

Original Research Article

Baicalein administration protects against pentylenetetrazole-induced chronic epilepsy in rats

Peiyu Li, Xiuping Wang, Junwu Zhang*

Department of Neurology, The Affiliated First Hospital Of Jiamusi University, Jiamusi, Heilongjiang, 154000, China

*For correspondence: **Email:** JulianneVselen@yahoo.com; **Tel/Fax:** 0086-0454-8623330

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Abstract

Purpose: To investigate the protective effect of baicalein against chronic seizures in pentylenetetrazole induced epilepsy in a rat model.

Methods: A rat model of chronic epilepsy was prepared by administration of pentylenetetrazole at a dose of 35 mg/kg to Sprague-Dawley rats. The animals were divided into 6 groups (5 rats/group): normal control, model (untreated epilepsy) and four treatment groups that received separately, intraperitoneal injection of 20, 30, 40 and 50 mg/kg baicalein, respectively, on alternate days for 30 days. On each day following baicalein treatment, behavioural alterations in the rats were assessed.

Results: Analyses of behavioural changes revealed significant ($p < 0.05$) decrease in pentylenetetrazole-induced convulsions by baicalein treatment at a dose of 50 mg/kg. Immunohistochemical studies revealed that treatment with baicalein caused significant ($p < 0.05$) dose-dependent reductions in the levels of inducible nitric oxide synthase (iNOS). Baicalein treatment inhibited alterations in cell morphology, and also inhibited pentylenetetrazole-induced increase in the proportion of glial fibrillary acidic protein (GFAP)-positive cells in a dose-dependent manner ($p < 0.05$). Real-time polymerase chain reaction (RT-PCR) analysis showed that baicalein significantly inhibited the expression of mRNA of NR1 subunit N methyl D aspartic acid (NMDA) receptor, without any effect on the expression of the NR2b (N-methyl D-aspartate receptor subtype 2B) subunit mRNA ($p < 0.05$).

Conclusion: These results indicate that baicalein inhibits pentylenetetrazole-induced chronic seizures in rats via reduction in astrocytes, inhibition of neuronal death and reduction of NR1 mRNA expression. Thus, baicalein has a potential for development into a new drug for the treatment of chronic epilepsy.

Keywords: Pentylenetetrazole, Epilepsy, Baicalein, Convulsion, Inhibition, behavioural changes, Hippocampus

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INTRODUCTION

The flavone, 5, 6, 7-trihydroxyflavone, known as baicalein (Figure 1), is a natural isolate obtained from *Scutellaria baicalensis* Georgi. Baicalein has for long been an important component of

traditional Chinese medicine [1]. It is used for treating ischemia and inflammatory diseases [1]. In vascular smooth muscle cells, baicalein attenuates endothelium intimal hyperplasia through inhibition of various pathways involved in inflammatory signalling, such as extracellular

signal-regulated kinase (ERK), serine threonine kinase (Akt) and nuclear factor- κ B (NF- κ B) [2]. In the mouse model of radiation-induced inflammation, baicalein treatment inhibited inflammation by targeting the Forkhead family of transcription factors and NF- κ B [3]. Baicalein also exhibits anticancer properties, and has been shown to inhibit colorectal cancer [4]. In prostate carcinoma cells, studies have shown that baicalein suppressed the rate of cell proliferation, and inhibited metastatic and migration potential [5]. Thus baicalein possesses promising anti-inflammatory properties.

The death of neurons due to chronic seizures increases the susceptibility of the hippocampal tissues to further degradation, resulting in the development of chronic epilepsy [6]. Studies have revealed that administration of pentylentetrazole leads to loss of neurons, resulting in chronic seizures [7]. The inflammatory factor, nitric oxide synthase is one of the prominent agents for induction of seizures [8, 9]. In the present study, the protective effect of baicalein against pentylentetrazole-induced chronic epilepsy in the rat was investigated.

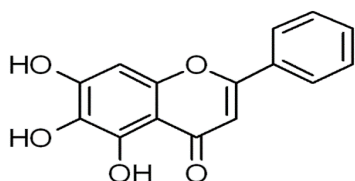


Figure 1: Structure of baicalein (5, 6, 7-trihydroxyflavone)

EXPERIMENTAL

Animals and treatment protocol

Thirty Sprague-Dawley rats (weighing 180 - 200 g) were obtained from the Shanghai Laboratory Animal Centre (SLAC, Shanghai, China). The animals were assigned randomly to 6 groups (5 rats/group): normal control, model and four treatment groups which were separately and intraperitoneally injected 20, 30, 40 and 50 mg/kg baicalein. The animal model of chronic epilepsy was prepared by intraperitoneal administration of 35 mg/kg pentylentetrazole for 28 consecutive days. Baicalein was injected on alternate days for 30 days. This study was performed in line with the guidelines contained in the *Guide for the Care and Use of Laboratory Animals* of the National Institute of Health [10]. All the experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the First Affiliated Hospital of

Zhengzhou University, Zhengzhou, China (approval no. 109/ZUC).

Analysis of alterations in rat behaviour

Changes in the behaviour of the experimental rats were analysed on the day following each treatment with baicalein, for the 30 days of the study. A known five-point scale was used for the assessment of the stage of chronic seizure [8]. In this scale, no response, twitching of ear and face, body convulsion, jerking, side turn and seizure were assigned stages 1, 2, 3, 4 and 5, respectively.

Extraction of hippocampus

On day 31, the animals were sacrificed, and whole brains were excised. The brain material was divided into two halves. The right half was subjected to paraffin embedment, followed by thin sectioning. Half of the brain material from each animal was subjected to RT-PCR analysis.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA from the hippocampus tissue was extracted using TRIzol reagent (Invitrogen Co., Carlsbad, CA). The extracted RNA was digested for 20 min using RNase-free DNase (Boehringer Mannheim, Indianapolis, IN) at 37 °C. The RNA samples (2 μ g-samples) were used for the synthesis of cDNA by incubation for 1 h at 37 °C with Avian Myeloblastosis Virus (AMV) reverse transcriptase (Amersham Corp., Arlington Heights, IL). Sequence amplification to desired primers was performed using polymerase chain reaction (PCR). For the purpose of amplification, Mastercycler (Eppendorf, Hamburg, Germany) was used. The cycling consisted of denaturation for 4 min at 94 °C, followed by 30 cycles of 30 sec at 94 °C, 30 sec at 58 °C, 40 sec at 72 °C and then 10 min at 72 °C. After amplification, the samples were put in 1.5 % agarose gels followed by visualization using ethidium bromide.

Histopathological examination

The paraffin-embedded hippocampal tissue sections were de-paraffinized in boiling xylene. The sections were then stained with haematoxylin and eosin (H & E). The stained sections were examined under light microscope (BX51M; Olympus, Tokyo, Japan; at a magnification of x 10) for changes in tissue morphology.

Immuno-histological examination

The paraffin-embedded hippocampus tissue sections were subjected to de-paraffinization in boiling xylene, followed by re-hydration in ethyl alcohol gradient. The sections were then treated with hydrogen peroxidase for blocking the activity of endogenous peroxidase enzyme. The tissue sections were then analysed for the expression of the activity of iNOS using rabbit anti-mouse primary antibodies (Santa Cruz Biotechnology, Inc.). The sections were examined and imaged using a Leica microscope equipped with a digital camera (Leica, Solms, Germany).

Statistical analysis

Statistical analysis was performed using SPSS 18.0 statistical software (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean \pm standard error of the mean (SEM). Comparisons among multiple groups were performed using a one-way

analysis of variance (ANOVA), and a least significant difference post hoc test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effect of baicalein on chronic seizures induced by pentylenetetrazole in rats

A known five-point scale [11] was used for the analysis of the effect of baicalein on convulsions caused by pentylenetetrazole in the rats. The seizure grades in the group treated with 50 mg/kg baicalein were significantly lower than those in the model control group ($p < 0.02$; Figure 2). Although reduction in seizure grades was observed with baicalein at a dose of 30 mg/kg, the effect was maximum at the dose of 50 mg/kg.

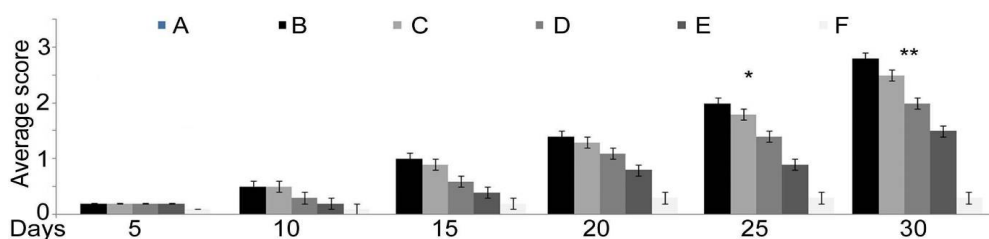


Figure 2: Inhibitory effect of baicalein on pentylenetetrazole-induced convulsions in rats. * $p < 0.05$ and ** $p < 0.02$ (untreated vs. 30 and 50 mg/kg groups, respectively). A, B, C, D, E and F stand for normal, model, 20 mg/kg, 30 mg/kg and 50 mg/kg treatment groups, respectively

Effect of baicalein on iNOS in pentylenetetrazole-treated rats

There was a significant increase in iNOS level in the pentylenetetrazole-treated group, when compared to the normal control group ($p < 0.05$). However, baicalein treatment for 30 days led to significant and dose-dependent reductions in iNOS ($p < 0.05$, $p < 0.02$), with maximum reduction at a baicalein dose of 50 mg/kg (Figure 3).

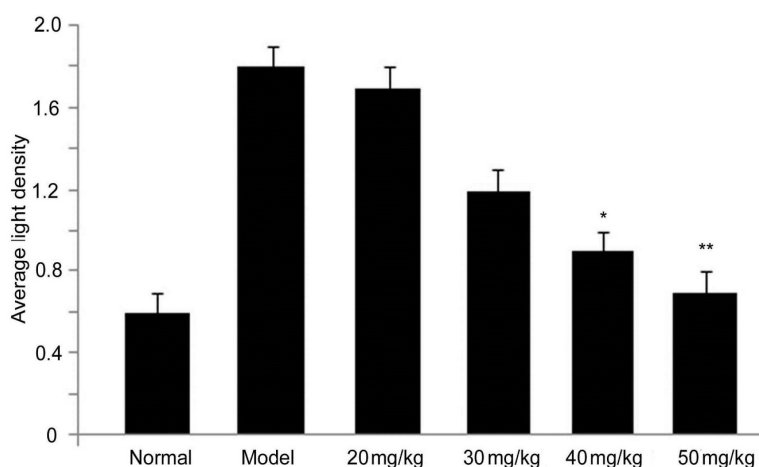


Figure 3: Effect of baicalein treatment on the expression of iNOS in pentylenetetrazole-treated rats. The rats were treated with 20, 30, 40 and 50 mg/kg of baicalein on alternate days for 30 days. * $p < 0.05$ and ** $p < 0.02$ baicalein groups vs. model group

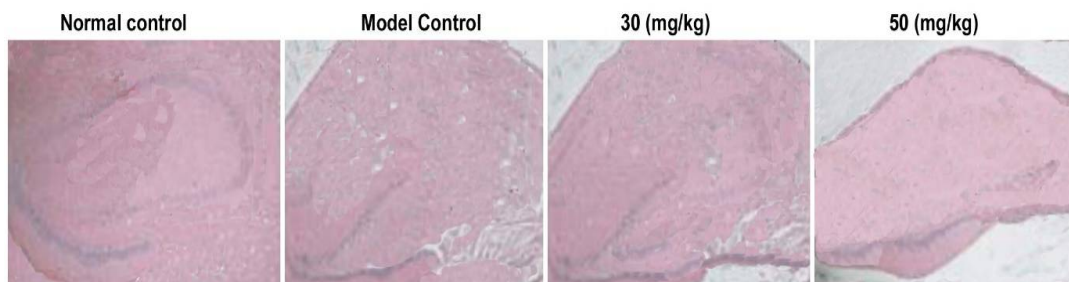


Figure 4: Effect of 30 mg/kg and 50 mg/kg doses of baicalein on hippocampal neuronal injury in pentylenetetrazole-treated rats. (H & E stain, x 250)

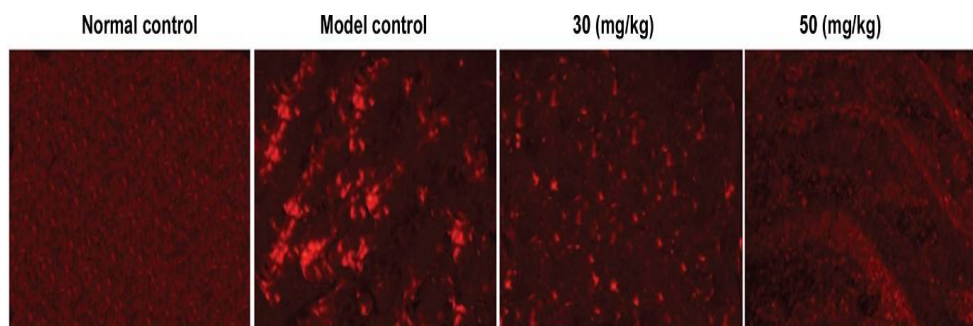


Figure 5: Effect of baicalein treatment on the population of GFAP-positive cells in rat hippocampus. Baicalein significantly reduced pentylenetetrazole-induced increases in hippocampal population of GFAP-positive cells. (x100)

Effect of baicalein on pentylenetetrazole-induced cell injury in rats

Microscopic examination of the rat hippocampal tissues revealed presence of wide intercellular spaces, irregular cellular morphology and apoptotic cells in the pentylenetetrazole (model) group. However, baicalein treatment inhibited the alterations in cell morphology in a dose-dependent manner (Figure 4). The morphologies of the hippocampus tissues of the rats in the normal control group were similar to those in the 50 mg/kg baicalein treatment group.

Effect of baicalein on astrocytic hyperplasia

Pentylenetetrazole significantly increased the proportion of GFAP-positive cells in the rat hippocampus, when compared to the control group. However, treatment of the rats with baicalein dose-dependently inhibited the pentylenetetrazole-induced increases in the proportion of GFAP-positive cells ($p < 0.05$; Figure 5). The proportion of GFAP-positive cells in the normal control and in the 50 mg/kg baicalein treatment groups were similar.

Effect of baicalein on NMDA receptor level

Results from RT-PCR analysis revealed a significant increase in the expression of NR1 mRNA in the pentylenetetrazole group, when

compared to the normal control group ($p < 0.05$). However, treatment of the rats with baicalein significantly inhibited the expression of NR1 mRNA (Figure 6). Analysis of the NR2b mRNA level showed no changes in the pentylenetetrazole group relative to the normal control group (Figure 6).

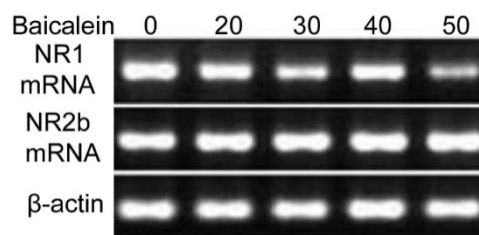


Figure 6: Effect of baicalein on expression of NR1 mRNA in the pentylenetetrazole-treated rats. RT-PCR analysis showed a significant reduction in the expression of NR1 mRNA in baicalein-treated rats, when compared to the model group

DISCUSSION

In the present study, the protective effect of baicalein on chronic seizures induced in rats by administration of pentylenetetrazole was investigated. The results revealed that baicalein treatment inhibited increases in proportion of astrocytes, and also inhibited neuronal loss and expression of NR1 mRNA in the rat hippocampus. It has been reported that the

development of chronic seizures as a result of neurological disorders in the rat model can be inhibited by administration of chemotherapeutic agents [8,12].

The results from the present study demonstrate that baicalein inhibited pentylentetrazole-induced convulsions in the rat model at comparatively low doses. This is important, because existing anti-convulsive agents exhibit their effects at much higher doses [12]. Furthermore, in the rat model of pentylentetrazole-induced chronic epilepsy, baicalein treatment markedly reduced seizure grades. These findings suggest that baicalein is an efficient anti-convulsive agent, relative to already-established anti-convulsive compounds. Studies in animal models have shown that the development and progression of chronic seizures is associated with the expression of iNOS [8,9].

The expression of iNOS is higher in the tissues of animals with chronic epilepsy [9]. In the present study pentylentetrazole administration to rats increased the expression of iNOS. This is consistent with earlier reports. However, treatment of the rats with baicalein inhibited pentylentetrazole-induced up-regulation of iNOS expression. These results suggest that baicalein inhibited development of chronic seizures in the rats through suppression of iNOS expression.

During chronic seizures, neuronal death enhances the susceptibility of the hippocampal tissues to further degradation, leading to the development of chronic epilepsy [9]. Pentylentetrazole administration to rats triggers neuronal death and leads to development of chronic seizures [13]. Results from the present study showed presence of large intercellular spaces and deranged cellular morphology in the rat hippocampal tissues after pentylentetrazole administration. However, baicalein treatment reversed these changes in cellular morphology induced by pentylentetrazole.

Astrocytes are responsible for increasing the expression of cytokines which in turn initiate inflammatory processes in neuronal cells [14]. The population of astrocytes in the hippocampus of pentylentetrazole-treated rats has been reported to be elevated, indicating the onset of epilepsy [15]. In the present study, pentylentetrazole increased the proportion of GFAP-positive cells in rat hippocampus. However, the pentylentetrazole-induced increase in the proportion of GFAP-positive cells was annulled by baicalein.

CONCLUSION

The results obtained in this study strongly suggest that baicalein treatment is effective in the prevention of chronic seizures caused by administration of pentylentetrazole in rats. It exerts this effect through reduction in astrocytes, inhibition of neuronal death and reduction of NR1 mRNA expression. Thus, baicalein has a potential for use in the treatment of chronic epilepsy but this requires further investigations.

DECLARATIONS

Conflict of Interest

The authors declare that they have no competing interests with regard to this work.

Author contributions

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Peiyu Li and Xiuping Wang performed the experimental work and compiled the results. Junwu Zhang designed the study and wrote the manuscript. The manuscript was read thoroughly and approved by all the authors.

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