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Inhibition Of Pentylenetetrazole-Induced Seizures And Neuronal Injury By Brilliant Blue G: Role Of Oxidative Stress, And Brain Derived Neurotrophic Factor

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Abstract

We aimed to investigate the effect of the P2X7 purinergic receptor antagonist brilliant blue G (BBG) on epileptic seizures, brain oxidative stress and neuronal injury induced in rats by pentylenetetrazole (PTZ). Rats received repeated intraperitoneal (i.p) injections of PTZ till the development of status epilepticus. BBG (50 or 100 mg/kg) was i.p. administered 30 minutes before starting PTZ injections. Seizure scores, the latency time and the PTZ dose for each group required to reach status epilepticus were determined. Brain kevels of malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), paraoxonase-1 (PON-1), brain derived neurotrophic factor (BDNF), interleukin-6 (IL-6) and chemerin were determined and histopathological examination done. PTZ induced significant increases in brain MDA and NO by 72.5% and 194.1%, respectively compared with the saline control. GSH, PON-1 activity and BDNF decreased by 41.4%, 39.8% and 33.4% while IL-6 and chemerin increased by 13.3% and 26.8%. The PTZ-induced changes in MDA, NO, GSH and PON-1 were significantly reduced by treatment with BBG which also significantly decreased IL-6 and BDNF levels. BBG (50 or 100 mg/kg) decreased the mean total seizure score by 82% and 87%, respectively. Scores for myoclonic jerks were reduced by 94.0% and 97.6% and that for generalized tonic-clonic seizures by 75%. BBG increased the threshold dose of PTZ by 66.7% and 106.7% and the latency time for status epilepticus by 118%. BBG exerts anticonvulsant and neuroprotective effects, at least in part by decreasing brain oxidative stress, BDNF and neuroinflammation.

Keywords: brilliant blue G; epilepsy; pentylenetetrazole; paraoxonase; acetylcholinsterase; interleukin-6; oxidative stress; purinergic P2X7 receptors.

1. Introduction

Epilepsy is one of the most common neurological disorders affecting about approximately 0.7% of the population. The disorder thus has important health, social and economic consequences. In epilepsy there is abnormal excessive synchronous firing of cortical cerebral neurons resulting in the clinical manifestations of seizures that includes disturbances in consciousness, sensation, movements, and mental function [1]. Seizures may originate simultaneously in both cerebral hemispheres (generalized seizures) originate in a part of one cerebral hemisphere, usually the temporal lobe (partial seizues) [2]. Despite the availability of several drugs that control seizures, it is estimated that about 30% of individuals with epilepsy are not controlled by two or more appropriately chosen antiepileptic drugs or other therapies, the so

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called “refractory epilepsy”, necessitating the search for or the development of new drugs or other treatment approaches [3]. The cascade of events during epileptogenesis involves activation of glutamate receptors, glutamate release, and an increase in the production of proinflammatory cytokines eg., IL-1β, IL-2, IL-6 [4] and reactive oxygen species (ROS) [5] which contributes to seizure-induced brain damage.

The purinergic P2X7 receptors are adenosine 5′-triphosphate (ATP)-sensitive ligand-gated cation channels. The sustained and excessive stimulation of P2X7 receptors by high extracellular concentrations of ATP released as a consequence of inflammation or tissue damage, results in opening of a transmembrane pore permeable to large molecular weight molecules and ultimately leading to cell death. P2X7 receptors are expressed in several brain areas and spinal cord on both neuronal and non-neuronal cells such as astrocytes, microglia, oligodendrocytes monocytes, macrophages, epithelial and endothelial cells [6]. P2X7 receptor activation causes the up-regulation and release of monocyte chemoattractant protein (MCP-1), interleukin-1β (IL-1 β), glutamate release from astrocyes, microglia, macrophages and monocytes [7,8]. It follows that blockade of these receptors, therefore, could represent a potential therapeutic mechanism in neuroinflammatory and neurodegenerative conditions including Alzheimer’s disease, multiple sclerosis and traumatic brain injury [9,10]. There is also an evidence to implicate P2X7 receptors in the pathogenesis of epileptic seizures [11]. Increased expression of the P2X7 receptor occurs in patients who have intractable temporal lobe epilepsy [12] and after status epilepticus in experimental animals [13].

Brilliant blue (BBG), also known as Coomassie brilliant blue which is widely used in biological studies in staining of proteins and in ophthalmic procedures in humans. Its derivative FD&C Blue No. 1 (Brilliant Blue FCF [BB FCF]) is a widely used food additive [14]. In the nanomolar range, BBG is a potent, non-competitive selective antagonist at the P2X7 receptor [15]. Studies showed that pharmacological blockade of P2X7 receptor with BBG exerts neuroprotective effects in experimentally induced cerebral ischaemia [16], brain trauma [17], spinal cord injury [18], Parkinson’s disease (19), and in Alzheimer’s disease transgenic mouse model [20]. P2X7 receptor antagonism reduced seizure severity in kainic acid-induced status epilepticus [21] while in the PTZ-kindling in rats, BBG was reported to a have moderate retarding on kindling development [22].

In this study, the effect of the ATP-gated P2X7 receptor blocker BBG on the development of seizures, brain inflammatory processes, oxidative stress, and seizure-induced brain damage evoked by the epileptogenpentylenetetrazole (PTZ) was studied. The γ-aminobutyric acid (GABA) (A) receptor antagonist PTZ is widely used to study the pathogenetic mechanisms underlying seizures development and in useful in screening of new compounds with antiepileptic activity [23].

2. Materials and methods

2.1. Animals

The study was conducted on male Sprague-Dawley rats weighing 180-200 g. Rats were group-housed under temperature- and light-controlled conditions and allowed standard laboratory rodent chow and water ad libitum. The experiments were done at 9 O’clock to avoid changes in circadian rhythm. The study was done in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) and the Institutional Ethics Committee. Seven rats were used per group.

2.2. Chemicals and reagents

Pentylenetetrazole (PTZ) and Brilliant blue G were purchased from Sigma Aldrich (St. Louis, USA) and dissolved in normal saline. Other chemicals and reagents used in the study were of analytical grade (Sigma, St. Louis, USA).

2.3. *Study design*

Rats were randomly divided into four different groups (6 rats each). Groups 1-3 were injected intraperitoneally with PTZ at a dose of 30 mg/kg and is followed by repeated doses each of 10 mg/kg every 10 minutes until the development of status epilepticus. Group 1 received saline 30 min prior to the start of PTZ injections and served as PTZ control group while groups 2 and 3 received BBG at doses of 50 or 100 mg/kg, 30 min prior to the begining of PTZ injections (treatment groups). A fourth group was treated with only saline (no PTZ) and served as a negative control. Seizures scores were recorded all over the experiment. Two hours after the last PTZ injection, rats were quickly euthanized by decapitation, their brains removed on ice-cold glass

plate, and stored at -80 °C until the biochemical assays. One half of each brain was kept in 10% formol saline for histopathological processing.

2.4. *PTZ-induced status epilepticus procedure*

Status epilepticus was induced by repeated i.p. injection of PTZ. Rats were given an initial dose of 30 mg/kg, followed by additional doses of 10 mg/kg PTZ i.p. every 10 minutes. Status epilepticus was defined as seizure lasting at least 10 minutes and consisting of prolonged episodes of seizures interrupted by post-ictal depression phases with no return to the quadruped posture or to consciousness [24]. Seizure behaviors during the study were classified into 5 stages and scored as follows: 0: no response; 1: ear and facial twitching; 2: convulsive waves through the body; 3: myoclonic jerks, rearing; 4: turn over onto one side position; 5: turn over onto back position, generalized tonic-clonic seizures [25].

The average individual seizure scores and the average total scores over the study period were determined and compared between the PTZ control and brilliant blue-treated groups. In addition, the average latency time and the average PTZ dose for each group to reach status epilepticus were determined.

2.5. Biochemical analyses

2.5.1. Determination of lipid peroxidation

Malondialdehyde (MDA), a product of lipid peroxidation was determined in tissue homogenates according to the method of Nair and Turne [26]. In this assay thiobarbituric acid reactive substances (TBA) react with thiobarbituric acid to form TBA-MDA adduct which can be measured colorimetrically at 532 nm.

2.5.2. Determination of lipid peroxidation

Nitric oxide was determined using colorimetric assay where nitrate is converted to nitrite via nitrate reductase. Griess reagent then act to convert nitrite to a deep purple azo compound that can be determined using spectrophotometer [27].

2.5.3. *Determination of reduced glutathione*

Reduced glutathione (GSH) was determined in tissue homogenates using the procedure of Ellman [28]. The assay is based on the reduction of Ellman´s reagent (DTNB; 5, 5'-dithiobis (2-nitrobenzoic acid)) by the free sulfhydryl group on GSH to form yellow colored 5-thio-2-nitrobenzoic acid which can be determined using spectrophotometer at 412 nm.

2.5.4. *Determination of paraoxonase-1*

The arylesterase activity of PON-1 was determined by a colorimetric method using phenyl acetate as a substrate. In this assay, PON-1 catalyzes the cleavage of phenyl acetate resulting in phenol formation. The rate of formation of phenol was measured by monitoring the increase in absorbance at 270 nm at 25°C. The working mix consisted of 20 mMTris/HCl buffer, pH 8.0, containing 1 mM CaCl2 and 4 mM phenyl acetate as the substrate. Samples diluted 1:3 in buffer were added to the above mix and the changes in absorbance were recorded following a 20 s lag time. One unit of arylesterase activity is equal to 1 μmole of phenol formed per minute. The PON1 activity is expressed in kU/L, based on the extinction coefficient of phenol of 1310 M–1cm–1. Blank samples containing water were used to correct for the spontaneous hydrolysis of phenyl acetate [29].

2.5.5. Quantification of interleukin-6

2.5.6. *Quantification of brain derived neurotrophic factor*

BDNF protein levels were detected in homogenates using a sandwich-type immunoassay kit (Glory Science, Del Rio, TX, USA) according to the manufacturer's instructions.

2.5.7. *Quantification of chemerin*

Chemerin levels were quantified using an ELISA kit according to the manufacturer instructions (Boster's Human/Chemerin kit, B Valley Ave, Pleasanton, CA, USA).

2.6. Histopathological studies

Brain samples of all animals were dissected immediately after death. The specimens were then fixed in 10 % neutral-buffered formalin saline for at least 72 hours. All the specimens were then washed in tap water for half an hour, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 5μm thick were cut and stained with haematoxylin and eosin for (H&E) for the histopathological investigation.

2.7. Statistical analyses

Data presented as mean ± standard error of the mean (SEM). Statistical significance for data from the biochemical assays was assessed using one-way ANOVA with Duncan's multiple range test post hoc test. Data of the behavioral study were analyzed by Kruskal-Wallis test followed by uncorrected Dunn's

test using Graphpad Prism software, version 6 (GraphPad Prism Software Inc., San Diego, CA, USA). A probability value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effect of brilliant blue G on PTZ-induced biochemical changes

3.1.1. Lipid peroxidation

In PTZ-treated rats, brain malondialdehyde was significantly increased by 72.5% compared with the control group (29.3 ± 0.62 vs. 17.0 ± 0.66 nmol/g.tissue). BBG given at doses of 50 or 100 mg/kg resulted in -19.2% and -26.70% decreases in brain malondialdehyde compared with the PTZ only treated group (23.7 ± 0.42 and 21.5 ± 0.47 vs. 29.3 ± 0.62 nmol/g.tissue) (Figure 1A).

3.1.2. Nitric oxide

After PTZ injections, there was marked and significant rise in brain nitric oxide by 194.1% compared to the saline control value (53.73 ± 0.94 vs. 18.27 ± 1.35 µmol/g.tissue). We observed no significant effect of BBG at 50 mg/kg on brain nitric oxide. BBG given at 100 mg/kg resulted in a significant decrease in brain nitric oxide by -24.1% compared with the PTZ only group (40.8 ± 1.59 vs. 53.73 ± 0.94 µmol/g.tissue) (Figure 1B).

3.1.3. Reduced glutathione

PTZ injections caused a significant depletion of brain reduced glutathione by 41.4% as compared to the saline control group (1.98 ± 0.13 vs. 3.38 ± 0.10 µmol/g.tissue). Treatment with BBG at 50 mg/kg had no significant effect on reduced glutathione levels in PTZ-kindled rats (2.28 ± 0.12 vs. 1.98 ± 0.13 µmol/g.tissue). The higher dose of BBG, however, induced a significant increase in reduced glutathione level by 36.9% compared with the PTZ control group (2.71 ± 0.06 vs. 1.98 ± 0.13 µmol/g.tissue) (Figure 1C).

3.1.4. Paraoxonase-1

A significant inhibition of PON-1 activity by 39.8% was observed in brain of PTZ-treated rats compared with their saline control group (7.65 ± 0.59 vs. 12.72 ± 0.83 kU/l). In PTZ-treated rats, BBG given at 100 mg/kg resulted in significant increase in brain PON-1 activity by 33.3% compared with the PTZ only group (10.2 ± 0.73 vs. 12.72 ± 0.83 kU/l).

3.1.5. Interleukin-6

In PTZ-treated rats, brain IL-6 increased significantly by 13.3% (16.62 ± 0.11 vs. 14.67 ± 0.22 pg/ml). In PTZ-treated rats, IL-6 decreased by 12.1% & 31.2% after administering BBG at 50 or 100 mg/kg (14.6 ± 0.37 and 11.44 ± 0.44 vs. 16.62 ± 0.11 pg/ml).

3.1.6. Brain derived neurotrophic factor

PTZ injections resulted in a significant decrease in brain BDNF by 33.4% compared with the saline control group (385.6 ± 15.9 vs. 578.4 ± 32.3 pg/ml). A further and significant decrease in brain BDNF by 17.8% & 21.6% was observed after treatment with BBG at 50 or 100 mg/kg (316.8 ± 10 and 302.2 ± 13.7 vs. 385.6 ± 15.9 pg/ml).

3.1.7. Chemerin

A significant increase in the brain level of the adipokinechemerin by 26.8% was observed in PTZ-treated rats compared with the saline control group (19.40 ± 0.42 vs. 15.30 ± 0.49 pg/ml). BBG at 50 or 100 showed no significant effect on brain chemerin in PTZ-treated rats (Fig. 3C).

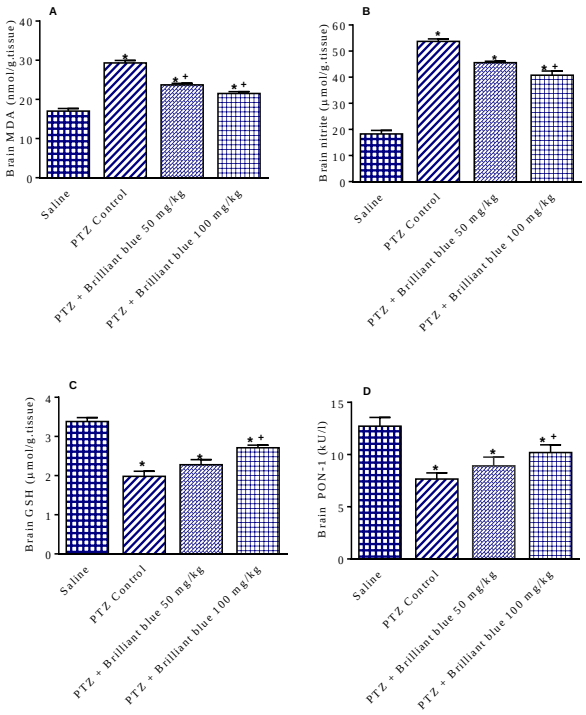

Figure 1. Effect of BBG on brain (A) Malondialdehyde (MDA), (B) Nitric oxide, (C) Reduced glutathione (GSH) and (D) Paraoxonase-1 activity (PON-1) in pentylenetetrazole (PTZ)-induced status epilepticus. *: p< 0.05 vs. saline-treated group. +: p< 0.05 vs. PTZ control group.

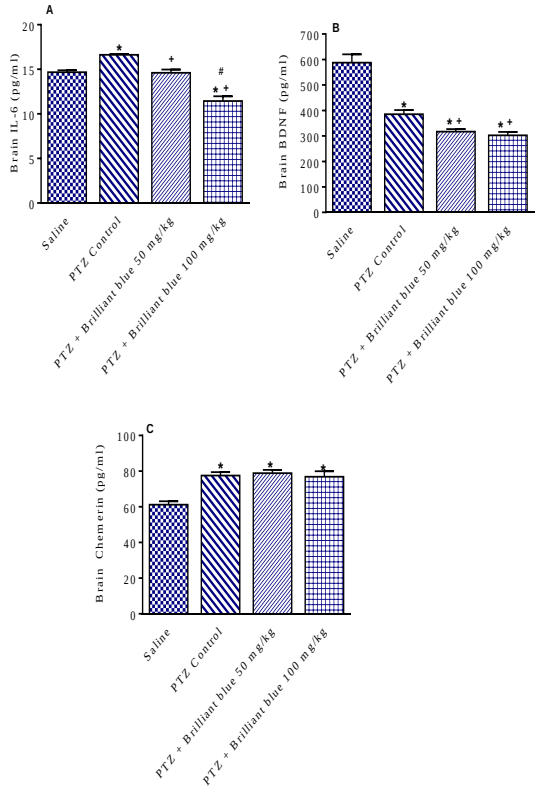

Figure 2. Effect of BBG on brain (A) Interleukin-6 (IL-6), (B) Brain-derived neurotophic factor (BDNF), and (C) Chemerinin in pentylenetetrazole (PTZ)- induced status epilepticus. *: p< 0.05 *vs.* saline-treated group. +: p< 0.05 *vs*. PTZ control group. #: p< 0.05 *vs.* PTZ + BBG 50 mg/kg group.

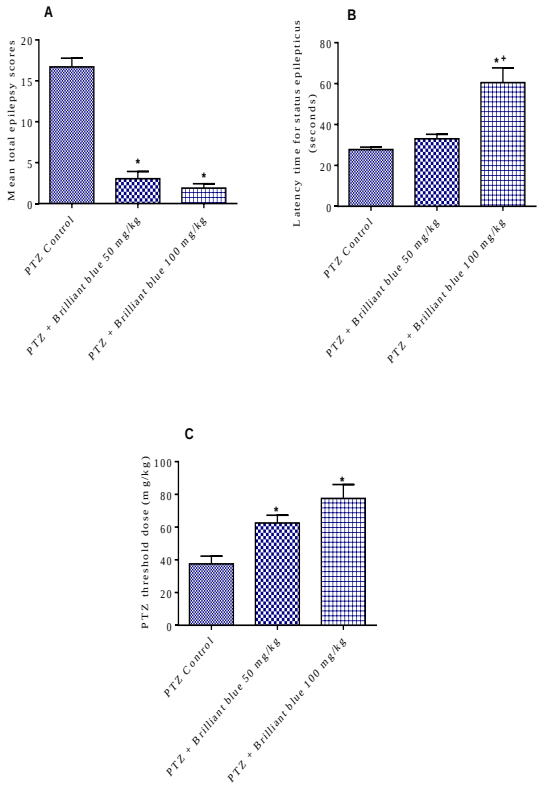

Figure 3. (A) Mean scores of all epilepsy stages in PTZ-treated rats. (B) Mean latency time for status epilepticus. (C) PTZ threshold dose to reach status epilepticus. Each bar represents mean ± S.E. of 7-8 experiments. Kruskal-Wallis test and uncorrected Dunn's test. *: p <0.05 vs. PTZ control. +: p <0.05 vs. PTZ + BBG at 50 mg/kg.

3.2. Effect of brilliant blue G on PTZ-induced convulsions

Figure 3A shows the total seizure scores during PTZ seizures following treatment with BBG (50 and 100 mg/kg, i.p.). BBG inhibited seizure development, decreasing mean seizure scores over the study period by 82% and 87%, respectively compared with the PTZ control group that received saline. The mean seizure score was 16.7 ± 1.0 in the PTZ control group and 3.05 ± 0.87 and 1.9 ± 0.52 in the 50 and 100 mg/kg BBG-treated groups, respectively. Figure 3B shows the corresponding changes in the latencies to develop status epilepticus. Only BBG at 100 mg/kg significantly increased the latency time by 118% (33.0 ± 2.2, 60.5 ± 7.1 vs. PTZ control value of 27.75 ± 1.2). Figure 1C shows that both doses of BBG resulted in significant increase in the threshold dose of PTZ needed to reach status epilepticus by 66.7% and 106.7%, respectively (62.5 ± 4.8, 77.5 ± 8.5 vs. PTZ control value of 37.5 ± 4.7 mg/kg).

Figure 4 shows the changes in the mean seizure scores for the stages 1-5 during the study. Compared with the PTZ control group, treatment with BBG (50 or 100 mg/kg) significantly decreased the development of stage 1 (ear and facial twitching) by 45.4% and 67.5%, respectively (10.5 ± 0.64, 6.25 ± 0.47 vs. PTZ control 19.25 ± 1.25). The occurrence of convulsive waves through the body (stage 2) was inhibited by 79.50% and 87.2% (2.0 ± 0.4, 1.25 ± 0.25 vs. PTZ control 9.75 ± 0.75) and myoclonic jerks and rearing (stage 3) by 94.0% and 97.6% (1.25 ± 0.25, 0.5 ± 0.29 vs. PTZ control 20.75 ± 0.85). The scores for stage 4 (turn over onto one side position) were inhibited by 92.8% by both doses of brilliant blue (0.25 ± 0.25 and 0.25 ± 0.25 vs. PTZ control value of 3.5 ± 0.28). The changes in the scores for generalized tonic-clonic seizures (stage 5) are shown in Figure E. Both doses of BBG were equally effective in inhibiting tonic-clonic seizures by 75% compared with the PTZ control value (1.25 ± 0.25 and 1.25 ± 0.25 vs. 5.0 ± 0.41).

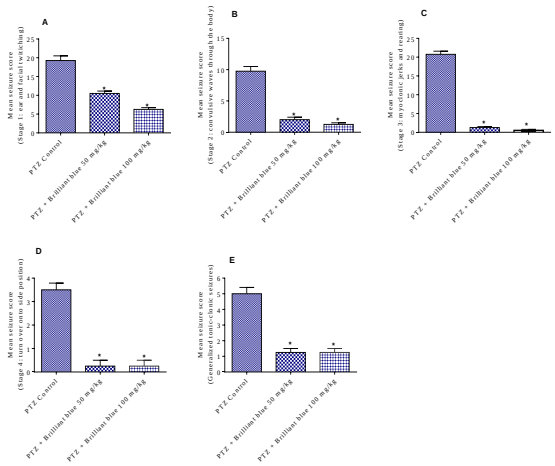

Figure 4. Mean scores of individual epilepsy stages in PTZ only, PTZ + BBG (50 mg/kg) and PTZ + BBG (100 mg/kg)-treated rats. Each bar represents mean ± S.E. of 7-8 experiments. Kruskal-Wallis test and uncorrected Dunn's test. *: p <0.05 vs. PTZ control.

3.3. Effect of brilliant blue G on PTZ–induced brain histologic damage

Sections from the hippocampus,of saline-treated rats showed normal structure (Fig. 5a). Rats treated with only PTZ showed the presence of many darkly stained neurons with small dark nuclei (Fig. 5b). Cerebral cortex and substantia nigra also suffered neurodegeneration (Figs. 6 & 7). The administration of BBG resulted in alleviation of the PTZ-induced neurodegeneration in hippocampus, cerebral cortex and substantia nigra (Figs. 5, 6, and 7).

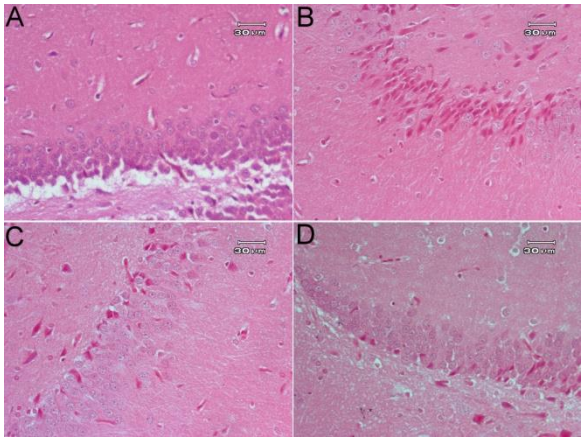

Figure 5. Representative photomicrographs of hippocampus sections in different groups. **(A)** Saline: shows normal normal orientation of neurons. **(B)** PTZ only: shows many cells appear darkly stained with small dark nuclei. **(C)** PTZ + 50 mg/kg brilliant blue: shows some dark neurons are still observed in between normal neurons. **(D)** PTZ + 100 mg/kg brilliant blue: shows only a few neurons appear dark in color.

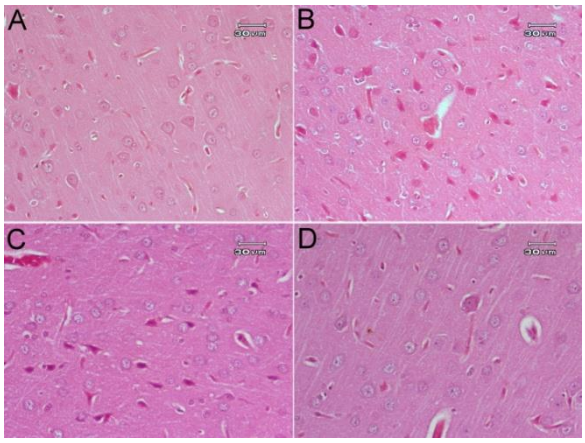

Figure 6. Representative photomicrographs of cerebral cortex in different treatment groups. **(A)** Saline: shows normal neurons with large vesicular nuclei and well-defined nucleoli. **(B)** PTZ only: shows dark stained neurons with small dark nuclei. **(C)** PTZ + 50 mg/kg brilliant blue: shows amelioration of many neurons, although some dark neurons are still observed. **(D)** PTZ + 100 mg/kg brilliant blue: shows normalization of almost all neurons.

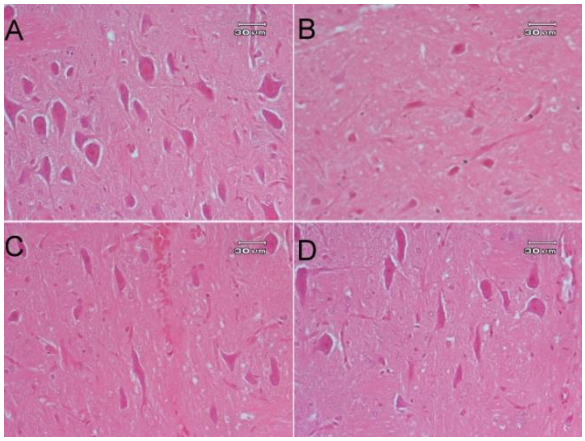

Figure 7 Representative photomicrographs of substantia nigra sections in different groups. (A) Saline: shows normal large, pigmented neurons with large vesicular nuclei. (B) PTZ only: shows dramatic decrease in both size and number of pigmented neurons. (C) PTZ + 50 mg/kg brilliant blue: shows slight increase in number of neurons. (D) PTZ + 100 mg/kg brilliant blue: shows a noticeable increase in neurons size.

4. Discussion

This study investigated the effect of the P2X7 antagonist BBG in an experimental model of status epilepticus using behavioral, biochemical and histological measures. We found that treatment with BBG was able to inhibit the PTZ-induced seizures, alleviate brain oxidative stress, IL-6 and afford neuronal protection.

Seizures evoked by the GABA(A) receptor antagonist PTZ are largely thought to involve attenuation of inhibitory function of GABA-ergic neurons [30,31]. There is also upregulation of NMDA glutamate receptors in hippocampus and cortex [32]. The hyperexcitability is associated with neuronal loss [33,34]. Using the PTZ-kindling model in rats, Fischer et al. [22] found a moderate, yet significant retarding effect for BBG on seizure development. PTZ was i.p., injected at an initial sub-convulsive dose of PTZ (35 mg/kg) and then once every 48 h. Pretreatment with BBG (50 mg/kg, i.p.) reduced seizure severity from the 5th to the 11th PTZ-injection. Engel [35] induced SE in mice using intra-amygdala injection of kainic acid and reported reduced seizure duration and neuronal death by treatment with BBG (1 pmol) given via intracerebroventricular route. Our results thus confirm previous studies and indicate an attenuating effect for BBG on the PTZ-induced SE. BBG inhibited all the individual epilepsy stages in a dose-dependent manner. The mean seizure score over the study period was markedly suppressed by 82% and 87% by 50 and 100 mg/kg of BBG, respectively, indicating potent antiepileptic potential for the dye. BBG also appear to interfere with the cascade of events leading to neuronal loss.

The present study indicated significant increase in brain oxidative stress after PTZ as indicated by an increase in the lipid peroxidation end product malondialdehyde [36], and the decrease in the antioxidant and free radical scavenger reduced glutathione [37]. This effect of PTZ is supported by other studies which showed decreased activity of the antioxidant enzymes superoxide dismutase and catalase in brain of PTZ-treated rats [34,38]. The increase in ROS during epileptogesis is considered to contribute to both seizure initiation and seizure-induced brain damage [39]. In this context, it has been shown that in electrically-induced SE in rats, reducing oxidative stress during epileptogenis with agents that increase intracellular glutathione level beneficially affected both the onset and progressions of seizures and decreased hippocampal CA1 cell loss [40]. Our results also indicated markedly increased brain nitric oxide, the over production of which is toxic to neurons via the reaction with superoxide to generate the highly reactive peroxynitrite (ONOO–) capable of protein and thiol nitrosylation and/or nitration as well as oxidation reactions [41]. The increase in brain NO could be involved in the initiation of seizures. The above notion is supported by studies in which inhibitors of nitric oxide synthases suppressed PTZ-induced seizures in rats [42] and low Mg^{++}-induced epileptic activity in hippocampal and entorhinal cortex slices in vitro [43]. Moreover, in PTZ-induced seizures, peroxynitrite derived from neuronal nitric oxide synthase accounted for endoplasmic reticulum stress and oxidative tissue damage [44]. Our results indicate an attenuating effect for BBG on the PTZ-induced increase in brain oxidative stress and nitric oxide levels. These actions of BBG are likely to be involved, at least in part in its anti-epileptic and neuroprotective effects. Under pathological conditions as in inflammation, cell damage, epileptic seizures, large amounts of ATP are released into the extracellular space, resulting in the activation of P2X7 receptors expressed on microglia cells, stimulating the production of superoxide and nitric oxide [45,46]. It is thus conceivable that blockade of P2X7 receptors under these conditions will have favorable effect reducing oxidative/nitrosative stress and consequently tissue damage.

We in addition demonstrated decreased brain PON-1 activity following PTZ injections which is consistent with our previous observations. The PON-1 enzyme is a calcium-dependent esterase that is involved in the hydrolysis of organophosphates and other xenobiotics [47] and is considered to have an important role in neurodegenerative disorders because of its antioxidative and anti-inflammatory capabilities [48]. Oxidative stress has been shown to inactivate PON-1 [49] which might provide an explanation for the decrease in the activity of the enzyme in conditions where there is an increased in ROS. This decrease in PON-1 activity would further increase the vulnerability of the cell to oxidative stress resulting in neurodegeneration. Here, we showed that treatment with BBG was associated with an increase in PON-1 activity refection either lower levels of oxidative stress or a neuroprotective effect.

The release of proinflammatory cytokines eg., IL-1β contributes to the initiation of epileptic seizures and the resulting brain damage [50,51]. Our present results demonstrated significant increase in IL-6 by PTZ which was reduced after treatment with 100 mg/kg BBG. IL-6 is a pro-inflammatory cytokine, expressed throughout the brain by both glial and neuronal cells and is upregulated following CNS infection or injury [52]. By decreasing the level of

IL-6, BBG thus is able to inhibit the inflammatory response. Brilliant blue G was shown to modulate the inflammatory response and provide neuroprotection in a number of central nervous system injury models. Thus, in spinal cord injury in the rat, intravenously (i.v) administered BBG (50 mg/kg) decreased the activation of astrocytes and microglia, infiltration of neutrophils near the site of injury and attenuated vacoulation and cell death in the injured tissue [18]. In rats with traumatic brain injury, BBG (50 mg/kg, iv.) reduced the levels of IL-1β and brain oedema [17]. BBG alleviated the increase in activated nuclear factor kappaB (NF-κB) and prevented neuronal atrophy in brain in experimentally-induced Parkinson's disease in the rat [19]. Moreover, its i.p. administration during mild systemic inflammation evoked by lipopolysaccharide endotoxin in the rat was shown to prevent neuronal loss in cerebral cortex and hippocampus and caspase-3 activation in cortex [53].

To further explore the possible mechanisms underlying BBG actions, we measured both BDNF and chemerin levels. The neurotrophin BDNF is widely distributed in the CNS being involved in neuronal survival and differentiation during development and modulating neuronal synaptic strength in adulthood [54]. BDNF is upregulated in the hippocampus during epileptogenesis and interference with BDNF signaling pathway inhibited epileptogenesis in animal models [55]. Other researchers, however, provided evidence that provision of BDNF suppresses seizures and hippocampal neuronal loss in experimental epilepsy [56]. Herein, levels of BDNF decreased in brain of PTZ-treated rats and showed further significant and dose-dependent decrease after treatment with BBG. Since BDNF increases neuronal excitability [55], there is an intriguing possibility that this effect of BBG is of relevance to its seizure suppressive action.

Chemerin functions as an inflammatory chemokine acting on the G protein-coupled receptors Cmklr1(chemokine receptor-like 1), chemokine (C-C motif) receptor-like 2 (Ccrl2) and G protein-coupled receptor 1 (Gpr1) on macrophages and dendritic cells enabling chemotaxis of these cells to sites of injury [57]. Chemerin also functions as an adipokine that has a role in inflammation and obesity [58]. The present study provided the first evidence for the increase in the level of chemerin in brain of rats with PTZ-induced SE, indicative of link between inflammation and seizure initiation. Our results, however, indicated no significant effect for BBG on brain chemerin in PTZ-treated rats.

The PTZ-induced kindling has been shown to result in neuronal cell loss in the hippocampus [33]. Our histologic study indicated the development of neuronal atrophy not only in the hippocampus but also cerebral and substantia nigra of PTZ-treated animals, suggestive of spreading excitotoxic neuronal injury inflicted by PTZ. These pathological changes were prevented by treatment with brilliant blue, suggesting that interference with the cascade of events that result in neuronal injury in this model of epilepsy.

5. Conclusions

The present study indicated marked suppressive effect for BBG on the development of seizures and neuronal degeneration evoked by the epileptogen PTZ. This effect of BBG was accompanied by decreased oxidative stress, IL-6 and BDNF in brain of PTZ-treated rats. These results suggest that BBG could prove of benefit in some forms of epilepsy.

6. Conflicts of interest

There are no conflicts to declare.

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8. References

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