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Dipeptidyl peptidase-4 inhibitor ameliorates status epilepticus seizures and cognitive disturbances in a rat model of temporal lobe epilepsy

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ABSTRACT

Background and Objective: In temporal lobe epilepsy (TLE), recurrent seizures accompany with cognitive deficit. In some patients, the current medications cannot provide satisfactory control of seizures, therefore new drugs that act through different mechanisms are required. In the present study, the useful effect of dipeptidyl peptidase-4 inhibitor was evaluated in experimental model of temporal lobe epilepsy in male rats.

Materials and Methods: In this study, the effects of administration of dipeptidyl peptidase-4 inhibitor, linagliptin, on seizures score according to Racine's scores and learning and memory impairment induced by intrahippocampal injection of kainic acid (4 □g) using Y-maze and passive avoidance test were studied in rats. Linagliptin thirty minutes before kainic acid injection was administrated intracerebroventricularly.

Results: In this study, the kainic acid-induced recurrent seizures, reduced alternation level in Y-maze test ($p<0.001$) and lowered step through latency (STL) in the passive avoidance test ($p<0.001$). Administration of linagliptin to epileptic rats reduced the score of status epilepticus seizures ($p<0.001$), increased alternation score ($p<0.05$) and learning capability in the passive avoidance test ($p<0.05$). The difference between the effect of valproic acid and linagliptin on STL and Racine's scores was significant ($p<0.05$-$p<0.01$).

Conclusion: The obtained data indicate that linagliptin in kainate rats mitigates seizure severity and develops short-term memory.

1. Introduction

Temporal lobe epilepsy (TLE) is a chronic nervous system disorder that is represented with prevalent seizures (1). These epileptic seizures are the result of excessive and abnormal activity of cortical neurons. These activity spread to other area of the brain and cause pathological changes and hippocampal neuronal loss (2). For studying epilepsy and its treatment, animal model of pilocarpine or kainic acid-induced epilepsy are used. In animal model of pilocarpine or kainic acid-induced epilepsy that is similar to TLE in human, temporal lobe and hippocampus damage develops. There are currently no definitive prevention and treatment methods for epilepsy, and about one-third of people with epilepsy are resistant to existing treatments. Current medications only alleviate symptoms of disease with relatively little effect on seizures (3).

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Therefore, it seems that the use of drugs with different mechanisms are necessary. DPP-4 is a proteolytic enzyme that is expressed at the surface of most cells and is associated with signal transduction, apoptosis, and cell death. This enzyme plays a key role in glucose metabolism by reducing the levels of incretin hormones including glucagon like peptide-1(GLP-1) and glucagon like peptide -2(GLP-2), and consequently lowering insulin (4,5). DPP-4 inhibitors increase insulin levels and decrease glucagon secretion consequently decrease blood glucose levels (6). Although the brain was considered an organ that is insensitive to insulin but recent reports of insulin and its receptors in the brain open new pathways for the function of insulin in the brain. Because the central nervous system plays an important role in homeostasis, reproduction, cognition and memory (7), insulin activity in the brain varies with its activities in other tissues. In this study, the effects of administration of dipeptidyl peptidase-4 inhibitor, linagliptin, on status epilepticus seizures and cognitive deficiency in kainic acid-induced epileptic model were investigated in rats.

2. Materials and Methods

2.1. Animals

In this study, adult male Wistar rats (n = 32) providing from IUMS animal house weighing 200-250 g were used. The controlled temperature and light rooms were utilized for housing of animals. Rats had free access to water and standard chow food.

2.2. Experimental procedure

Rats were divided into four groups (n=8): Sham-operated (SH); Kainate; linagliptin (10 µm)-treated kainate and valproic acid – treated rats. After anesthetizing rats with a combination of ketamin (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), they placed in a stereotaxic apparatus (incisor bar -3.3 mm, ear bars positioned symmetrically).

For induction of epileptic model, kainic acid (Sigma Chemicals, USA, 10 µl of normal saline containing 0.4 µg/µl of kainic acid) was unilaterally injected in the dorsal hippocampus animals. Valproic acid (200 mg/kg) was intraperitoneally administered for seven days before induction of epilepsy.

Thirty minutes before kainic acid injection, linagliptin (10 µl; Sigma Chemicals, USA) dissolved in 30% Cremophor was intracerebroventricularlly administered.

The Racine's score for revealing the progression of kainate-induced seizures was no reaction 1; stereotype mounting, eye blinking, and/ or mild facial clonus 2; head nodding and/or several facial clonus 3; myoclonic jerks in the forelimbs, clonic convulsions in the forelimbs with rearing 4; and generalized clonic convulsions associated with loss of balance 5 (8).

2.3. Y-maze task

In this study, two weeks after vehicle or kainic acid injection, using alternation behavior in Y-maze, spatial memory was assessed (8). Y-maze is an apparatus consist of three black Plexiglas arms. Each rat after putting at the end of one arm, move for an 8-min session into the maze freely. The total number of arms entered – 2 is as the maximum number of possible spontaneous alternations, and the alternation percentage was calculated as the ratio of actual to possible alternations × 100

2.4. Single-trial passive avoidance test

Two to three days after Y-maze, single-trial passive avoidance test was done (10). The single-trial passive avoidance apparatus includes light and dark chambers that were separated by a guillotine door. In this test, first rats were habituated with the apparatus, then with putting the rats in the light chamber, the guillotine door was opened, as soon as the rat entered into the dark chamber, the door was brought down and a single electric shock with intensity of 1 mA for 1 second was delivered. In this trial, the initial latency (IL; The amount of time rat takes to enter the dark chamber) was recorded. In retention trial, after placing each rat in the light chamber, step-through latency (STL) as the interval between placement in the light chamber and entry into the dark chamber was measured.

3. Results

3.1. Behavioral study

The acute period of seizure scores was recorded 24 hours after intrahippocampal kainic acid injection. Average of seizure scores in kainate group was 4.57±0.13. After administration of linagliptin and valproic acid separately, average of seizure scores were significantly decreased to 2.66±0.23 and 1.28±o.52, respectively comparing to kainate group ($p<0.001$). According to present results, there was significant difference between kainate+linagliptin and kainate+valproic acid ($p<0.05$) (Fig. 1).

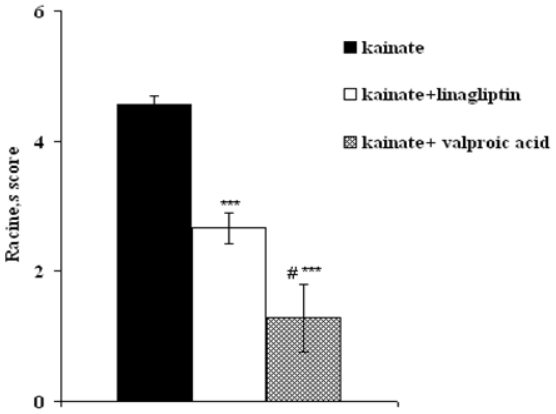

Fig. 1. Recorded status epilepticus seizures according to Racine's scores in experimental groups. Values are means ± SEM.
*** P< 0.001 (vs. kainate); # p < 0.05 (vs. kainate + linagliptin).

3.2. Spatial recognition memory in Y-maze

Recording of alternation behavior in Y-maze task was used for evaluation of spatial memory. In the epileptic and sham rats, average of alternation percent was 45.04±8.83 and 130.03± 11.34 respectively that had significant difference with each other ($P<0.001$). Average of alternation percent in linagliptin or valproic acid–treated rats improved significantly comparing to kainate group (85.2±14.03 and 69.15±3.86, respectively; $p<0.05$). Total number of arms entered by rats in different groups showed non- significant difference (Fig. 2).

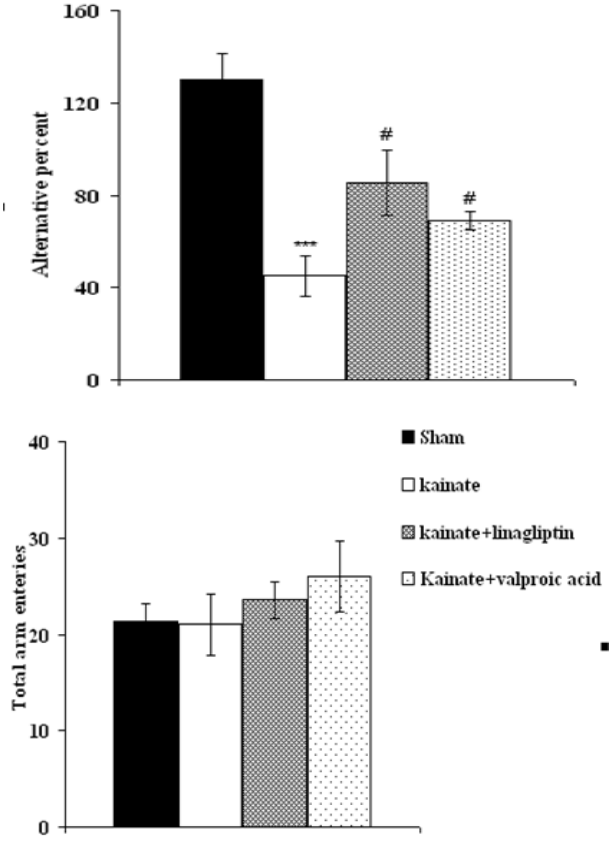

Fig. 2. Total entrance (A) and alternation behavior (B) displayed in the Y-maze by rats. Values are means ± SEM.
*** P< 0.001 (vs. Sham); # p < 0.05 (vs. kainate)

3.3. Passive avoidance test

Fig. 3 shows average of IL and STL in experimental groups. Based on these results, IL shows non-significant difference in different groups. Comparing of STL average in kainate (13.86±1.32) and sham (256.66±27.4) groups revealed that it significantly reduced ($p<0.001$) in kainate rats. Average of STL in linagliptin or valproic acid–treated rats became greater significantly comparing to kainate group (66.8±30.57 and 154.16±21.82, respectively; $p<0.05$-$p<0.001$). The effect of valproic acid on STL had considerable difference with linagliptin–treated group ($p<0.01$). Initial latency in different groups showed non- significant difference (Fig.3).

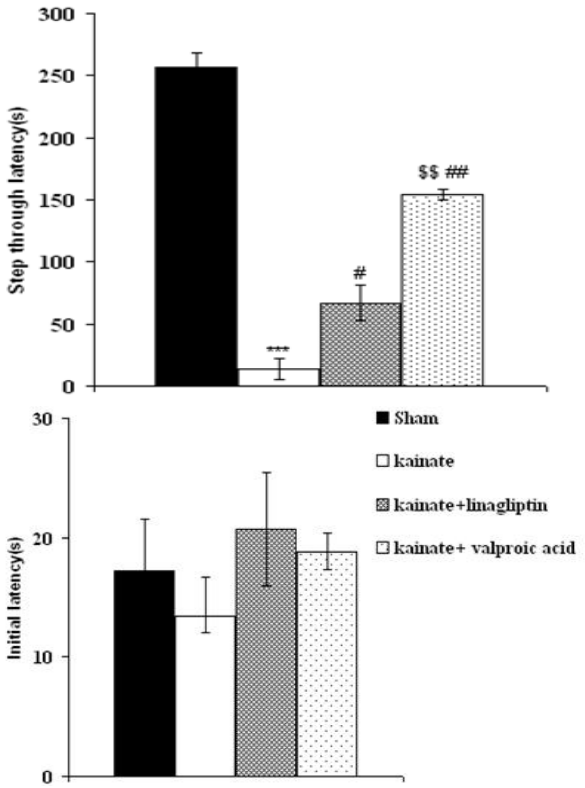

Fig. 3. Initial latency (A) and step-through latency (B) recorded in a single-trial passive avoidance test for rats. Values are means ± SEM

*** p< 0.001 (vs. Sham); # p < 0.05, ### p < 0.001 (vs. kainate); $$ p < 0.01(vs. kainate+ linagliptin)

4. Discussion

Present study examined the effect of linagliptin on status epilepticus and cognitive disturbances in kainic acid –induced epilepsy model in rat. According to the findings of this study, it was revealed that two weeks after injection of kainic acid, the epileptic rats had status epilepticus seizures, reduced spatial short-term memory. Treatment of epileptic rats with linagliptin or valproic acid could reduce severity of status epilepticus seizures and improve partially alternation percentage and STL.

Recent studies indicate that insulin signaling pathways have protective role in central nervous system. DPP-4 inhibitors including linagliptin get better secretion and signaling of insulin. Thus, linagliptin with improvement of insulin signaling pathway blocks neuronal death (11). Today, DPP-4 inhibitors are used for treatment of type-2 diabetes. In addition, some studies showed that linagliptin could attenuate cerebral injury after stroke-induction in rats. Also it improves the proliferation of neuronal stem cells in diabetic rats after stroke induction (12). It has been shown that another DPP-4 inhibitor, sitagliptin, reduced epileptic ripples in rat (13). This function of sitagliptin can be attributed to stabilization of homeostasis of cellular calcium ions (14). By the way, DPP-4 inhibitors increase the expression of incretins such as GLP-1 that has a direct and effective role in learning and memory, neuronal protection and neurogenesis. It has been demonstrated that lack of hippocampal GLP-1 receptors in rats exacerbates the kainic acid-induced seizures and brain damage (15). This peptide as an endogen agent in central nervous system, causes normal function, plasticity, neuronal survival, neuritis growth and neuroprotection against exotoxic substances and oxidative stress (16).

In diabetes, agonists of GLP-1 receptor not only improve glucose homeostasis, but also exert neuroprotection effects (17). GLP-1 can easily pass through blood-brain barrier and enters the brain and binds to its receptors in cortex, hippocampus and cerebellum. Recently, it has been reported that GLP-1 is capable to stimulate neurogenesis in substantia nigra. Injection of GLP-1 into the brain improves long term potentiation in the hippocampus (18). Vascular deficiency is one of the most common causes of dementia. Based on recent studies, it has revealed that selective DPP-4 inhibitors can ameliorate experimental vascular dementia (19).

Due to neuroprotection effect of GLP-1, it seems that in the present study, DPP-4 inhibitor, linagliptin, acts in part through increasing GLP-1 production. But to clarify the exact mechanism of DPP-4 inhibitor, linagliptin, additional studies are required.

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