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Original article

Sitagliptin Modulated Hippocampal Inflammation, Endoplasmic Reticulum Stress and Inhibitory Interneurons in a Rat Model of Epilepsy

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ABSTRACT

Background: Sitagliptin is a specific dipeptidyl peptidase-4 inhibitor that has established neuroprotective properties. **Aim of this study:** To investigate the potential anti-epileptic effect of sitagliptin and its role in attenuating the neuronal changes that take place in the hippocampus. **Material and methods:** Adult male wistar rats were used and alienated into; control group and pilocarpine group. The later was injected with a single intraperitoneal dose of pilocarpine (350 mg/kg) and was monitored for 4 weeks for established chronic epilepsy. The rats of pilocarpine group were further subdivided into; the untreated pilocarpine group and sitagliptin (30 mg/kg/day, orally, for 8 weeks) treated group. After the experimental duration, the hippocampus was dissected for biochemical and structural analysis. **Results:** The current data revealed a significant decrease in the hippocampal levels of nuclear factor-kappa-B (NF-kB) and malondialdehyde (MDA) in sitagliptin treated group in comparison to untreated Pilocarpinegroup. Sitagliptin also induced a significant increase in the hippocampal levels of Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1α (PGC1α), reduced glutathione (GSH) and (C/EBP)-homologous protein (CHOP). Additionally, sitagliptin counteracted the damaging effect of pilocarpine on the cellular structure of hippocampus, where most of The CA1 pyramidal cells maintained the normal rounded vesicular nuclei with less degree of vacuolation compared to untreated Pilocarpinegroup. Moreover, sitagliptin treated group showed a significant increase in parvalbumin and somatostatin-positive interneurons which are the main GABAergic inhibitory interneurons involved in epilepsy. **Conclusion:** The measured parameters exposed the neuroprotective role of sitagliptin against the hippocampal inflammation, endoplasmic reticulum stress and the histopathological alterations, that suggest therapeutic applications of sitagliptin in cases of epilepsy.

KEYWORDS: Epilepsy; Endoplasmic reticulum stress; Hippocampus; Histopathology; Interneurons;Sitagliptin

INTRODUCTION

Epilepsy is one of the commonest neurological disorders that affects the brain with a tendency to have recurring seizures[1]. The hippocampus is considered the most liable brain area for seizure-induced functional and pathological changes with excitotoxic neuronal injury [2]. Several mechanisms have been proposed to be responsible for the development of epilepsy. Among these mechanisms are the inflammatory reactions in the brain as in cases of traumas, malignancies, and infections. They are associated with different levels of CNS inflammation that affects a range of Ca^{2+}-regulated neuronal processes including excitation and synaptic transmission which in turn predisposes to seizures occurrence [3].

Another possible mechanism for epileptogenesis is the excessive free radical generation and oxidative stress that leads to functional disturbance, tissue injury and endoplasmic reticulum (ER) stress [4]. The endoplasmic reticulum is highly sensitive organelle to intracellular and extracellular changes, Where the variations in the protein-folding environment lead to accumulation of

misfoldedproteins in the ER that strongly affects a variety of cellular signaling processes, such as inflammation, oxidative stress, and apoptosis [5]. Furthermore, prolonged ER stress activates the (C/EBP)-homologous protein (CHOP) which is a principal regulator of ER stress-induced apoptosis through, induction of oxidative stress [6], inhibition of pro-survival protein Bcl-2 [7] and increasing several pro-apoptotic factors [8].

Another potential factor in the development of epilepsy is the Peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1α (PGC-1α). It is a member of a transcription coactivators family that plays a central role in the regulation of cellular energy metabolism. It is mainly expressed in mitochondria-rich tissues of high energy demand such as; the cortex, substantianigra, and the hippocampus [9]. PGC-1α has a crucial role in regulating the oxidation processes via its role in mitochondrial biogenesis [10].At the cellular level, the hippocampus consists of two basic neuronal types: the excitatory principal cells which mostly release glutamate and the inhibitory interneurons which are mainly GABAergic interneurons [11]. The imbalance between the excitation and the inhibition circuit plays an important factor in the development of epilepsy [12].

Parvalbumin (PV) and somatostatin (SOM) are the main types of GABAergic inhibitory interneurons that are pivotal homeostatic regulators throughout the hippocampus [13]. PV affects a range of Ca^{2+}-regulated neuronal processes including excitation and synaptic transmission. It defenses transient increases in cytosolic Ca^{2+} from extracellular and intracellular sources [14, 15]. SOM has multiple inhibitory cellular actions in the CA1 and CA3 region of the hippocampus through activation of somatostatin-2 receptors [16]. Abnormal function of somatostatin interneurons in the dentate gyrus contributes to epileptogenesis and increased susceptibility to seizure [17].

Even though, the currently available antiepileptic drugs (AEDs) can symptomatically suppress the epileptic seizures, there is little evidence that it could correct the underlying predisposing pathology [18]. Moreover, the use of AEDs is associated with severe adverse effects such as; behavioral disturbances, cognitive impairment, and bone marrow suppression [19]. Therefore, there is an urgent need for the development of new disease-modifying neuroprotective drugs with better safety profile.

Sitagliptin is a new dipeptidyl peptidase-4 (DPP-4) inhibitor that is used in controlling the type II diabetes through its glucose lowering effect. Sitagliptin also reduce the degradation of endogenous glucagon-like peptide1(GLP-1) and thereby increases its concentration with prolonging its cytoprotective action [20]. Recent studies have shown that GLP-1 is also produced in the hippocampus and caudate brain nuclei with the presence of GLP-1 receptors (GLP-1R) in the neurons [21, 22]. Moreover, previous study revealed the relation between the epilepsy and GLP-1receptors, in which GLP-1R deficit mice representing lower seizure threshold, increased seizure severity and neuronal injury in animal model of epilepsy [23]. Recently, sitagliptin have shown neuroprotective effects in rodent models of cognitive dysfunction, cerebral ischemia and traumatic brain injury [24, 25]. Therefore, the aim of the current study was to evaluate the possible role of sitagliptin in ameliorating the neuroinflammation, oxidative stress, and ER stress and cellular alterations in the rat model of pilocarpine-induced epilepsy.

MATERIALS AND METHODS

Animal and experimental design

The animal work has been conducted according to the guideline of the Animal Care Ethical Committee, Faculty of Medicine, Tanta University, Egypt that complies with the international guidelines set by National Institute of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

The current study was carried out on forty adult male Wistar rats with an average weight 180–200 g each. The animals were housed in standard rat cages with maximum of five/cage, at 22–26°C and 12h dark/light cycle with free access to water and food. After one week of acclimatization in the animal house, the rats were divided into the following experimental groups;

Group I (Control group, n=10) where the rats received a single intraperitoneal injection of 0.9% saline followed by drug-free period for 28 days. After that, the rats received a daily oral administration of 0.9% saline for 8 weeks.

Group II(Pilocarpine group, n=30), where the rats received a single intraperitoneal injection of pilocarpine (Macfarlan Smith Ltd, Edinburgh, UK) at a dose of 350 mg/kg [26] dissolved in 0.9% saline (140 mg/ml). After 30 min, the rats became hypoactive then displayed oro-facial movements, salivation, eye-blinking, twitching of vibrissae and yawning following by the development of generalized convulsions and limbic status epilepticus (SE). However, ten rats of pilocarpine-injected groups did not recover from status epilepticus (SE) stage and died within 1 hour. The rats that survived SE (n=20) were monitored for additional 4 weeks to establish the chronic phase of the spontaneous recurrent seizures (SRS) and they were further subdivided into the following subgroups:

Group IIa (untreated pilocarpine, n=10) where the ratsreceived a daily oral administration of 0.9% saline for 8 weeks.

Group IIb (sitagliptin-treated, n=10) where the ratsreceived a daily oral administration of sitagliptin (Sigma) in a dose of 30 mg/kg/day dissolved in 0.9% saline (12 mg/ml) for 8 weeks [27].

At the end of the experimental duration, all the rats were scarified according to ethical committee roles and the brains were rapidly dissected. One hemisphere from each brain was quickly fixed overnight in 10% formalin for histological and immunohistochemical investigation. While, the hippocampus of the other hemisphere was dissected, weighted and stored in -80°C freezer for the biochemical analysis.

Biochemical analysis

The frozen hippocampus was divided into two parts; the first part was homogenized as 10% (w/v) in PBS 50mM pH 7.4 and centrifuged at 4000 r.p.m. for 30 minutes and the supernatant was used for measurement of tissue levels of malondialdehyde (MDA), reduced glutathione (GSH), nuclear factor kappa-B (NF-κB) and peroxisome proliferator-activated receptor-γ-coactivator 1α (PGC-1α).

While the other part of hippocampus was used for nuclear extraction to measure the tissue level of C/EBP homologous protein (CHOP) using membrane, nuclear and cytoplasmic protein extraction Kit (Bio Basic INC., Canada). Total protein content was measured in both tissue homogenates and nuclear fractions using Lowry method [28].

The tissue level of MDA was assayed calorimetrically using the principle and method described by Yagi assay [29]. Reduced glutathione (GSH) was measured according to Beutler et al [30]. ELISA Kits were used for measuring the levels of NF-κB (EIAab Science Co., Wuhan, China), PGC-1α (Shanghai Sunred Biological Technology, Subreddit) and CHOP level (Glory Science Co., Ltd).

Histological analysis

Formalin-fixed brain samples were rinsed with 0.1 M phosphate buffer solution (PBS) and were embedded in paraffin. For hematoxylin and eosin (H&E) staining, 5 µm thick brain sagittal sections were deparaffinized through two changes, 10 minutes each, of histoclear and rehydrated by incubation in the descending grades of alcohol (100%, 90%, and 70%) and water. After that, the sections were stained with Mayer's hematoxylin for 10 minutes and then rinsed with tap water. Next, the sections were stained with 1% eosin for 2 minutes and rinsed with water. The sections were dehydrated in the ascending grades of alcohol/water (v/v): 70%, 90%, and 2 changes of 100% and cleared in histoclear for 15 minutes. Finally, the sections were mounted with DPX.

Parvalbumin (PV) and somatostatin (SOM) immunohistochemistry

The paraffin sections were deparaffinized in histoclear and rehydrated by incubation with the following concentration of alcohol/water (v/v): 100, 90, and 70% respectively. Afterward, brain sections were incubated in preheated 0.1 M sodium citrate buffer (pH 6.0) for 5 min in the pressure-cooker for antigen retrieval. The sections were cooled down at room temperature for 20 minutes and then were rinsed with 0.1 M phosphate buffer saline. The endogenous peroxidase was inactivated by incubating the sections with 3% H_2O_2 in methanol for 30 minutes.

The non-specific reaction was blocked by incubating the sections first in 5% normal goat serum (NGS) in 0.3M phosphate buffer saline with Triton X-100 (PBS/TritonX-100) for 1hour at room temperature. Next, the sections were incubated either with mouse anti-parvalbumin (1:3000, PV 235, Swant) or rabbit anti-somatostatin-14 (1:1000, T-4103, Peninsula Laboratories) overnight in a humidity chamber at 4°C. The sections were rinsed with PBS (3x15 minutes) and were incubated with the secondary antibodies (goat anti-mouse/anti-rabbit, 1:200, Jackson ImmunoResearch) for 1hour at room temperature. The sections were then treated with the avidin-biotin complex (ABC) reagent (Vector) for 30 minutes, as per manufacturer's instructions. The peroxidase reaction was developed using diaminobenzidine (DAB) as chromogen (Vector). The sections were then dehydrated, cleared, and cover-slipped with DPX. The images were captured using AxioCamMRc (Zeiss, Germany) mounted on the light microscope (Zeiss, Germany).

Quantitative assessment

Image J (National Institutes of Health, Bethesda, Maryland, USA, 1.48v) software was used for the analysis the images and quantitate assessment of following parameters from 3 non-overlapping brain sections from each animal;

- The total number pyramidal cells (normal and abnormal shaped) of CA1 region of hippocampus/ field from the hematoxylin and eosin stained brain sections at (400x) magnification. The normal-shaped pyramidal cells were identified by: rounded contour, intact cytoplasmic membrane, clear visible nuclear membrane with absence of any nuclear condensation or distorted feature [13,31].
- The density (number/area) of parvalbumin-positive interneurons in CA1 region of hippocampus from the PV immunolabeled brain sections at (200x) magnification.
- The density (number/area) of somatostatin-positive interneurons in the hills of dentate gyrus (DG) from the somatostatinimmunolabeled brain sections at (200x) magnification.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism 5.0 software. One-way ANOVA with Tukey's post hoc test was used to compare experimental groups. The data were presented as mean ± standard error of the mean (SEM). $P< 0.05$ was used to define statistical significance.

RESULTS

Biochemical analysis.

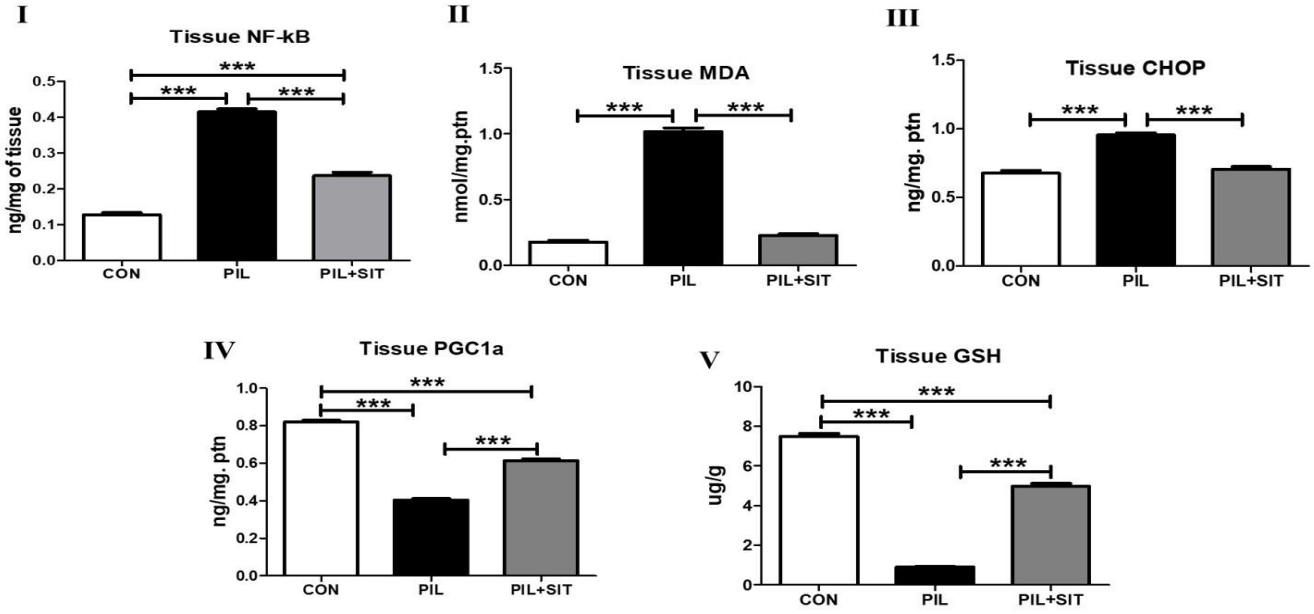
The untreated Pilocarpinegroup showed a significant increase ($p<0.0001$) in the hippocampal levels of nuclear factor kappa B (NF-κB) (0.42±0.01) as an inflammatory marker and malondialdehyde (MDA) (1.02±0.03) as a lipid peroxidation marker, in comparison with their levels in the normal control group (0.13±0.01 and 0.18±0.01, respectively). On the other hand, sitagliptin-treated group displayed a significant decrease ($p<0.0001$) in the tissue level of NF-κB (0.24±0.01) and in the tissue level of MDA (0.23±0.01) as compared to untreated Pilocarpinegroup. However, the tissue level of (NF-κB) in sitagliptin treated group still significantly higher ($p<0.0001$) than its level in the control group (Fig 1: I, II).

The Untreated Pilocarpinegroup also displayed a significant increase ($p<0.0001$) in hippocampal level of (C/EBP)-homologous protein (CHOP) (0.96 ± 0.02) that induces endoplasmic reticular stress as compared to the control group (0.68 ± 0.02). However, treatment with sitagliptin promoted a significant decrease ($p<0.0001$) in (C/EBP)-homologous protein (0.71± 0.02) in comparison to untreated Pilocarpinegroup (Fig 1: III).

Moreover, the untreated Pilocarpinegroup showed signs of tissue oxidative stress as verified by a significant decrease ($p<0.0001$) in the hippocampal levels of peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α) (0.40±0.01) and reduced glutathione (GSH) (0.89 ± 0.03) when compared to their levels in control group (0.82±0.01 and 7.48±0.15, respectively). Interestingly, sitagliptintreated

group showed a significant increase ($p<0.0001$) in the tissue levels of (PGC1α) (0.61±0.01) and (GSH) (4.97±0.14). However, their levels were still significantly lower ($p<0.0001$) than that of control group (Fig 1: IV, V).

Figure 1: Sitagliptin modulates hippocampal inflammation, lipid peroxidation, endoplasmic reticulum stress and oxidative stress in experimental rat model of epilepsy.



Tissue levels of [I] nuclear factor kappa-B (NF-κB), [II] malondialdehyde (MDA), [III] (C/EBP) homologous protein (CHOP), [IV] peroxisome proliferator activated receptor-gamma coactivator 1alpha (PGC-1alpha) and [V] reduced glutathione (GSH) in the hippocampus of the different studied groups. Statistical analysis is carried out using one-way analysis of variance (ANOVA) with Tukey's post-hoc test. Values are presented as mean ± SEM. ******* $P < 0.0001$.

Histological analysis.

The hematoxylin and eosin stained hippocampal sections of control group revealed three main layers in CA1 subfields; stratum oriens, stratum radiatum and the stratum pyramidal. The pyramidal cells are the principal cells of the stratum pyramidal layer, where most of its cells presented with large rounded pale nuclei, prominent nucleoli and distinct nuclear boundary (Fig 2: Ia). The untreated Pilocarpinegroup showed misalignment in the pyramidal cell layer. There is variation in the size and the shape of cells with different degree of cell degeneration. Most of the neurons are pyknotichyperchromatic surrounded by vacuolated cytoplasm (Fig 2: Ib). On the other hand, most of the pyramidal cells in sitagliptin treated epileptic animals keep its normal morphological character of the CA1 pyramidal cell layer. The cells have pale rounded nuclei, prominent nuclei with a well-defined nuclear membrane. Few cells still presented with pyknotichyperchromatic nuclei surrounded by vacuolated neuropil (Fig 2: Ic).

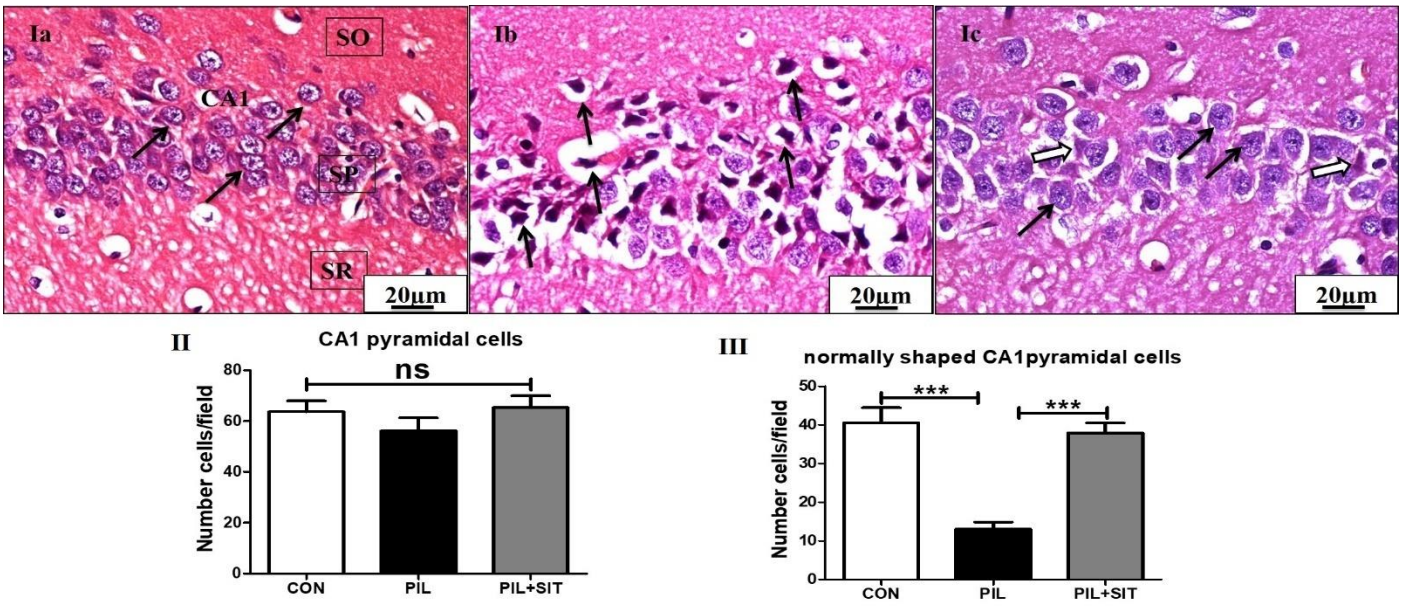
Quantitatively, there was insignificant difference in the total number of pyramidal cells per field in CA1 region of the hippocampus within the experimental groups (control; 63.73±4.27, pilocarpine untreated; 56.17±5.11 and sitagliptin-treated group; 65.27±4.72) (Fig 2: II).

However, the number of normally shaped pyramidal cells per field in the hippocampal CA1 region was significantly decrease ($P<0.0001$) in the untreated Pilocarpinegroup (13.0±1.9) as compared to the control group (40.60±3.9). Sitagliptin administration induced a significant increase ($p<0.0001$) in the number of normally shaped pyramidal cells (37.9±2.64) in comparison with untreated Pilocarpinegroup that was comparable to their number in the control group (Fig 2: III).

Parvalbumin (PV) immunohistochemistry.

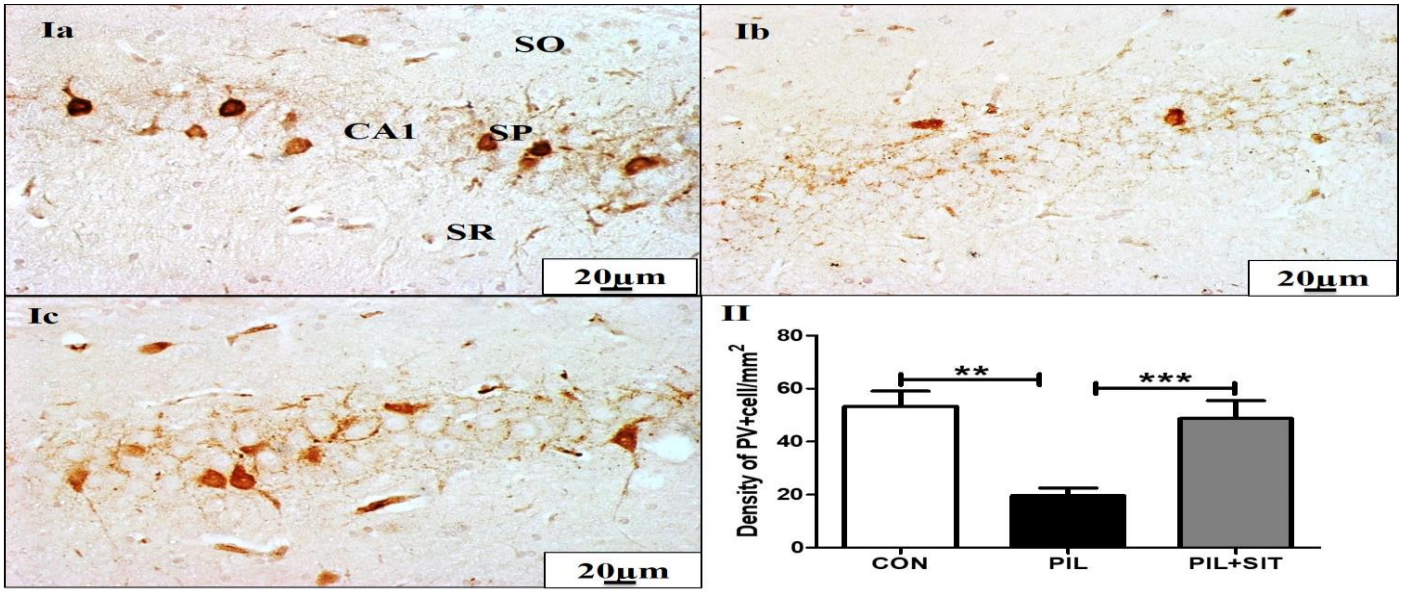
The brain tissues immunolabelled with anti-parvalbumin antibody showed PV-expressed interneurons in the CA1 region of the hippocampus of control group with high numbers in the stratum oriens and stratum pyramidal. The positive staining was mainly localized in the cell body with less expression in the neuronal processing (Fig 3: Ia). Analysis of the brain sections of untreated Pilocarpinegroup showed apparent decrease in the number of PV interneurons (Fig 3: Ib), however their number was apparently increase in sitagliptin-treated group (Fig 3: Ic). These findings were in consistent with the quantitative analysis of the density (number/area) of PV positive cell bodies in CA1 region of hippocampus. There was a significant decrease ($P<0.001$) in the density of PV positive interneurons in untreated Pilocarpinegroup (19.61±2.9) compared to the control group (53.33±5.8). However, sitagliptin-treated group showed a significant increase ($p<0.0001$) in the density of PV positive interneurons (48.77±6.8) incomparable to the untreated Pilocarpinegroup (Fig 3: II).

Figure 2: Sitagliptin ameliorated pilocarpine-induced histopathological alterations in CA1 region of the hippocampus.



Representative photomicrographs of hematoxylin and eosin-stained CA1 region of the hippocampus of the experimental groups;[Ia] control group showing stratum oriens (SO), stratum radiatum (SR) and stratum pyramidal (SP). The pyramidal cells are the principal cells with large rounded pale nuclei, prominent nucleoli and distinct nuclear boundary (arrows). [Ib] untreated Pilocarpinegroup showing pyramidal cells with pyknotichyperchromatic nuclei surrounded by vacuolated cytoplasm (arrows). [Ic] sitagliptin-treated group showing normal pyramidal cells with pale nuclei and visible nucleoli (black arrows) as well as few pyknotic cells (white arrows). Scale bar=20 µm. [II] Quantitative analysis of the total number of CA1 pyramidal cells of the hippocampus. [III] Quantitative analysis of normally shaped CA1 pyramidal cells of the hippocampus. Statistical analysis is carried out using one-way analysis of variance (ANOVA) with Tukey's post-hock test. The values are presented as mean ±SEM. ***P<0.0001. ns; non-significant.

Figure 3: Sitagliptin significantly increased the parvalbumin-positive interneurons in the hippocampus CA1 region in a rat model of epilepsy.



Representative photomicrographs of anti-parvalbuminimmunostaining of CA1 region of the hippocampus of [Ia] control, [Ib] untreated Pilocarpineand [Ic] sitagliptin-treated groups. The immunolabelled positive interneurons are mainly localized in the stratum oriens (SO) and stratum pyramidals (SP) of CA1 region of the hippocampus. Scale bar = 20 µm. [II] Quantitative analysis of the density of PV positive interneurons in CA1 region of the hippocampus. The data are analyzed by one-way analysis of variance (ANOVA) with Tukey's post hock test. The values are presented as mean ± SEM. **P≤0.001, ***P≤0.0001.

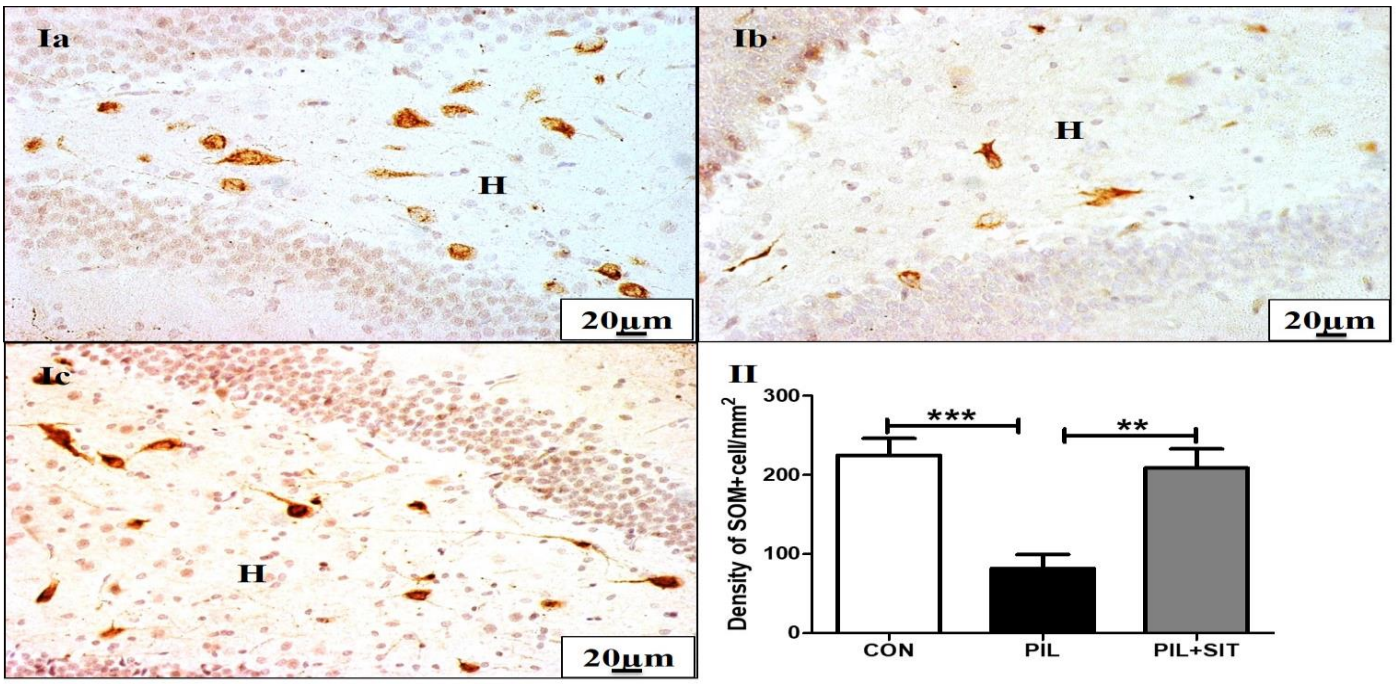
Somatostatin (SOM) immunohistochemistry

Analysis of the brain sections immunolabelled with anti-somatostatin antibody showed the somatostatin-positive interneurons are mainly restricted to the hilus region of hippocampus of control group (Fig 4: Ia) with obvious decrease in the number of SOM interneurons in the hilus region of hippocampus of the untreated Pilocarpinegroup (Fig 4: Ib). However, the brain sections of sitagliptin-treated group displayed an increase in the number of SOM positive cells (Fig 4: Ic) compared to the untreated Pilocarpinegroup.

These results were supported by quantitative analysis of SOM interneurons in the hilar region of hippocampus of experimental groups, that demonstrated a significant decreased (p<0.0001) in the density (number/area) of SOM interneurons in the hilus region of untreated Pilocarpinegroup (81.50±17.93) as compared to the control

group (224.8±21.43). While, sitagliptin-treated group displayed a significant increase ($p<0.001$) in the density of SOM-positive interneurons (208.9±23.88) compared to the untreated Pilocarpinegroup (Fig 4: II).

Figure 4: Sitagliptin significantly increased the somatostatin-positive interneurons in the hilus region of hippocampus in a rat model of epilepsy.



Representative photomicrographs of anti-somatostatinimmunostaining of hippocampus dentate gyrus of [Ia] control, [Ib] untreated Pilocarpineand [Ic] sitagliptin-treated group. The immunolabelled positive interneurons are mainly localized in the hilus region (H) of the hippocampus. Scale bar =20 µm. (II) Quantitative analysis of the density of somatostatin-positive interneurons in the hilus region of the hippocampus. The data are analyzed by one-way analysis of variance (ANOVA) with Tukey's post hock test. The values are presented as mean ± SEM. **P≤0.001, ***P ≤0.0001.

DISCUSSION

Epilepsy is one of the most common neurological conditions that profoundly affects many aspects of life quality [32]. The epilepsy might have associated with various structural and functional changes in different brain regions [33, 34], however, the exact mechanism is elusive. Until now, there is no efficacious protective treatment against the development of epilepsy in patients having neurological insults such as brain trauma, or stroke [18]. Efforts should be directed not only to abort status epilepticus with anticonvulsants but also to break the independently progressing mechanisms of cellular injury and brain circuitry reorganization that are believed to be responsible for development of epilepsy [35].

In this study, we used a well-established rat model of epilepsy to reveal the potential neuroprotective effect of sitagliptin on the biochemical and structural alterations in rat hippocampus.

The current data showed different biochemical and structural alterations in the hippocampus of epileptic rat model. Pilocarpine induced a significant increase in the inflammatory and lipid peroxidation biomarkers. Pilocarpine also encouraged endoplasmic reticulum stress as well as oxidative stress in the hippocampus. Varieties of cellular alterations in the pyramidal cells with significant decrease in GABAergic interneurons in the hippocampus were also detected in rat model of epilepsy induced by pilocarpine.

The significant increase of hippocampal level of NF-κB of untreated Pilocarpinerats supported by the fact that NF-κBis playing a strategic role in promoting the cellular inflammatory response patterns that considered one of the most damaging seizures insults [36]. NF-κB stimulates Toll-like receptors (TLRs) that induce several pro-inflammatory factors encoding genes such as cytokines, cyclooxygenase-2 (COX-2) and nitric oxide resulting in severe inflammation. Neuroinflammation deleteriously affects neurons via alteration in neuronal excitability, production of toxic mediators and increased leakage of the blood-brain barrier [37].

Our data also revealed the reduction in PGC-1α level in untreated Pilocarpinerats. That could potentially have linked to the increased level of NF-κB through the interaction between PGC-1α and the p65 subunit of NF-κB [38] or through reduction of peroxisome proliferator-activated receptor-gamma (PPARγ) activity [39, 40].

In the present study, the untreated Pilocarpinerats also showed evidence of increased oxidative stress in the form of increased level of malondialdehyde production and reduced level of Glutathione. This could be belong to increased level of NF- κB and the inflammatory response in the brain that induce excessive free radicals generation [41]. This is further supported by the fact that; the brain is enriched with high levels of unsaturated fatty acids which are good substrates for peroxidation reactions. Moreover, brain's

endogenous antioxidant defense mechanisms are poor which further augment the damaging effects of lipid peroxidation and free radicals on the neurons and could potentially contribute to the pathogenesis of epilepsy [42, 43].

Another consequence of the pilocarpine-injection is the increased level of CHOP in the hippocampus that could be belong to the oxidative stress [5] as well as overexpression of CHOP triggers cell cycle arrest and down-regulates the pro-survival molecule Bcl-2 that promote apoptosis and the production of reactive oxygen species [43, 44].

At the cellular level, this study also showed that pilocarpine promoted morphological alterations in the CA1 pyramidal cell layer of the hippocampus that was in agreement with Navarro Mora and his colleagues who reported similar histopathological alterations in the pyramidal cells of CA1 in the epileptic rat models [45]. The pyramidal cells are the main excitatory neurons in the hippocampus and are controlled by a diverse population of GABAergic inhibitory interneurons such as parvalbumin and somatostatin [46].

The current data revealed a significant decrease in the number of parvalbumin- positive interneuron in the hippocampus of epileptic rat that agreed with the findings of previous researchers. Who reported the reduction in the number of parvalbumin-positive cells in human patients with epileptogenic focal cortical dysplasia [47, 48].Parvalbumin deficiency could be one of the possible underline mechanisms for the development of epilepsy through creating a state of depolarizing post-synaptic potential in the hippocampus. Where it alters the inhibitory control at the local level with consequence simulating the hypersynchronous neuronal activity that disseminates over a large network facilitating seizure spreading [49, 50].

Another important type of inhibitory interneurons is somatostatin interneurons which affect electrophysiological properties of neurons and modulates classical neurotransmission [51]. In this study, we showed that number of somatostatin-positive interneurons was reduced in untreated Pilocarpinerats. this settled with previous researchers who reported the relation between SE-induced cell death and degeneration of somatostatinergichilar interneurons in experimentally induced epileptic mice [52].

Interestingly, administration of sitagliptin, as one of the DPP-4 inhibitors, was able to rescue the inflammation, oxidative stress and endoplasmic reticulum stress induced by pilocarpine. Sitagliptin also improved the cellular changes reported in the pyramidal cells as well as it significantly increases the number of parvalbumin and somatostatin inhibitory interneurons in rat hippocampus.

The neuroprotective role of sitagliptin was previously reported in traumatic brain injury, where it helped reduce the lesion size [24]. Moreover, more recent study revealed the role of sitagliptin in enhancing neuroprotective effect of pregabalin against pentylenetetrazole-induced acute epileptogenesis in mice [53].

Several mechanisms could explain the neuroprotective effects of sitagliptin. The most important one is the role of sitagliptin in activation and stabilization of Glucagon-like peptide-1 with consequence stimulating cAMP response element binding protein (CREB) system and production of neuroprotective proteins [24] as well as inhibition of free radical production by influencing NADPH oxidase and activating the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway [54]. Glucagon-like peptide-1 could promote mitochondrial biogenesis by downregulating microRNA 23a (miR-23a) resulting in expression of mitochondrial protective gene PGC-1α in vitro [55].

Moreover, our data stated the significant role of sitagliptin in reducing the hippocampal level of NF-κB. That was agreed with previous researchers who revealed the role of sitagliptin in preventing NF-κB activation. It decreased phosphorylation of p65 subunit of NF-κB leading to a significant reduction in the level of the inflammatory cytokines. With subsequent attenuating in the severity of febrile seizures in rats [56]. Additionally, El-Sahar and his colleagues showed that sitagliptin alleviated cerebral ischemia/reperfusion hippocampal injury through suppressing NF-κB activation, reducing lipid peroxides, increasing GSH level, and reducing mitochondrial matrix component cytochrome-C and the key downstream executioner caspase-3 [57].

The neuroprotective effect of sitagliptin could also belong to its ability to increase the mitochondrial antioxidant protein manganese superoxide dismutase (MnSOD), which is one of the major ROS detoxifying enzymes [24]. In addition to its role in decreasing the ER stress biomarkers such as CHOP as well as cleavage activating transcription factor 6 (ATF6) through ameliorating the oxidative stress [58].

CONCLUSION

This study demonstrated the effective role of sitagliptin against the neuroinflammation, oxidative stress, the ER stress as well as the reduction of GABAergic interneurons and the histopathological alteration in the rat hippocampus of experimental model of pilocarpine-induced epilepsy. The current data reflect the neuroprotective properties of sitagliptin which suggest the possible therapeutic applications of sitagliptin in cases of epilepsy.

Conflict of interest

The authors declare that there are no conflicts of interest.

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