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Research Article
Alogliptin, DPP4 Inhibitor, Improved Cognitive and Depressive Function in Obese ApoE-/- Mice with Modification of BDNF in Hippocampus

[1]Masayuki Mori, [2]Wentao He, [3]Yasuhiro Kawasaki, [4]Nobuo Kato, [1]Yuji Kasamaki and [1]Tsugiyasu Kanda

[1]Department of Community Medicine, Kanazawa Medical University, Uchinada, 920 Ishikawa, Japan
[2]Department of Endocrinology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 430030 Wuhan, China
[3]Department of Neuropsychiatry, Kanazawa Medical University, 920-0293 Ishikawa, Japan
[4]Department of Physiology, Kanazawa Medical University, 920-0293 Ishikawa, Japan

Abstract

Background and Objective: Obesity has been associated with cognitive deficits and even dementia. The purpose of this study was to identify the beneficial effects of anti-diabetic agent, alogliptin benzoate (ALG), dipeptidyl peptidase-4 inhibitor to cognition deficits, anti-depressive state and metabolic abnormality. **Materials and Methods:** Three months oral administration of ALG (30 mg kg^{-1}/day) were performed in ApoE-/- mice with high-fat diet (HFA, n = 15). The non-treated mice with high-fat diet (HFD, n = 15) became obese. Mice were fed from the age of 8 weeks until 20 weeks. As a control, non-exercised mice without high-fat diet (NOR, n = 15) were prepared. Morris water maze test as spatial learning and novel object recognition test as recognition memory were performed. Forced swimming test as depressive state was also performed. Histopathological analysis was performed in the hippocampus and the liver of three groups. Comparisons among groups were performed using two-way ANOVA followed by Fisher's least significant difference analysis ($p<0.05$). **Results:** Mice in HFD showed cognition deficits, depressive condition and metabolic abnormality. The ALG treatment did not reduce the body weight compared with untreated mice with high-fat diet. The liver weight/body weight ratio was significantly ($p<0.05$) reduced in HFA compared with HFD. The circulating levels of liver enzyme and lipids were significantly ($p<0.05$) lower in HFA compared with HFD. Both of Morris water maze test and novel object recognition test were significantly ($p<0.05$) recovered in HFA compared with HFD. The forced swimming test was also better in HFA compared with HFD. The ALG treatment significantly ($p<0.05$) induced brain-derived neurotrophic factor (BDNF) mRNA and reduced calcineurin 1 regulator (RCAN1) mRNA in hippocampus. Histopathological analysis showed reduced pyknotic neurons of the hippocampus and steatosis of the liver by the ALG treatment. **Conclusion:** These findings suggest that ALG treatment could attenuate cognitive deficit and depressive function in the association with metabolic advantages, through the protection of pyknotic neurons in the hippocampus and steatosis in the liver.

Key words: Obesity, brain-derived neurotrophic factor, regulator of calcineurin 1, ApoE-/-, alogliptin

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Corresponding Author: Tsugiyasu Kanda, Department of Community Medicine, Kanazawa Medical University, Uchinada, 920 Ishikawa, Japan Tel: 09016171790

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Obesity has been associated with cognitive deficits and dementia, as well as a reduction in brain-derived neurotrophic factor (BDNF)[1]. The calcineurin 1 regulator (RCAN1) controls neuronal plasticity and depressive states by regulating BNDF[2]. Similarly, RCAN1 expression is enriched in both the nervous system[3].

Alogliptin benzoate (ALG), a selective inhibitor of dipeptidyl peptidase-4 (DPP-4), is in clinical use worldwide for patients with diabetes mellitus type 2. ALG was not clear to improve cognitive dysfunction[4,5]. Recently, ALG was reported to reduce cognitive decline in diabetic rats[6]. ALG treatment may have the potential to be used in treating Alzheimer's patients[7]. Neuroinflammation has emerged as an important cause of cognitive decline during aging and in Alzheimer's disease (AD)[8]. Chronic low-grade inflammation is observed in obesity, which are important risk factors for AD. The expression of oxidative stress and inflammatory genes are related to AD development. Chronic, prophylactic treatment with AGL increased BDNF levels in the brain and protected the brain. DPP-4 inhibitors are used as neuroprotectants or neurotrophins. AGL may be useful as a neuroprotectant, or an enhancer of BDNF production in the brain. It is hypothesized that ALG could provoke an alternation of BDNF and RCAN1 in the hippocampus, thereby affecting cognitive deficit and depressive states.

Synaptosomal dysfunction is enhanced in ApoE-/- mice compared to control animals[9]. Moreover, ApoE-/- gene polymorphism is the strongest and most robust genetic risk factor for AD. Brain RCAN1 in patients with Down's syndrome and AD is over expressed and may be linked to the pathogenesis of neurodegeneration[10]. It is known to have a bearing on learning and memory and has also been identified as a key component of body mass control and energy homeostasis[11]. Finally, BDNF appears to play a major role not in both central metabolic pathways[12].

The purpose of this study was to investigate the chronic administration of ALG to obese ApoE-/- mice, introducing cognitive dysfunction, depressive state and metabolic abnormality. This is hypothesized that ALG could provoke the modification of BDNF and RCAN1 in the hippocampus, thereby affecting cognitive deficit, depressive states and physiological adaptation.

MATERIALS AND METHODS

Animals: Thirty-six female ApoE-/- mice (Charles River, Wilmington, USA) were kept at room temperature (22°C) and under a 12 h dark-light-cycle. Drinking water and rodent chow were available *ad libitum*. Laboratory animal use was in accordance with the institutional guidelines of Kanazawa Medical University. The HF diet comprised 56.7% fat and 25.5% protein, as described previously[13]. All the experiments were performed in accordance with the guiding principle of the Physiological Society of Japan and were approved by the Animal Care Committee of Kanazawa Medical University. This experiment was carried out in Animal Care Centre of Kanazawa Medical University since 2015-2016.

Methods: ALG treatment was monitored in ApoE-/- mice on a high-fat diet (HFA, n = 15) for a period of three months. Non-exercising mice on the high-fat diet (HFD, n = 15) became obese. All mice were fed from the age of 8 weeks until 20 weeks. Mice in the voluntary exercise group ran on a wheel for 5 days/week over the same period. Non-exercising mice not on a high-fat diet (NOR, n = 15) were used as a control. The morris water maze test was used to evaluate spatial learning, the novel object recognition test to evaluate recognition memory and the forced swimming test to evaluate depressive state.

Histological analysis: At termination, the brain and liver were excised and fixed in 10% neutral-buffered formalin. The brains were removed and processed as previously described[14]. Histological examination was performed in ApoE-/- mice of three groups. Liver tissue was paraffin embedded, sectioned (5 μM) and mounted taking care to select similarly sized sections representative of both the tissue edge and center. Hematoxylin and eosin stains were used for morphological analyses.

Morris water maze test: The morris water maze test was used to evaluate the spatial memory of the animals. Training in the maze took place between 8:00 AM and 2:00 PM, which was during the light phase of the daily cycle. The morris water maze comprised a large circular pool with a transparent plexiglas platform submerged 1 cm below the surface of the water. The animals were subjected to four swimming trials per day for five consecutive days. The trial was initiated by placing mice into one of the four quadrant positions on the edge of the pool. Each trial was terminated as soon as the mouse reached the platform or the maximum trial time of 60 sec had elapsed. After the final trial on the fifth day, the mice were subjected to a probe trial for 60 sec with the platform removed. The swimming trajectory of each mouse was analyzed using SMART[15].

Novel object recognition test: This training relies on the rodent's spontaneous tendency to explore objects, spending more time exploring the novel than the familiar. P20 subjects were habituated individually to an empty exploratory box (40×25×20 cm) for 5 min. Immediately after habituation, a 5 min training session was conducted by adding two equal objects (object A and A_1), positioned in two adjacent corners, 10 cm from the walls. In the short-term retention test, 1 h after training, mice explored the open field for 5 min in the presence of one familiar and one novel object. In the long-term retention test, 24 h after training, mice explored the box for 5 min in the presence of one familiar and one novel object. The same groups of animals were subjected to NOR testing trials at 1 and 24 h post-training. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Data were analyzed by calculating a recognition index for each animal, which was expressed as the ratio $T_B/(T_A+T_B)$ [T_A = time spent exploring the familiar object A; T_B = time spent exploring the novel object. A recognition index of 0.5 shows no preference for either object, as was always tested in the training session[16].

Forced swimming test: For forced swimming, we used the test paradigm originally designed to test rat behavior rather than the five-day paradigm previously employed to induce depression-like behavior in mice The mice were forced to swim for 10 min daily for 2 consecutive days (on days 1 and 2) in a transparent acrylate cylinder (24 cm ⌀, 60 cm high) filled with water at 25°C (25 cm deep).

Statistical analysis: Data were analyzed using ANY-Maze (Stoelting, Wood Dale, IL, USA)[16]. Data were recorded as Mean±SED. For all comparisons, the significant differences in body weight, peripheral biochemical parameters and gene expressions were calculated using student's t-test. Comparisons among groups in morris water test, novel object cognition, force swimming tests were performed using two-way ANOVA followed by Fisher's least significant difference analysis. A value of $p<0.05$ was considered to be statistically significant[16].

RESULTS

Organ weight: Body weight (BW) in the HFD group was significantly higher than that in the NOR group ($p<0.05$, Table 1). The BW in the HFA group was not changed compared with that in the HFD group. The brain weight in the HFD group was similar among three groups. Liver weight in the HFD group was predominantly higher than that in the NOR group and was significantly ($p<0.05$) reduced in the HFA group compared with that in HFD group.

Table 1: Effects of alogliptin on body weight, liver weight and laboratory data for ApoE -/- mice on a high fat diet

	NOR	HFD	HFA
BW (g)	30.40±1.6	49.70±2.7	46.50±5.9
Brain weight (mg)	430.00±20	410.00±10	430.00±20
Liver weight (g)	1.45±0.328	3.80±0.99	2.81±0.93 †
Liver/body weight ratio 10^{-3}	48.00±9	76.00±18	59.00±17 †
ALT (U L^{-1})	92.00±34	557.00±183	262.00±147 †
TG (mg dL^{-1})	44.00±13	66.00±28	30.00±17 †
LDL-C (mg dL^{-1})	78.00±22	187.00±18	157.00±24 †
Blood sugar (mg dL^{-1})	151.00±17	146.00±20	155.00±31

NOR: Non-exercised mice not on a high-fat diet: HFD, ApoE-/- mice on a high-fat diet: HFE: Voluntary exercise in ApoE-/- mice on a high-fat diet. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, TG: Triglyceride, LDL-C: Low density lipoprotein cholesterol. †$p<0.05$ vs HFD. Mean±SD

Laboratory data: Blood sugar levels did not differ between the three groups. In contrast, liver enzyme such as aspartate aminotransferase (AST), triglyceride and low-density lipoprotein (LDL) levels in the HFD group were significantly ($p<0.05$) higher than those in the NOR group. The AST, triglyceride and LDL cholesterol in the HFA group were significantly ($p<0.05$) reduced than those in the HFD group (Table 1).

Histological features: The hematoxylin and eosin staining showed significant atrophy and the pyknotic neurons were frequently observed in the hippocampus of HFD compared with NOR. Mice in HFA showed reduced pyknotic neurons compared with HFD (Fig. 1a). Histological examination of the liver sections revealed the development of severe steatosis and ballooning hepatosites in the HFD group, whereas reduced steatosis was present in HFA mice (Fig. 1b).

Gene expression: BDNF mRNA expression in the HFA group was significantly higher in the hippocampus than in the HFD group ($p<0.05$). The expression of RCAN1 mRNA in the hippocampus did not differ significantly between the HFD and NOR groups, whereas that in the HFA group was significantly ($p<0.05$) lower than that in the HFD group (Fig. 2).

Morris water maze test: The escape latency of morris water maze test in the HFD was significantly slower than that for the NOR group ($p<0.05$). The escape latency in HFA were significantly better than that in HFD group, but not better than that in NOR ($p<0.05$, Fig. 3).

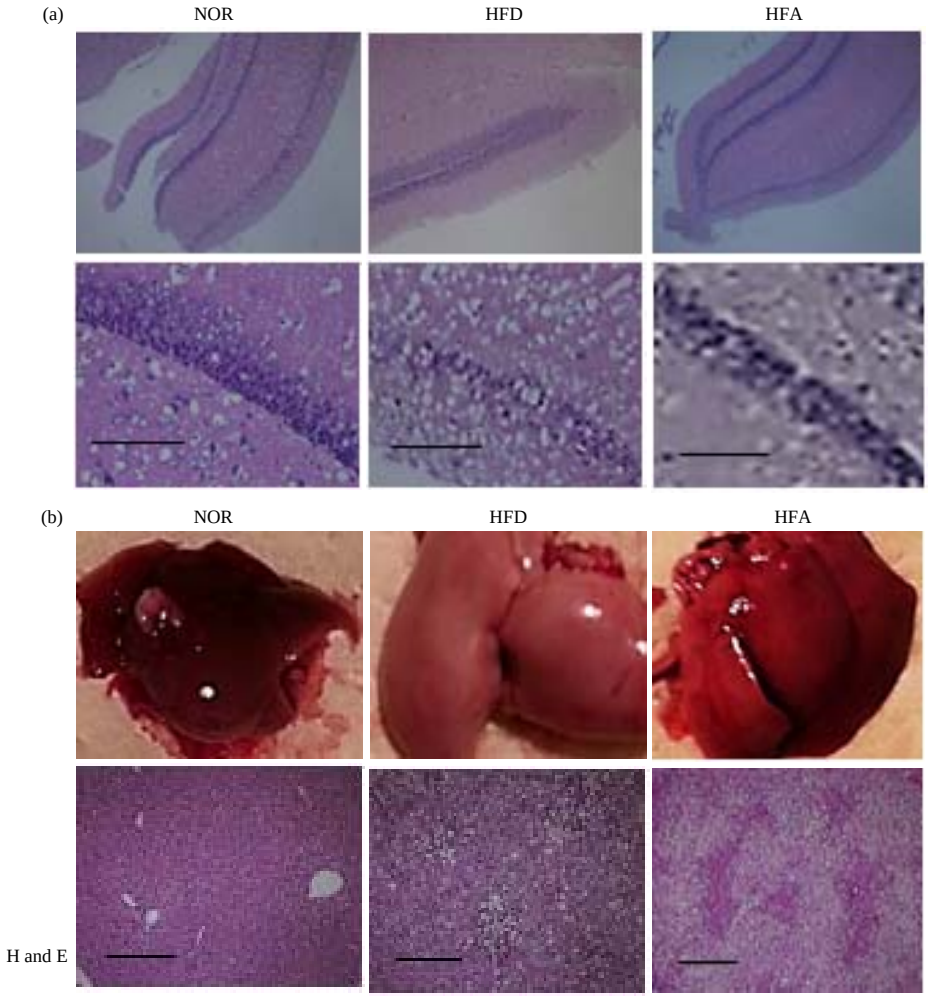

Fig. 1(a-b): Alogliptin reduced pycnotic neurons in the (a) Hippocampus and (b) Reduced steatohepatitis in the liver. Original magnification, x40 (upper A), x200 (lower A), x100 (lower B), Scale bar = 100 μm. A; The neurons in HFD were pycnotic. Those of HFA were reduced of pycnosis compared with HFD. B; The liver in HFD revealed steatohepatitis. That in HFA was reduced steatohepatitis

Novel object recognition test: The exploration time for familiar and novel objects varied but did not differ significantly between the three groups on days 1 and 2 ($p<0.05$). However, the recognition index on day 2 for the HFA group was significantly ($p<0.05$) higher than that for the HFD group (Fig. 4).

Forced swimming test: The distance travelled and immobility time for the HFD group were significantly impaired compared to those for the NOR group. Mice in HFA significantly normalized the distance travelled and shortened the immobility time ($p<0.05$, Fig. 5).

DISCUSSION

This study suggests that ALG improves cognitive and depressive function, with increased expression of BDNF mRNA and decreased RCAN1 mRNA expression in the hippocampus of obese ApoE-/- mice. They also reveal that ALG reduces liver weight, liver enzymes and lipid levels while no change of body weight and brain weight in comparison with high-fat obese mice. Histological examination showed that ALG protected pyknotic neurons in the hippocampus and steatosis in the liver.

Brain cells from ApoE-/- mice are more sensitive to excitotoxic and age-related synaptic loss[17] and β-induced

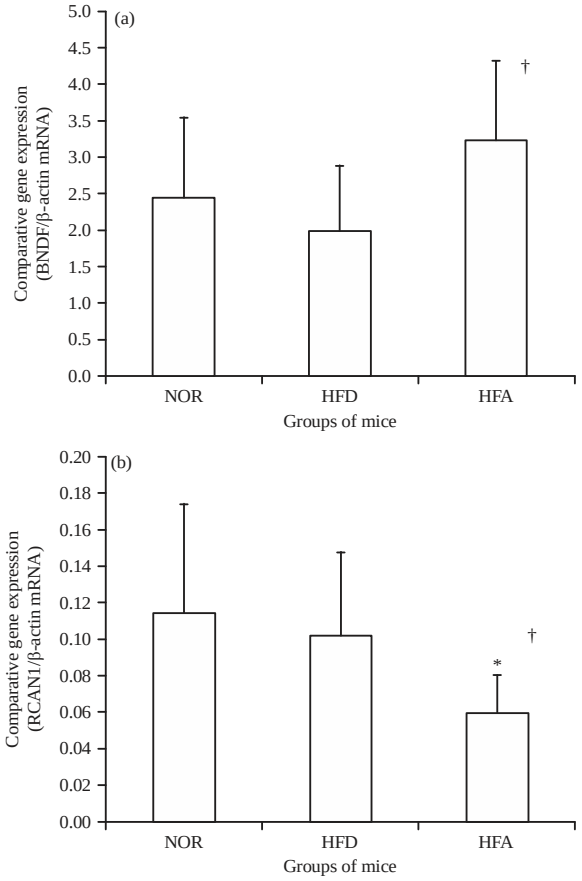

Fig. 2(a-b): Effects of alogliptin treatment on (a) BDNF and (b) RCAN1 expression in the hippocampus. *p<0.05 vs NOR, †p<0.05 vs HFD
Error bars represent SED

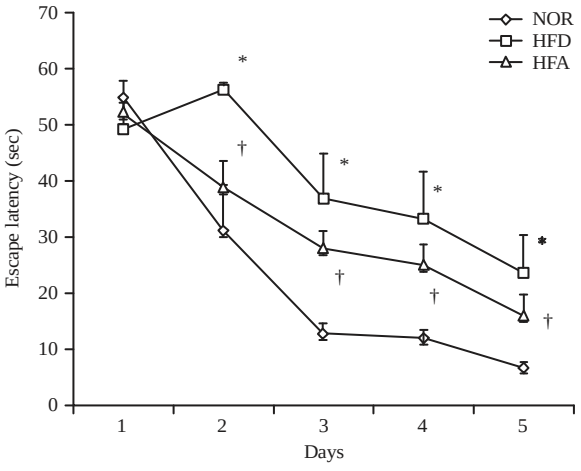

Fig. 3: Alogliptin treatment normalized morris water maze test. *p<0.05 vs NOR, †p<0.05 vs HFD
Error bars represent SED

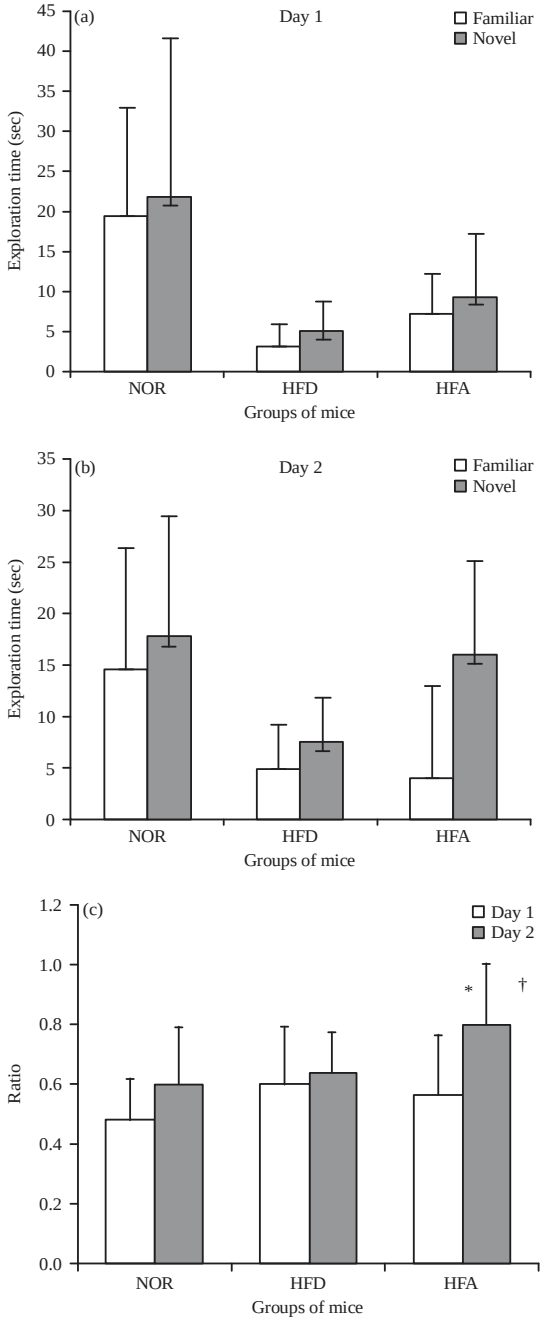

Fig. 4(a-c): Alogliptin was beneficial in novel object recognition task. Discrimination index was calculated as described in the Methods. *p<0.05 vs NOR, †p<0.05 vs HFD
Error bars represent SED. X-axis shows group of mice

synaptosomal dysfunction in these mice is also enhanced compared to control animals[9]. The gene dose of APOE ε4 is a major risk factor for AD, with many studies reporting an association between gene dose, age at onset[17,18] and cognitive

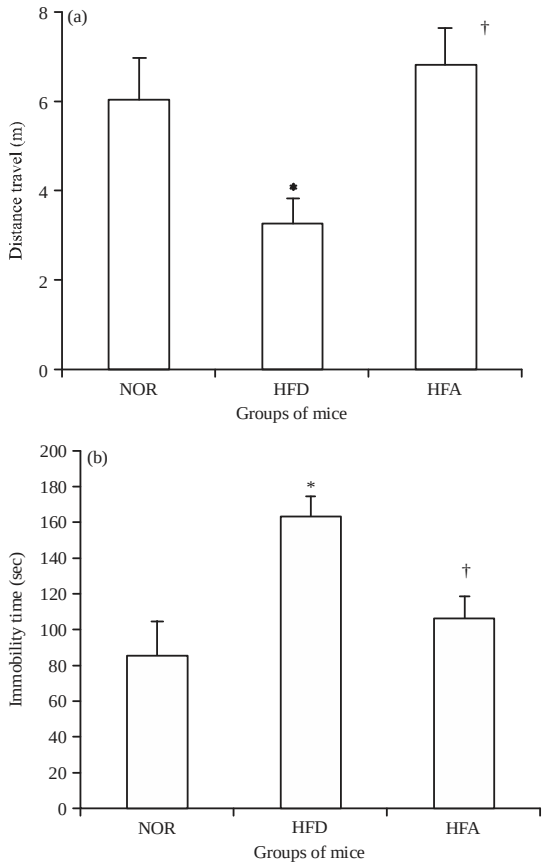

Fig. 5(a-b): Alogliptin lengthens the (a) Distance traveled and (b) Shortens the immobility time. *p<0.05 vs NOR, † p<0.05 vs HFD
Error bars represent SED. X axis shows group of mice

decline[19]. Indeed, association of the APOE polymorphism with late-onset AD is one of the strongest and most robust genetic risk factors for a common disease.

HFD consumption has been demonstrated to cause peripheral and neuronal insulin resistance and brain mitochondrial dysfunction in rats. DPP-4 inhibitors are known to improve peripheral insulin sensitivity, improved brain mitochondrial dysfunction and cognitive function. DPP-4 inhibitors effectively restored neuronal insulin function, increased glucagon-like-peptide 1 levels and prevented brain mitochondrial dysfunction, thus attenuating the impaired cognitive function caused by HFD[20]. Obesity reduces cognitive and motor function and alters brain plasticity[21]. Similarly, a high fat diet increases oxidative stress in the brain and reduces BDNF, which is known to mediate the effect of obesity on cognition and behavior, in the hippocampus[22]. This study showed that ALG induces BDNF gene expression in the hippocampus. Reduced RCAN1 protein levels by ALG treatment could be neuroprotective under acute stress conditions, including oxidative stress. As such, long-term RCAN1 gene overexpression may help us to understand the molecular mechanism of neurodegeneration in diseases such as AD and Down's syndrome[23].

The Morris water maze test and novel object recognition test results were significantly ($p<0.05$) improved by ALG treatment in obese ApoE-/- mice. Similarly, the forced swimming test, as an indicator of depressive state, was also improved by ALG administration. ALG treatment can improve both cognition and mood, with evidence suggesting that BDNF activity may mediate these effects[9,24]. Moreover, chronic consumption of high-fat food and obesity induces plasticity-related changes in reward circuitry that are associated with a depressive-like phenotype, with an alternation in BDNF activity beings implicated in depressive behavior[10].

The limitations of this study were the three-month period of ALG treatment, not yet verified. The more metabolic approaches were not investigated in the hippocampus in this experiment. Further studies are required in this field.

CONCLUSION

The present study has been shown that anti-diabetic drug alogliptin, dipeptidyl peptidase-4 inhibitor, improves cognitive and depressive function. Alogliptin treatment induced the expression of BDNF mRNA and decreased RCAN1 mRNA expression in the hippocampus of obese ApoE-/- mice. Application of alogliptin could be considered to prevent dementia and/or depression in metabolic dysfunction.

SIGNIFICANCE STATEMENT

This study discovered the anti-diabetic agent, Alogliptin benzoate, dipeptidyl peptidase-4 inhibitor, can be beneficial for cognition deficits, anti-depressive state and metabolic abnormality. This study will help the researcher to uncover the critical areas of obesity and cognitive deficits that many researchers were not able to explore. Thus in this study suggested a new theory on beneficial effects of dipeptidyl peptidase-4 inhibitor for dementia and/or depression.

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