In chronic or sustained brain injury, microglial cells are gradually transformed from resting, ramified form to an activated or

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amoeboid form (Tambuyzer et al., 2009). Activation of microglia and astrocytes releases an array of inflammatory molecules that include cytokines and chemokines which can influence neuronal survival (Walker and Sills, 2012). These cells also influence the synaptic transmission which further results in altered excitability in epilepsy (Vezzani et al., 2012). Long term enhancement of excitability has been observed in the mice over-expressing cytokine levels following an inflammatory challenge (Akassoglou et al., 1997). Subsequently, several studies have detected changes in the levels of various pro-inflammatory cytokines in epileptic condition (Rao et al., 2009). The proliferation, immunophenotypical and other functional changes in microglial cells are induced by cytokines that affect neuronal functions (Ricci et al., 2009).

Compelling evidence demonstrates that compounds such as alpha-tocopherol, edaravone, alpha-asarone having antioxidant properties can reduce neuroinflammation and neuronal degeneration in various models of epilepsy (Betti et al., 2011; Miyamoto et al., 2008; Shin et al., 2014). Curcumin (diferuloyl methane) is a polyphenolic diketone found in *Curcuma longa* Linn. (Family Zingiberaceae) has anti-inflammatory and antioxidant properties (Motterlini et al., 2000). Curcumin has been found to have neuroprotective effects in Alzheimer's disease, stroke, head trauma (Cole et al., 2007), alcohol-induced neurotoxicity (Kandhare et al., 2012), Huntington's disease (Sandhir et al., 2013). Curcumin also shown to have an antioxidant effects in different models of epilepsy including kainic acid induced model of epilepsy, amygdala kindling and post-kindled model of epilepsy (Agarwal et al., 2011; Choudhary et al., 2013; Du et al., 2009; Gupta et al., 2009). Recently, curcumin has been shown to directly bind to and limit aggregation of the β-sheet conformations of amyloid characteristic of many neurodegenerative diseases, restore homeostasis of the inflammatory system, boosts the heat shock response to enhance clearance of toxic aggregates, scavenges free radicals, chelates iron and induces anti-oxidant response elements (Hu et al., 2015). It has also been reported that curcumin treatment significantly prevents the generalization of electroclinical seizure activity and hence has the potential anti-epileptogenic ability in iron-induced model of epileptogenesis (Jyoti et al., 2009). We have reported beneficial effect of curcumin on mitochondrial dysfunctions and oxidative stress in kindled model of chronic epilepsy (Kaur et al., 2014). However, the role of curcumin supplementation on glial cell response in chronic epilepsy has not been examined which might contribute to neuroinflammatory response in chronic epilepsy. Pentylenetetrazole (PTZ) induced kindling is well accepted and widely used model of chronic epilepsy to discover novel anti-epileptic compounds (Dhir, 2012). PTZ is a $GABA_A$ antagonist which when administrated (repeatedly) at sub-convulsive doses for a specific period of time, slowly results in the development of kindled model of chronic epilepsy (Hansen et al., 2004). Therefore, this study was designed to evaluate the anti-inflammatory potential of curcumin in PTZ induced kindled model of chronic epilepsy.

2. Material and methods

2.1. Chemicals

PTZ was purchased from Sigma Chemicals (St. Louis, MO, USA) and curcumin from HiMedia Laboratories (Mumbai, India). All the other chemicals used in the present study were of analytical grade and were procured from either Sigma Chemical Co. (St. Louis, MO, USA) or Sisco Research Laboratories (Mumbai, India). Anti-GFAP (Glial fibrillary acidic protein) and Anti-Iba-1 (Ionized calcium-binding adapter molecule 1) antibodies used for western blotting were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA) respectively. Rabbit polyclonal antibodies GFAP and Iba-1 used for immunohistochemistry were procured from Dako Corp. (Carpenteria, CA, USA) and Wako chemicals (Osaka, Japan) respectively. ELISA kits (OptEIA Set with specific antibody) for TNF-α (Tumor necrosis factor-alpha), IL-1β (Interleukin 1- β) and IL-6 were purchased from BD Biosciences (San Jose, CA, USA). Monocyte chemoattractant protein-1 (MCP-1) ELISA kit was purchased from Ray Biotech Inc. (Norcross, GA, USA). cDNA synthesis kit was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA USA). Primers were obtained from Invitrogen Corporation (Carlsbad, CA, USA) and Integrated DNA Technologies (Coralville, Iowa, USA).

2.2. Animals and treatment schedule

Adult male wistar rats (220–250 g) were procured from the Central Animal House of Panjab University, Chandigarh. The animals were housed under standard environmental conditions and had free access to standard pellet diet (Ashirwad Industries, Ropar, India) and water *ad libitum*. All the procedures were performed in accordance with ethical guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Ethics Committee (IAEC). All efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were randomly segregated into the following four groups with minimum eight animals in each group. The schematic representation of the treatment paradigm is provided in Fig. 1.

Control	Animals were administered with vehicle (normal saline) daily for the duration of treatment.
PTZ	Animals were administered PTZ intraperitoneally, (dissolved in saline) at a dose of 40 mg/kg every alternate day for a period of 30 days and a single dose of PTZ was administered on day 40 (challenge dose) to ensure kindling success.
Curcumin	Animals were administered with curcumin (suspended in 1% carboxymethylcellulose in distilled water) daily at a dose of 100 mg/kg body weight, orally through gavage for 40 days.
PTZ + Curcumin	Animals were administered with curcumin at a dose of 100 mg/kg orally daily, 30 min prior to PTZ administration for 40 days and PTZ was administered for 30 days only.

The dose of curcumin was selected based on reports from literature suggesting the average intake of turmeric is 2–2.5 g per day that corresponds to an intake of 60–100 mg of curcumin daily. Further, no adverse effects have been reported at this dose (Basnet and Skalko-Basnet, 2011). Carboxymethylcellulose, a hydrophilic polymer, was used as a vehicle for curcumin to improve its solubility and stability (Li et al., 2013).

2.3. Kindling procedure

Kindled model of epilepsy is extensively studied and routinely used model for the pre-clinical evaluation of anti-epileptic drugs in animals (Schroeder et al., 1998). In present study, kindling was induced in rats with repeated administration of pentylenetetrazole