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Research Paper

Effect of γ-glutamylcysteine ethylester on the levels of *c-fos* mRNA expression, glutathione and reactive oxygen species formation in kainic acid excitotoxicity

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Abstract

Objectives The aim of this study was to investigate the effect of γ-glutamylcysteine ethylester (GCEE), a precursor of glutathione biosynthesis, on the levels of glutathione, formation of reactive oxygen species and c-fos mRNA expression in rat hippocampus and cortex in kainic acid-induced excitotoxicity.

Methods Sprague–Dawley rats were used and divided into four groups: control, kainic acid (10 mg/kg), GCEE (10 mg/kg) and kainic acid (10 mg/kg) + GCEE (10 mg/kg). Kainic acid and GCEE were administered to the rats intraperitoneally. The levels of glutathione and the expressions of c-fos mRNA in hippocampus and cortex tissues were determined using spectrophotometric and reverse transcription followed real-time PCR methods, respectively. Formation of reactive oxygen species was determined using dichlorofluorescin fluorescence in brain synaptosomes treated with kainic acid or GCEE *in vitro*.

Key findings Kainic acid treatment significiantly upregulated the expression of c-fos mRNA in the hippocampus and cortex when compared to the control group. GCEE treatment significantly decreased the levels of *c-fos* mRNA in the cortex when compared to the kainic acid-treated group. GCEE treatment against kainic acid significantly increased the levels of glutathione in the cortex and hippocampus, and decreased the levels of formation of reactive oxygen species when compared to kainic acid-treated synaptosomes.

Conclusions The increased levels of glutathione and the reduced levels of reactive oxygen species formation lead us to conclude that GCEE may be beneficial as a potential antioxidant against neurodegenerative processes where excitotoxicity is involved.

Keywords γ-glutamylcysteine ethylester; c-fos mRNA; glutathione; kainic acid; rat brain; reactive oxygen species

Introduction

Glutamate is a primary excitatory neurotransmitter in the central nervous system. It is well established that the glutamate receptors play a critical role in the neuronal survival and refinement of neuronal connections during brain development, as well as in the synaptic plasticity underlying learning and memory.[1,2] Over-activation of glutamate receptors leads to neurodegeneration and this phenomenon, called excitotoxicity, has been implicated in major areas of brain pathology.[3,4] Glutamate induced excitotoxicity is a triggering factor that causes neurodegeneration underlying central nervous system pathologies such as epilepsy, stroke and Alzheimer's disease.[5] Kainic acid (KA) is a glutamate analogue. Systemic administration of KA to rodents at convulsant doses induces epileptic seizures and excitotoxic cell death in neurons of the entorhinal cortex, amygdala, CA1 and CA3 regions, and hilus of the hippocampus.[6]

It has been hypothesised that increased levels of intracellular calcium trigger the excitotoxic process. In addition glutamate-mediated excitotoxicity activates certain enzymes by calcium-dependent pathways.[7] These enzymes are calpains, protein kinase C, lipases, phospholipases, endonucleases, xanthine oxidase, nitric oxide synthase and the arachidonic acid cascade. Oxidative stress and the formation of reactive oxygen species (ROS) are other key components of neurotoxicity induced by glutamate.[8,9] KA-induced neuronal death in certain brain regions such as the hippocampus and cortex shows an apoptotic character, which is accompanied by a specific pattern of immediate early gene (IEG) induction.[10,11]

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Proto-oncogene *c-fos*, a member of the immediate early gene family, is highly expressed in specific types of neurons during brain development, although the levels are lower in adulthood.[12] Following a variety of physiological stimuli, chemical agents and transmitter agonists, *c-fos* gene expression is rapidly and transiently induced in many cell types for signalling late-response genes that generate functional proteins.[12,13] *c-fos* activation has been proposed as a marker of neuronal injury since its induction is promoted by abnormal brain function.[14] *c-fos* mRNA expression triggered by KA in rat brains shows specific patterns. The *c-fos* expression is mainly observed in the hippocampus and cerebral cortex. Calmodulin is involved in the process of *c-fos* induction.[15,16]

Previous studies examining the role of oxidative stress in neurodegenerative diseases, ageing and carcinogenesis have been concentrated on the characterisation of the cellular antioxidant defence systems. The primary defences of eukaryotic cells against oxidative stress include antioxidant enzymes and glutathione (GSH)-utilising systems.[17,18] The ratio of intracellular thiol reductants and ROS plays a pivotal role in determining whether cells undergo growth or apoptosis.[18,19] Because of the inadequate antioxidant capacity of brain, GSH is highly important as an endogenous antioxidant. Intravenous or oral administration of GSH provides only constituent amino acids. It is known that GSH is poorly taken up by cells and is rapidly degraded in the circulation. GSH is also not able to cross the blood–brain barrier.[20,21]

It has been shown that GCEE can upregulate GSH levels in rat brains as a precursor of glutathione biosynthesis. GCEE treatment prevents feedback inhibition of γ-glutamylcysteine synthase (the rate limiting enzyme) activity and provides cysteine (the limiting substrate) for GSH synthesis in order to upregulate the levels of GSH.[22,23]

The present study aims to investigate the effect of GCEE on the levels of GSH, ROS formation and the expressions of *c-fos* mRNA in rat brain cortex and hippocampus tissues treated with KA.

Materials and Methods

Animals and treatments

Twenty adult male Sprague–Dawley rats, weighing 200–250 g, were used in the present study. All animals were maintained on a 12 : 12 h light : dark cycle and given continuous access to food and water. The protocol for the experiment was approved by the Appropriate Animal Care and Use Committee of Ege University.

Animals were divided into four groups: (1) the control group ($n = 4–6$), 1 ml/kg saline; (2) KA group ($n = 4–6$), 10 mg/kg kainic acid only; (3) GCEE group ($n = 4–6$), 10 mg/kg GCEE only; (4) KA+GCEE group ($n = 4–6$), 10 mg/kg GCEE plus 10 mg/kg KA. Saline, KA and GCEE injections were administered to the rats intraperitoneally (i.p.).

GCEE was purchased from Bachem (Torrance, CA, USA) and KA was obtained from Sigma-Aldrich (Vienna, Austria). GCEE and KA were dissolved in saline. Animals injected with KA were kept under observation for 3 h to score seizure sensitivity and behavioural alterations. At the end of the observation period, rats were decapitated and the brains were removed. Cortex and hippocampus tissues were dissected on ice. All samples were stored at −80°C until use.

Measurement of glutathione levels

GSH levels in the hippocampus and cortex were measured enzymatically by using a modified version of the DTNB-GSH reductase assay[24,25] as described by Drake *et al.*[23] The method is based on the determination of a chromophoric product, 2-nitro-5-thiobenzoic acid, resulting from the reaction of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) with GSH. In this reaction, GSH is oxidised to oxidized glutathione (GSSG), which is then reconverted to GSH in the presence of GSH reductase and NADPH. The formation of 2-nitro-5-thiobenzoic acid was followed at 412 nm using a Shimadzu UV-160 spectrophotometer.

Rat brain cortex and hippocampus samples were precipitated with 10% 5-sulfosalicyclic acid. Samples were centrifuged for 5 min at 10 000g and the supernatant was neutralised with 6–9 μl of neat triethanolamine. Both 6 mM DTNB and 0.3 mM NADPH were made in the stock buffer (143 mM sodium phosphate and 6.3 mM Na_4-EDTA, pH 7.5) daily. A stock solution of 50 units of GSH reductase per millilitre was stored at 4°C. GSH standard stock solution was made in double-distilled water. To the NADPH solution (140 μl), 20 μl of DTNB solution and 40 μl of sample or standard were added in polyethylene dark tubes and incubated at 37°C for 10 min. Ten microlitres of a 10-times diluted GSH reductase stock solution was added to each tube. The samples were vortexed and the absorbance was read at 412 nm. The GSH levels in the brain cortex and hippocampus were expressed as nanomoles per milligram of protein. Protein concentrations were measured using Lowry's method.[26] All chemicals were purchased from Sigma-Aldrich (Vienna, Austria).

Synaptosomal preparation

Synaptosomes were prepared as previously described by Bonnet and Costentin,[27,28] with minor modifications. Total brain was homogenised in 10 volumes of 0.32 M sucrose using a Teflon–glass homogeniser (800 rpm). The homogenates were then centrifuged at 1000*g* for 10 min to give a nuclear pellet containing nuclei, cell bodies and axon fragments. Supernatants were stored at 4°C, and the pellet was resuspended in 10 volumes of 0.32 M sucrose and centrifuged for 10 min at 1000*g*. Then supernatants were pooled and centrifuged at 17 500*g* for 30 min at 4°C, after which the supernatant was discarded and the final pellet resuspended in ice-cold Krebs–Ringer buffer, pH 7.6 (NaCl 120 mM; KCl 4.8 mM; $CaCl_2$ 1.3 mM; $MgSO_4$ 1.2 mM; KH_2PO_4 1.2 mM; $NaHCO_3$ 25 mM; glucose 6 mM). Protein concentrations in the pellets were measured by the method of Lowry, and the protein content of each sample was adjusted to 1 mg/ml.[26] All chemicals were purchased from Sigma-Aldrich (Vienna, Austria).

Measurement of formation of reactive oxygen species

The dicholorofluorescein (DCF) assay was used to measure the levels of ROS according to the procedure previously described by Wang and Joseph.[29] The cell-permeable 2,7-

dichlorofluorescin diacetate (DCF-DA) crosses into the synaptosomes, where it is deesterified by cellular esterases, resulting in DCFH. DCFH in turn is converted on oxidation to the highly fluorescent DCF.

A 10 mM stock solution of 2,7-dichlorofluorescin diacetate (DCF-DA) in ethanol was stored at −70°C. 0.5 mM and 1 mM KA or GCEE solutions were prepared in saline. Synaptosomes were pre-incubated for 3 h at room temperature with KA or KA and GCEE in concentrations of 0.5 mM and 1 mM according to the procedure previously described by Camins *et al*. with minor modifications.[30] Synaptosomes were incubated with 10 mM DCF-DA for 30 min at room temperature, then were spun at 3000*g* for 5 min at 4°C and resuspended in phosphate-buffered saline (PBS). Synaptosomes that were pre-incubated with saline were used as control. DCF fluorescence was measured at $\lambda_{ex} = 495$ and $\lambda_{em} = 530$ nm. The measurements were performed on a Molecular Devices SpectraMax microtiter plate reader. All chemicals were purchased from Sigma-Aldrich (Vienna, Austria).

RNA isolation, reverse transcription and real-time polymerase chain reaction

Total RNA was isolated from the hippocampus and cortex tissues using Trizol reagent (Gibco BRL Life Technologies, Grand Island, NY, USA) followed by phenol–chloroform exraction and isopropanol precipitation.[31] Total RNA (1 μg/μl) was used for first-strand cDNA synthesis by Moloney-murine leukemia virus (MMuLV) reverse transcriptase enzyme (MBI Fermantas, Slovenia).

The forward and reverse primers for *c-fos* (Gene bank: X06769) and glyceraldehyde-3-phoshate hydrogenase (*GAPDH*) (Gene bank: AF106860) were derived from earlier publications.[32,33] The forward primers were 5′-AAT AAG ATG GCT GCA GCC AA-3′ for *c-fos* and 5′-AAG GTC ATC CCA GAG CTG AA-3′ for *GAPDH*. The reverse primers were 5′-TTG GCA ATC TCG GTC TGC AA-3′ for *c-fos* and 5′-ATG TGA GCC ATG AGG TCC AC-3′ for *GAPDH*. Conditions for PCRs were optimised in a gradient cycler relative to Taq DNA polymerase (MBI Fermantas, Slovenia), primers (Thermo Electron GmbH, Germany), $MgCl_2$ concentrations and various annealing temperatures. Optimised settings were transferred to real-time PCR protocols on a Stratagene Mx3000P real-time detection system (Stratagene, USA). Amplification of 1 μl of cDNA (1/5 diluted) was carried out using 1 μl of 15 pmole forward and reverse primers, 12.5 μl of 2X Brilliant SYBR Green QPCR Master Mix (Stratagene, USA) and 9.5 μl of water in a total volume of 25 μl. The amplification protocol was followed as an initial melting step at 95°C for 10 min, followed by 40 cycles of a 95°C melting step for 30 s, a 60°C annealing step for 1 min, a 72°C elongation step for 1.5 min and an additional 3 min at 72°C. Following amplification, a dissociation curve analysis was performed to confirm the purity of PCR products. Cycling parameters for melting curve analysis were 1 min at 95°C, ramping down to 55°C, then ramping up temperature from 55 to 95°C with a default rate of 0.2°C/s.

Statistical analyses

Data were analysed using analysis of variance (ANOVA). In the case of a significant ANOVA, post-hoc analysis was performed using Tukey's or the least significant differences (LSD) test. Values are expressed as mean ± standard error (SEM). A level of $P < 0.05$ was considered to be statistically significant. Statistical analyses were performed with the Statistical Package for the Social Sciences for Windows (SPSS, Version 13.0).

Results

Seizure sensitivity

Systemic KA administration produced clear behavioural changes. KA-treated rats showed an increased locomotor activity, rapid wet-dog shakes and recurrent tonic–clonic convulsions. GCEE or GCEE+KA treatments did not completely abolish abnormal motor behaviours. In the KA group, wet-dog shakes were observed 30 min after KA administration. In the KA+GCEE group, wet-dog shakes were observed 50 min after treatment. The severity of the convulsions was not different in GCEE or KA+GCEE-treated animals when compared to control animals.

Glutathione levels

Cortex GSH levels in the control, KA, GCEE and KA+GCEE groups were determined as 9.62, 5.26, 9.73 and 7.23 nmole/mg protein, respectively (Figure 1). The increased levels of GSH in the GCEE-treated group were not significant when compared to control animals. KA treatment significantly decreased the levels of GSH when compared to the control or the KA+GCEE-treated group ($P < 0.05$). Hippocampus GSH levels in control, KA, GCEE and KA+GCEE groups were determined as 10.47, 4.08, 11.47 and 6.11 nmole/mg protein, respectively (Figure 1). GCEE treatment significantly increased the levels of GSH when compared to control ($P < 0.05$). KA treatment significantly decreased the levels of GSH when compared to control ($P < 0.05$). In addition, a significant increase in the levels of GSH was observed in the KA+GCEE group when compared to KA-treatment only ($P < 0.05$).

Formation of reactive oxygen species

ROS formation was measured using DCF fluorescence assay. DCF is formed by the reaction of DCFH with ROS. Figure 2 shows the ROS levels in synaptosomes incubated with KA or GCEE. Synaptosomes incubated with saline were used as the control treatment. DCF fluorescence in all treatment groups was expressed as a percentage of the control. The levels of ROS formation were significantly increased in synaptosomes incubated with 0.5 or 1 mM KA when compared to control ($P < 0.05$). The levels of ROS formation were significantly decreased in synaptosomes incubated with 0.5 or 1 mM GCEE against KA when compared to 0.5 or 1 mM KA treatments ($P < 0.05$).

c-fos expressions

The relative gene expressions of *c-fos* were quantified according to the comparative C_t method.[34,35] C_t values indicate a PCR cycle number at which the measured fluorescence of the indicator dye (SYBR Green I), corresponding to the quantity of amplified products, is increasing in an exponential fashion

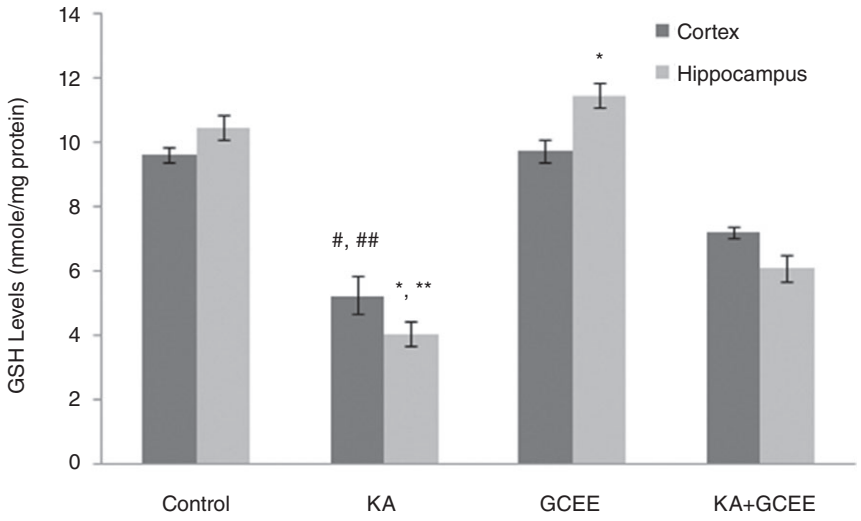

Figure 1 Levels of glutathione in rat brain cortex and hippocampus in all treatment groups. Results are given as nmole/mg protein. Data are mean ± SEM. Each group has four animals. GCEE, γ-glutamylcysteine ethylester; GSH, glutathione; KA, kainic acid. $^{\dagger}P < 0.05$ vs control (cortex); $^{\dagger\dagger}P < 0.05$ vs KA+GCEE (cortex); $^{*}P < 0.05$ vs control (hippocampus); $^{**}P < 0.05$ vs KA+GCEE (hippocampus).

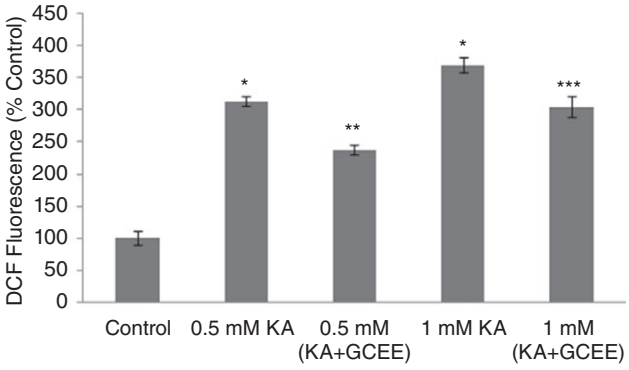

Figure 2 Reactive oxygen species production in synaptosomes incubated with KA and GCEE. Incubation with 0.5 mM and 1 mM KA, with or without 0.5 mM or 1 mM GCEE. DCF, dicholorofluorescein; GCEE, γ-glutamylcysteine ethylester; KA, kainic acid. DCF fluorescences are expressed as a percentage of control. Data are mean ± SEM, $n = 3$. $^{*}P < 0.05$ vs control; $^{**}P < 0.05$ vs 0.5 mM KA; $^{***}P < 0.05$ vs 1 mM KA.

above background. In this study *GAPDH* was used as an endogenous control for normalisation and untreated control group samples were used as the calibrator for quantification. The comparative C_t ($2^{-\Delta\Delta Ct}$) method is a mathematical model that calculates changes in gene expression as a relative fold difference between an experimental and calibrator sample as follows:

$$\Delta C_t = \text{average } c\text{-}fos\ C_t - \text{average } GAPDH\ C_t$$

$$\Delta\Delta C_t = \text{average } \Delta C_t \text{ untreated sample} - \text{average } \Delta C_t \text{ treated sample}$$

The fold change in $c\text{-}fos = 2^{-\Delta\Delta Ct}$

Figure 3 shows the relative expressions of *c-fos* mRNA in cortex and hippocampus tissues. According to our results, KA treatment significantly increased the expressions of *c-fos* both in the cortex (3.64 ± 0.44) and hippocampus (2.89 ± 0.38) when compared to the control group ($P < 0.05$). In addition there was a significant increase in the expression levels of *c-fos* mRNA in cortex treated with GCEE when compared to KA treatment only ($P < 0.05$).

Discussion

Regulation of apoptosis is very important for nervous system integrity. Inappropriate neuronal death occurs in a number of clinically important neuropathological states in the adult nervous system, such as stroke, amyotrophic lateral sclerosis, and Parkinson's and Alzheimer's diseases.[36] Excitotoxicity is widely considered to be a contributing factor in neuronal death associated with several types of acute brain injury ranging from cerebral ischoemia to epilepsy and mechanical brain trauma.[37] Kainic acid, a potent glutamate agonist, is widely used to induce seizures to elucidate cell-death mechanisms.[38]

It has been shown that intraperitoneal injection of *N*-methyl-D-aspartate (NMDA) or KA can induce both *c-fos* mRNA and c-fos protein in cell extracts of the murine whole brain.[39,40] KA-induced seizures lead to c-fos expression in the hippocampus and cortex due to the increased levels of intracellular calcium.[38,39] It has been proposed that c-fos may function as a third messenger in an intracellular cascade linking extracellular stimuli to long-term adaptative processes, including neuronal plasticity and delayed neuronal death.[40–42] DNA damage in neuronal death and excitotoxicity is the result of oxidative stress triggered by KA. It has been shown that KA treatment causes DNA damage in hippocampal neurons and induces c-fos and c-jun expression.[43] Nuclear factor kappa B (NFKB) and activator protein-1 (AP-1) are regulated by the intracellular redox state. These transcripton factors are implicated in the inducible expression of a wide variety of genes involved in oxidative stress and

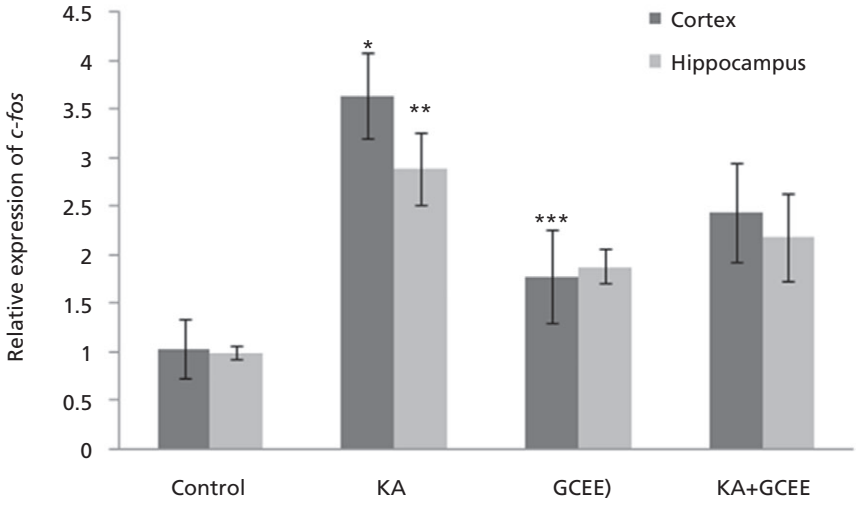

Figure 3 Relative expression of *c-fos* in rat brain cortex and hippocampus in all treatment groups. GCEE, γ-glutamylcysteine ethylester; KA, kainic acid. The results are given as mean ± SEM. Each group has four animals. $^{\dagger}P < 0.05$ vs control (cortex); $^{\#}P < 0.05$ vs KA (cortex); $^{*}P < 0.05$ vs control (hippocampus).

cellular response mechanisms. AP-1 is formed by the heterodimerisation of c-jun and c-fos proteins. Superoxide produced by a xanthine/xanthine oxidase system and hydrogen peroxide induce the expression of several early response genes, including *c-fos* and *c-jun*.[44–48] The cis-acting element mediates *c-fos* expression in response to activation by serum, certain growth factors, UV radiation and serum response element (SRE) in the *c-fos* promoter.[49] In addition, drug-induced seizure induces AP-1 activation in the hippocampus. Consistently, our results showed that KA treatment significantly increases the levels of *c-fos* mRNA expression both in the hippocampus and the cortex.

Interestingly, GCEE treatment significantly induced the expression of *c-fos* mRNA in the cortex but not in the hippocampus. The observed regulator effect of GCEE on the expression levels of *c-fos* may be explained by the antioxidant mechanisms regulating AP-1 activation. It has been reported that AP-1 activity is sensitive to an antioxidant effect. Certain antioxidant compounds are capable alone of influencing AP-1 activation. Phenolic antioxidants, e.g. butylated hydroxytoluene and butylated hydroxyanisole (BHA) alone, have been observed to substantially increase the expression of *c-fos* and *c-jun* mRNA and to induce AP-1 DNA binding on their own.[50] The AP-1 function is also responsive to thiol antioxidants. It has been found that intracellular thioredoxin and glutathione status influence AP-1 transactivation.[51,52] Direct interaction of oxidants and antioxidants with specific cysteine groups of c-fos and c-jun polypeptides may regulate the expression of these proteins.[53,54] The possible interaction of certain proteins with SRE during AP-1 activation may be sensitive to intracellular changes in the thiol redox state caused by antioxidants. Reducing thiol agents such as *N*-acetyl cysteine (NAC) enhances DNA binding activity and transactivation of AP-1. In addition, the role of c-fos and c-jun in the specific activation of gene expression is mediated by a complex site called the electrophile response element (EpRE), found in the mouse glutathione S-transferase Ya-subunit gene. Moreover, EpRE and the antioxidant response element are known to be bound by c-fos/c-jun and c-jun, respectively, indicating that AP-1 may be implicated in electrophilic and antioxidant responses.[49] Our results also indicate that KA+GCEE treatment downregulated the expression of *c-fos* mRNA, both in the hippocampus and in the cortex, but this regulation did not reach a significant level.

The molecular mechanisms leading to excitotoxicity are not known yet, but several pathways, including the activation of multiple enzyme cascades and the increased levels of free intracellular calcium, have been elucidated.[9] It is known that oxidative stress and ROS formation are also key components of glutamate-induced excitotoxicity.[55] Glutamate receptor activation, free-radical generation and mitochondrial energy metabolism are interconnected systems, linked through the intracellular actions of calcium.[56,57]

According to our data, KA treatment significantly increased the levels of ROS formation in brain synaptosomes, supporting the idea that ROS-generating mechanisms are involved in KA-induced excitotoxicity. GCEE and GCEE+KA treatments significantly decreased the levels of ROS formation, confirming the free-radical scavenger activity of GCEE. Drake *et al*. showed that rat brain synaptosomes *in vitro* or *in vivo* and treated with GCEE were less susceptible to ROS formation induced by peroxynitrite ($ONOO^{-}$) as assessed by the DCF fluorescence assay.[23]

The excitotoxicity also occurred indirectly, through the toxic effects of the depleted levels of intracellular glutathione. Cysteine is normally transported into cells via a cysteine carrier that also transports glutamate out of the cell. The action of this amino acid carrier is driven by the glutamate concentration gradient across the cell membrane. An increase in extracellular glutamate concentration can alter this gradient and reduce intracellular transfer of cysteine. Since cysteine is a vital precursor of glutathione, the intracellular concentration

of this important antioxidant and free-radical scavenger will be affected.[57,58] GSH effectively scavenges free radicals and plays important roles in antioxidant defence, metabolism and apoptosis.[59–61] GSH synthesis is primarily regulated by γ-glutamylcysteine synthase (GCS) activity, cysteine availability and non-allosteric feedback inhibition by GSH on GCS.[62–64]

GSH deficiency contributes to oxidative stress, which plays a key role in ageing and the pathogenesis of acute or chronic neurodegenerative diseases, including epilepsy, stroke, and Alzheimer's and Parkinson's disease.[61] The regulation of GSH metabolism is critical for the development of neuroprotective treatment strategies.[59,65] Brain cells are particularly vulnerable to oxidative stress because of their high oxygen utilisation, high iron content, presence of excess unsaturated fatty acids, and decreased activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione reductase.[66]

Thiol-dependent reactions provide an important antioxidant defence in the brain.[67] Factors (insulin and growth factors) that stimulate cysteine (cystine) uptake by cells generally may lead to an increase in intracellular GSH levels. It has been reported that administration of cysteine or its precursors such as cysteine, NAC and L-2-oxothiazolidine-4-carboxylate is able to increase GSH biosynthesis.[23,61] GSH esters have also been used as an alternative strategy to increase cellular GSH content since these substances are able to cross the blood–brain barrier easily. GSH diethylester is more rapidly transported into cells than GSH monoester.[68,69]

GCEE is an antioxidant agent and has the ability to increase GSH levels in the brain by providing the limiting substrate cysteine and preventing feedback inhibition of rate limiting enzyme GCS in GSH biosynthesis.[22] Neuroprotective effects of GCEE have also been demonstrated in several oxidative stress models, induced by peroxynitrite and amyloid-beta peptide 1–42.[23,70,71] GSH deficiency is induced in the brain when L-buthionine (*S,R*)-sulfoximine (BSO), a transition-state inhibitor of GCS enzyme, is administered to rats. Drake *et al*. showed that the administration of 150 mg/kg GCEE (i.p.) to the BSO-injected rats increased the levels of GSH in brain. In addition, a single i.p. injection of 150 mg/kg GCEE to gerbils caused a 41% increase in total GSH levels in the brain.[23]

Similarly, it has been shown that GCEE treatment significantly upregulates mitochondrial GSH content and provides protection against peroxynitrite-induced mitochondrial damage.[70] Our results showed that GCEE treatment with a dose of 10 mg/kg significantly changes GSH levels in the hippocampus but not in the cortex. However, GCEE treatment against KA significantly increases GSH levels both in the hippocampus and the cortex when compared with KA alone.

Antioxidants are known to help after insults that have induced neurodegeneration,[72] but they may supress endogenous defence mechanisms such as superoxide dismutase and glutathione peroxidase,[73] therefore it may be appropriate to emphasise that in situations when repeated occurence of oxidative damage cannot be prevented, administration of antioxidants may cause cumulative damage.

Conclusions

In conclusion, our results indicate that GCEE treatment is able to provide a protection against KA-induced excitotoxicity in rat brains due to the increased levels of GSH both in the hippocampus and cortex and the decreased levels of ROS formation in the synaptosomes. Further experiments are necessary to clarify the potential neuroprotective actions of GCEE in the brain at protein or mRNA levels since this agent has the potential to be an antioxidant therapy for neurodegenerative processes or diseases.

Declarations

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Conflict of interest

The Author(s) declare(s) that they have no conflicting interests to disclose.

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