Exenatide Stimulates Hippocampal Autophagic Activity in Alzeheimer Rat Model

Eman Abdo[1], Mohamed Saad[1], Alaa El-Din Firgany [1], Youssef mosaad[1], and Sara Eldusoky[1]

[1]Mansoura University Faculty of Medicine

June 1, 2020

Abstract

Aims: This study was designed to demonstrate potential neuroprotective and autophagic activity of exenatide in rodent AD model. Methods: Thirty adult Sprague-Dawley male rats were divided into 3 groups (10 rats each); Group 1; control normal group, Group 2; AD pathological group, Group 3; exenatide treated group. All drugs were given intraperitoneal (IP) for 42 days. Behavioral changes using Morris water maze test has been evaluated, gene expressions of beclin-1 and the mammalian target of rapamycin (mTOR) in the hippocampus were assessed. Examination of hippocampal tissue using hematoxylin & eosin (H&E) stain and ultrastructural analyses were also done. Data were analyzed by using the statistical package for the social sciences (SPSS). Results: Exenatide alleviated both behavioral and pathological changes compared to pathological group. Exenatide treated group was found to improve autophagic activity by increasing beclin-1 and decreasing mTOR gene expression. Exenatide treatment significantly prevented hippocampal neuronal degeneration demonstrated by H&E. Moreover, accumulation of autophagic vacuoles in ultrastructure study of hippocampus, alleviated in exenatide group compared to pathological group indicating enhanced autophagic activity by exenatide. Conclusion: The results of the present study clearly indicated exenatide might have beneficial effects on impaired cognitive performance and hippocampal neuronal viability in AD by increasing autophagic activity. Increased beclin-1 seems to be the initiating player in this disease modifying effect and this supports the assumption of a disease modifying activity of exenatide through the autophagic activity.

Abstract:

Aims: This study was designed to demonstrate potential neuroprotective and autophagic activity of exenatide in rodent AD model.

Methods: Thirty adult Sprague-Dawley male rats were divided into 3 groups (10 rats each); Group 1; control normal group, Group 2; AD pathological group, Group 3; exenatide treated group. All drugs were given intraperitoneal (IP) for 42 days.

Behavioral changes using Morris water maze test has been evaluated, gene expressions of beclin-1 and the mammalian target of rapamycin (mTOR) in the hippocampus were assessed. Examination of hippocampal tissue using hematoxylin & eosin (H&E) stain and ultrastructural analyses were also done. Data were analyzed by using the statistical package for the social sciences (SPSS).

Results: Exenatide alleviated both behavioral and pathological changes compared to pathological group. Exenatide treated group was found to improve autophagic activity by increasing beclin-1 and decreasing mTOR gene expression. Exenatide treatment significantly prevented hippocampal neuronal degeneration demonstrated by H&E. Moreover, accumulation of autophagic vacuoles in ultrastructure study of hippocampus, alleviated in exenatide group compared to pathological group indicating enhanced autophagic activity by exenatide.

Conclusion: The results of the present study clearly indicated exenatide might have beneficial effects on impaired cognitive performance and hippocampal neuronal viability in AD by increasing autophagic activity. Increased beclin-1 seems to be the initiating player in this disease modifying effect and this supports the assumption of a disease modifying activity of exenatide through the autophagic activity.

Key words: Alzheimer's disease, exenatide, Becline-1, mTOR, autophagy.

Introduction

Alzheimer's disease is a progressive, irreversible neurodegenerative disease, the most prevalent form of dementia and has a tremendous impact on the health care and on the affected individuals[1·] It is estimated that 35 million people have AD world- wide, and it is predicted to affect one of every 85 persons globally by 2050 without an efficient solution [2·] Alzheimer is a complex disease that causes accumulation of Aβ plaques and neurofibrillary tangles composed of tau amyloid fibrils associated with synapse loss and neurodegeneration [3].The pathogenesis of AD is complex, there are some hypotheses to explain it including; cholinergic, amyloid, tau hypothesis[4]. The autophagy pathway is the major degradation pathway of the cell for abnormal proteins and organelles so the active role of autophagy is the maintaining cellular homeostasis. Dysfunction of autophagy in AD associated with an accumulation of two abnormal protein aggregates forms, tau tangles and Aβ plaques[5]. Increasing evidence supports the notion that AD, a condition that presents heterogeneous pathological disturbances, is also associated to perturbed metabolic function affecting insulin and insulin-like growth factor I (IGF-I)[6].Currently available treatments are mainly symptomatic, aim to correct the neurotransmitter disrupted in AD. Treatment of mild to moderate AD by cholinesterase inhibitors while treatment moderate to severe AD by memantine[7]. These approved drugs in AD are considered symptomatic treatments and fail to achieve a definite cure, thus, there is a need to develop novel and effective medication[8].

Current drug development focuses on a new strategy in treatment of AD to block the progression or modify the course of AD through interference with the pathogenic steps responsible for the disease[9].

Exenatide, glucagon like peptide (GLP-1) analogue, have been developed for use in the treatment of type 2 diabetes mellitus through its effects on glucose homeostasis and facilitation of insulin signaling. Accumulating evidence suggests that GLP-1 analogues can cross the blood–brain barrier and may exert several extrapancreatic effects which may accounts for its positive effects in neurological disorders[10].

GLP-1 receptors exist widely throughout the brain, in the hypothalamus, thalamus, brain stem nucleus, cortex and hippocampus[11, 12]. GLP1 has been shown to act as a growth factor in the brain and promote neurite growth[13].GLP1 receptor agonists are reported to attenuate endogenous levels of Aβ in the brain and prevent amyloid plaque accumulation in the AD brain[14,15] . Furthermore, stimulating glucose metabolism in AD patients markedly improves cognitive dysfunction in the AD [16].

In the present study, we used $AlCl_3$ to develop experimental AD model and investigate whether exogenous exenatide supplementation has beneficial effect on cognitive performance through its stimulant effect of autophagy demonstrated by result of beclin-1 gene expression and ultrastructure study.

Materials and Methods

2.1 Drugs and chemicals

Aluminum chloride - hydrated (AlCl3.6H2O), was purchased from El-Gomhouria Company, Mansoura, Egypt. Exenatide injection pen (BYETTA) was purchased as a sterile solution for subcutaneous injection (Amylin Pharmaceuticals, Inc., San Diego).

2.2 Ethical consideration

The study was conducted in accordance with ethical guidelines of Faculty of medicine, Mansoura University, Egypt. Institutional Research Board under the code of MD.16.12.50.

2.3 Animals

Thirty adult male Sprague- Dawley rats weighing 200-250 grams each were used throughout this study. They were obtained from the medical experimental research center, Mansoura faculty of medicine. For seven days prior to the experiment, the animals were acclimatized to standard laboratory conditions and they were put in similar optimum housing condition with free access to food and water. The animals were housed in plastic cages lined with sawdust that was renewed daily and were observed for food intake. They kept in cages at a room with controlled temperature (23 ± 2°C), humidity (50 ± 5%) and 12-h light–dark cycle. All experiments were carried during fixed time (from 8 AM to 12 PM).

2.4 Experimental design

Three groups of rats (10/group) were used and received drugs daily for six weeks: Saline for control group, $AlCl_3$ (70 mg/kg, IP) for AD group [17], while the treated group received exenatide (20 µg/kg, IP) [18]. All drugs were administered at a dose volume 0.5 ml/250 g body weight.

In the current study, we evaluated AD by behavioral Morris water maze (MWM) test, weight detection in all groups at beginning and end of study, measurement of glucose levels, demonstration of beclin-1 and mTOR genes expression and histopathologically by light and electron microscope.

Morris water maze (MWM) test was done during study on day20, 21 and 42. At end of study rats were weighted, fasted overnight and were sacrificed 24 h after Morris test by sodium thiopentone (100 mg/kg, i.p. injection)[19]. Blood was withdrawn from the heart of rats by 5 ml syringe. The blood was collected in a dry test tube permitted to clump for 30 minutes prior to centrifuge at 4000 revolutions per minute for 15 minutes and then serum was separated and collected in Eppendorf tube and kept frozen at -20°C till the time of measurement serum glucose level. Craniotomy was performed to dissect out the intact brains and extraction of hippocampus for molecular, histological, and ultrastructure studies. Brains were removed and sagittaly divided into right and left hemispheres using a sharp blade for isolating the hippocampal tissues. Total RNA was extracted from 25-30 mg hippocampus tissue from right hemisphere immediately after shock freeze in liquid nitrogen and real time RT-PCR for study of beclin-1 and mTOR gene expressions. The hippocampi of left side were processed for histopathological and ultrastructure examination.

2.5 Behavioral experiment

The MWM is a useful test was described 30 years ago to study spatial learning and memory in laboratory rats because it is more specific for hippocampal function, one of the most affected regions in AD[20].

Animals will be trained to swim to a visible platform on Day 20 from starting study. Following training the time taken to find the hidden platform (retention latency, RL) on Day 21 and Day 42 was recorded.

Animals were trained in a circular pool (180 cm in diameter and 60 cm in height) located in a test room. The pool was filled with water to a height of 40 cm. During the acquisition phase, a movable circular platform (9 cm diameter) was placed in one of the quadrants of the pool approximately 2 cm above the water level, and during the retention phase, a similar platform was placed in the pool 2 cm below the water level. The water was made opaque by adding a nontoxic dye[21].

Maze acquisition phase (training phase)

Animals received a training session consisting of four trials with a gap of 5 minutes between the two trials on Day 20. Four different starting positions used during all four trials. Animal were released into the maze facing the wall of the pool and the latency to find the escape platform was record to a maximum of 90 seconds. The time taken by the animal to reach the platform was recorded as the initial acquisition latency (IAL).

Maze retention phase (retention latency phase)

Following training, the time taken to find the hidden platform (retention latency, RL) was recorded and assessed on Day 21 (24 hours after the last training session) and Day 42 and termed as first retention latency

(1^{st} RL) and second retention latency (2^{nd} RL), respectively. The change in RL from Day 21 to Day 42 used to evaluate the learned skill or memory.

Time in days

Day 0 Day 20 Day 21 Day 42

(Beginning of study) (Training phase) (1st retension phase) (2^{nd}retension phase)

Biochemical Parameters

2.6.1. Fasting blood glucose:

Fasting serum glucose was measured by glucose kits (BioMed-Glucose L.S, Eng Chem for lab technology, Hannover, Germany)[22].

2.6.2. Beclin-1 and mTOR gene expression in rat hippocampus by

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Total RNA was extracted from rat hippocampus tissue samples of all groups using RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to manufacturer's instruction. The Concentration and purity for each RNA sample were determined spectrophotometrically using 260 and 260/280 nm ratio respectively, by NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). The purity of RNA was checked and it ranged between 1.8 and 2.1 demonstrating the high quality. RNA was reverse-transcribed into complementary DNA (cDNA) using Arktik Thermal Cycler (Thermo Fisher Scientific Inc., MA, USA) and quantified using infra-red (IR)-specific primer by Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The primers were manufactured by Primers Biosearch technologies (Primers Biosearch technologies, CA, USA) and the specific gene sequences were obtained from Pubmed (Entrez Gene). Sequences of beclin-1 primers[23] were 5'-TTGAGATGAGAT-GCTTGT-3' (forward); and 5'-TAGAAGTGAGGACAGA-GT-3' (reverse), β-actin, used as internal control with sequences 5'-CCTGTGCTGCTCACCGAGGC-3' (forward) and 5'-GACCCCGTCTCTCCGGAGTCCATC-3' (reverse); mTOR specific primers were 5'-ATG ACG AGA CCC AGG CTA AG-3' (forward); and 5'-GCC AGT CCT CTA CAA TAC GC-3' (reverse), GABDH used as internal control, 5'-CCT CTG GAA AGC TGT GGC GT-3' (forward) and 5'-TTG GAG GCC ATG TAG GCC AT-3' (reverse) [24]. The thermal cycling conditions were adjusted as follow 15 min at 95 °C for activation of DNA polymerase followed by 40 cycles of 15 s at 95 °C, 20 s at 60 °C and 20 s at 72 °C.

Relative quantification (RQ) of mRNA expression was calculated with the 2-ΔΔCt method. The data were presented as relative quantity of target mRNA, normalized respect to ß-actin mRNA and relative to a calibrator sample. Normal control samples were used as calibrators. Where: ΔCt = (Ct of target gene – Ct of reference gene); ΔΔCt = (ΔCt of sample – ΔCt of control). Ct is defined as the fractional cycle number at which the fluorescence passes the fixed threshold.

2.6.3. Histopathological Examination

1-Haematoxylin and Eosin stain:

The hippocampi were fixed in 10% formalin for 24 h and then washed with tap water. For dehydration, serial dilutions of alcohol were used. Specimens were cleared in xylene embedded in paraffin at 56ºC in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by microtome[17].

2-Electron microscopic study:

The hippocampus cut into suitable pieces. 1 mm specimens were fixed immediately in a fixative containing 2.5% glutaraldehyde in 0.1 M cacodylate buffer (PH 7.4) at 4ºC for 24 hours. Specimens were then washed in two changes of 0.1 M cacodylate buffer and postfixed for 90 minutes in 2% osmium tetraoxide at 4ºC. The specimens were dehydrated in gradual series of ethanol, infiltrated and embedded in propylene-epon

sequences then polymerized at 60^{o}C for one day. Ultrathin silvery golden sections were cut by ultramicrotome and picked on uncoated grids then stained with uranyl acetate and lead citrate and viewed by transmission electron microscope [25].

2.7 Statistical analysis

Data were analyzed with SPSS version 21. The normality of data was first tested with Shapiro test. Continuous variables were presented as mean ± SEM (standard error of mean). ANOVA test followed by posthoc Tukey test was used for multiple comparisons. Paired t-test was used to compare paired data in the same group. A P value <0.05 was considered statistically significant in all analyses.

Results

The results of this study on Alzheimer rat model indicate effective therapeutic effect of d exenatide (table 1).

3.1 Morris water maze task

Rats treated with $AlCl_3$ took longer time to reach the visible platform (80.75±1.29) than those of the control group (48.25±1.09) on day 20, indicating memory deficits, whereas administration of exenatide significantly enhanced memory performance on day 20 (49.00±0.96) as compared to $AlCl_3$ treated group.

Moreover $AlCl_3$ treatment significantly diminished the 1st and 2nd retention latencies on day 21 and 42 respectively (70.25±0.88, 66.50±0.94) as compared to the control group (17.87±0.91, 12.87±0.71 respectively). Exenatide treatment significantly enhanced both the retention latencies (12.25±0.59, 18.87±0.51 respectively) as compared to $AlCl_3$ alone.

Exenatide attenuates $AlCl_3$ induced weight loss

The body weight changes in all experimental groups. Pathological $AlCl_3$ group showed a significant decrease in body weight at end of study (156.87±4.84) compared to beginning of study (239.87±2.66). Contrary, control normal group, exenatide treated group showed significant increase in body weight at end of study (281.00±5.38, 281.62±6.16) compared to beginning of study (207.50±2.52, 213.25±4.47) respectively.

3.3 Fasting serum glucose level

Aluminum chloride induced AD in rats produced highly significant increase in serum glucose level (130.37±2.24) compared to control normal rats (94.37±1.98).

Exenatide treated group showed significant decrease serum glucose level (97.00±1.47) compared to $AlCl_3$ induced AD in rats while didn't show significant increase in serum glucose level compared to control normal rats.

3.4 Hippocampal mTOR gene expression

Aluminum chloride induced AD in rats produced highly significant increase in mTOR gene expression (1.81±0.02) compared to control normal rats1 (00±0.02). Exenatide treated group (0.98±0.02) showed significant decrease mTOR gene expression compared to $AlCl_3$ induced AD in rats (figure 1A).

3.5 Hippocampal beclin-1 gene expression

Aluminum chloride induced AD in rats produced highly significant decreases in beclin-1 gene expression (0.27± 0.01) compared to control normal rats (1.01±0.04). Exenatide treated group showed significant increase beclin-1 gene expression (1.21±0.05) compared to $AlCl_3$ induced AD in rats (figure 1B)

3.6 Effects of exenatide on the histological structure of hippocampus

Aluminum chloride induced AD in rats produced highly significant increase in number of degenerated cells in hippocampus (23.00±0.70) compared to control normal rats (0.0±0.0) in H&E stain. Exenatide treated

group showed significant decrease in number of degenerated cells in hippocampus (2.25±0.25) compared to $AlCl_3$ induced AD in rats (Table 1, Figure 2, 3).

In ultrastructure examination by EM, there was improvement in exenatide treated rats compared to AlCl3 untreated rats (Figure 4); there was preservation of mitochondria, ER and no accumulation of autophagic vacuoles as observed with AD group.

4- DISCUSSION

As continuation to researches concerned with disease modifying drugs in AD, this research was done to demonstrate potential modifying effect of exenatide in rat model of AD.

In the present study, AD mimics model was induced by injection of $AlCl_3$. Aluminum is the most abundant metal on the earth crust. It gets access to the human body through drinking water, food, use of utensils, deodorants, and some pharmaceutical products so AL^3induced cognitive deficit has been widely used for the preclinical testing of promising molecules against AD[26]. IP $AlCl_3$ administration has benefit than oral rout as it predominantly accumulates in the hippocampus, the most susceptible region in AD and has important role in learning and memory functions, unlike oral route which resulted in a significant increase metal concentration in ventral, midbrain[27].

In MWM test, AD group took more time to reach the visible platform in IAL, 1st RL on day 21 and 2nd RL on day 42 than the control group, indicating memory deficits. The increase in time in pathological AD group is in agreement with previous studies[28, 29, 30.]

Increasing time to reach plate AD group due to AL^{+3}deposition and accumulation in sensitive areas in brain which is considered a contributing factor to the pathogenesis of AD[26]. Its deposition disturbs the amyloid precursor protein (APP), results in elevated APP leading to deposition and accumulation of Aβ-plaques in the hippocampus of the brain. Aluminum also causes tau hyperphosphorylation and intracellular neurofibrillary tangle formation and creates fatty acid abnormalities in the phospholipids [31].

As regard weight, AD model group has associated with decrease in body weight at end of study compered to weight at beginning of study. This result concurs with many previous researches [32, 33, 34]. Reduction in the body weight following $AlCl_3$ administration may be due to decrease in feed consumption by direct effect [35]. Aluminum administration has been shown to accumulate in many mammalian tissues and several organs which affect their functions[34]. Aluminum administration enhances catabolic processes which is the outcome of lack of cellular glucose in liver cells. The disturbance of the catabolic process in the liver was evidenced by a significant hyperglycemia and an altered lipid metabolism[36].

AD group has associated with increase in blood glucose level compared to control group. This is concurring with previous studies[36, 37, 38]. The rise in blood glucose may be caused by a disruption in carbohydrate metabolism, through enhancement of the breakdown of liver glycogen, possibly mediated by an increase in adrenocorticotrophic and glucagon hormones and/or reduced insulin activity [39].

In this study, AD group has associated with increased mTOR expression level in hippocampus compared to normal group. Aluminum can significantly increase the protein expression levels of PI3K, AKT, mTOR signaling pathway [40]. **Spilman et al** reported that mTOR increased in animal models of AD[41]. Several laboratories also have reported that mTOR hyperactivity is increased in postmortem AD human brains compared with age-matched controls [42, 43, 44]. mTOR signaling hyperactivity has damaging effects on learning and memory functions. mTOR hyperactivity characterized by widespread Aβ accumulation and increased tau levels and phosphorylation[45]. One of the primary regulators of the autophagy pathway is mTOR complex 1 (mTORC1). mTORC1 becomes inhibited as a result of the modulation of various upstream regulators, such as AMP-activated protein kinase (AMPK). This subsequently leads to the activation of the autophagy-initiating complex which triggers the activation and recruitment of primary autophagy[46].

In the current study AD group showed decrease in beclin-1 expression compared to normal group. This is matched with **Pickford et al**[47] who ensure about the precise role of beclin-1 in autophagy pathway and

its decrement in AD. Beclin-1 is an important multifunctional protein that also plays an important role in autophagy. Beclin-1 mediates the localization of autophagic proteins to nascent membranes and is required for the induction of autophagy[48]. Thus its inhibition is suggested to inhibit autophagic process. The findings in animal models of AD suggest that modulation of beclin-1 activity may be a therapeutic strategy for AD [49].

Histopathological studies using H&E stain of hippocampus in AD group showed significant increase in number of degenerated cells and alteration in histological structures compared to control normal group. This concurs with other previous studies [50, 51].

Electron microscopic study of AD model group shows dystrophic neurite displaying autophagic vacuoles. Several studies demonstrate a link between autophagy and AD pathology using EM [52, 53, 54]. Autophagy was first discovered through EM studies, due to a lack of specific protein markers, and for a long time and retained its fundamental position in the detection of autophagic process[55].

Increased numbers of autophagic vacuoles in dystrophic neurons in AD brains indicates dysfunction of the autophagic pathway[56]. A defect in autophagy vacuole clearance rather than an increase in autophagy induction is suggested to explain this accumulation of vacuoles [57].

Autophagy in AD may be misregulated by either beclin-1 deficiency, disruption of axonal transport or by dysfunction of presenilins, which are part of a γ-secretase complex which have been implicated in fusion of autophagosomes with lysosomes. The reduction in neuronal autophagy and disruption of the lysosomes in beclin-1 deficient may be explained by separate actions of beclin-1 both on autophagy initiation and on endosome/lysosome fusion [47].

Accumulated autophagic vacuoles contribute to the production of Aβ where they contain all necessary constituents for Aβ production, thus autophagic compartments were identified as a major reservoir of intracellular Aβ in the brain of Alzheimer patients[58].

An attention was focused on the molecular mechanisms underlying the autophagic fight against neurodegeneration and therapeutical approaches to up regulate protective neuronal autophagy[59].

As GLP-1 analogues can cross the blood brain barrier and may exert several beneficial extrapancreatic effects. Positive effects in neurological disorders as AD are expected through these effects[10].

Exenatide treated group significantly showed enhanced memory performance in MWM compared to AD group. Exenatide protective effect against aluminum-induced neurotoxicity and enhanced memory performance is in accordance with many studies [60, 61, 18].GLP-1 mimetics could readily cross BBB and promote progenitor cell proliferation in neurons of the brain [12].

Exenatide treated group showed significantly reduced serum glucose level when compared with AD group. GLP-1 agonists may affect AD indirectly by reducing blood glucose, insulin levels and subsequent reduced levels of inflammatory markers and reactive oxygen species[62]. Exenatide secrete insulin and exerts its secretagogue effect by activating GLP1R in the β-cells resulting in the release of insulin [15]. This effect by increasing β-cell gene expression resulted in β-cell proliferation and neogenesis [63].

In this study, exenatide treated group showed significantly reduced hippocampal mTOR gene expression when compared with AD group. GLP-1 analogue significantly inhibit mTOR phosphorylation at the serine/threonine kinase S6K1 which is one of the most well-known downstream targets of mTORC1 [64]. Exenatide decreased mTOR gene expression is in agreement with **Chen et al study** [65], who approved that GLP 1 analogue treatment, inhibits mTOR activation.

Also exenatide treated group in this study showed significantly increased beclin-1 expression level when compared with AD group. This increase in beclin-1 activity is in parallel with other studies[66, 67].

Through light microscope histological examination, exenatide treated group showed significant improvement of changes and decrease number of hippocampal degenerated cells compared to AD group.

In EM examination, exenatide group showed improvement compared to AD group. The effect of exenatide of healthy and complete autophagic activity may indicate a preserved interaction between the autophagic vacuoles and the autohagosome in one hand and the lytic enzymes of the vacuoles on the other hand. This can be in agreement with the assumption of **Cui et al** [68] of coordinating multivesicular body and autophagosome pathways of autophagy.

The important stimulant effect of exenatide on beclin-1 gene expression with a less proportional inhibitory effect on mTOR gene expression (figure-1), suggest a direct beclin-1 gene stimulatory mechanism for autophagy.

Caleb**et. al,** [69] suggested that AD treatment must target different mechanisms to treat this complex disease with multiple pathways; reducing oxidative stress, stabilizing mitochondria, activating autophagy or proteasome, and increasing energy levels of neurons. Ideally, GLP-1 agonists are safe and non-interfering with other processes.

Conclusion

Studying different important autophagic parameters in an Alzeheimer experimental animal model; Exenatide has important stimulant effect on beclin-1 gene expression which is significantly correlative to the improved alzeheimer model manifestations more than its decreasing effect on mTOR gene expression. Becli-1 hypoactivity is suggested to be the defect in alzeheimer. Beclin-1 activation by exenatide represents a disease modifying effect.

Exenatide treated rats during exposure to $AlCl_3$neurotoxicity showed more pronounced improving effect on learning and memory abilities, attenuated the $AlCl_3$ induced increases in serum glucose level, mTOR gene expression, also increase in beclin-1 gene expression. Exenatide can also protect against neuronal degeneration in the hippocampus demonstrated in histological examination and increase the autophagic activity demonstrated in EM examination.

Exenatide increased beclin-1 even significantly more than that of the normal rats. Increased beclin-1 seems to be the initiating player in this disease modifying effect and this supports the assumption of a disease modifying activity of exenatide through the autophagic activity.

If AD and the other neurodegenerative diseases can be considered as autophagic diseases; the widely cerebrally distributed GLP-1 receptors could be an important therapeutic target. Also, this study directs the attention of aluminum monitoring in some cases of the neurodegenerative diseases.

Acknowledgements

We are thankful to MERC, Faculty of Medicine, Mansoura University.

Authors' contribution

E.A.E. designed the study, conducted experiments, analysed and interpreted the data, and wrote the manuscript; Y.M.M. was responsible for analysing and evaluating chemical biomarkers and gene expressions. A.E.F. was responsible for preparation and examination of the histological samples. S.E. conducted experiments; animal maintenance and sample collection. M.A.A supervised the thesis, designed the experiments, analysed and interpreted the data and wrote and revised the manuscript.

Funding resources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

No conflict of interest.

Data availability statement

Author elects to share data.

ORCID:

Eman Abdo ID https://orcid.org/0000-0003-2567-9665

References:

1. Simsir, I. Y., Soyaltin, U. E., & Cetinkalp, S., 2018. Glucagon like peptide-1 (GLP-1) likes Alzheimer's disease. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 12(3), 469-475. https://doi.org/10.1016/j.dsx.2018.03.002
2. Brookmeyer, R., Johnson, E., Ziegler-Graham, K. and Arrighi, H. M., 2007. Forecasting the global burden of Alzheimer's disease. Alzheimer's & dementia, 3(3), 186-191. https://doi.org/10.1016/j.jalz.2007.04.381.
3. Anand, R., Gill, K.D. and Mahdi, A.A., 2014. Therapeutics of Alzheimer's disease: past, present and future. Neuropharmacology. 76, 27–50. https://doi.org/10.1016/j.neuropharm.2013.07.004.
4. Tanzi, R.E., Bertram, L., 2005. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 120: 545-555. https://doi.org/10.1016/j.cell.2005.02.008.
5. Funderburk S. F., Marcellino B. K., Yue, Z., 2010. Cell "Self Eating"(Autophagy) Mechanism in Alzheimer's Disease. Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine, 77(1), 59-68. https://doi.org/10.1002/msj.20161.
6. Zegarra-Valdivia, Jonathan, A., Andrea, S. Fernández de Sevilla, Estrella. M., Nuñez, Angel. N., Aleman, T., Ignacio, 2019. Serum insulin-like growth factor I deficiency associates to Alzheimer's disease co-morbidities. Journal of Alzheimer's disease. 69 (4), 979-987. DOI: 10.3233/JAD-190241
7. Konstantina, G. Yiannopoulou, and Sokratis, G. Papageorgiou, 2013**.** Current and future treatments for Alzheimer's disease. Therapeutic Advances in Neurological Disorders .6 (1), 19–33. https://doi.org/10.1177/1756285612461679.
8. Kumar, A. and Singh, A., 2015. A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacological Reports, 67(2), 195-203. https://doi.org/10.1016/j.pharep.2014.09.004.
9. Yiannopoulou, K. G., Papageorgiou, S. G., 2013. Current and future treatments for Alzheimer's disease. Therapeutic advances in neurological disorders. 6(1), 19-33. https://doi.org/10.1177%2F1756285612461679.
10. Athauda, D. and Foltynie, T., 2016. The glucagon-like peptide 1 (GLP) receptor as a therapeutic target in Parkinson's disease: mechanisms of action. Drug discovery today. 21(5); 802-818. https://doi.org/10.1016/j.drudis.2016.01.013.
11. Abbas, T., Faivre, E., Holscher, C., 2009. Impairment of synaptic plasticity and memory formation in GLP-1 receptor KO mice: Interaction between type 2 diabetes and Alzheimer's disease. Behav. Brain Res. 205, 265–271. https://doi.org/10.1016/j.bbr.2009.06.035.
12. Hamilton, A., Patterson, S., Porter, D., Gault, V.A., Holscher, C., 2011. Novel GLP-1 mimetics developed to treat type 2 diabetes promote progenitor cell proliferation in the brain. J. Neurosci. Res. 89, 481–489. https://doi.org/10.1002/jnr.22565.
13. Cork, S.C., Richards, J.E., Holt, M.K., Gribble, F.M., Reimann, F. Trapp, S., 2015. Distribution and characterization of Glucagon-like peptide-1 receptor expressing cells in the mouse brain. Mol. Metab. 4, 718–731. https://doi.org/10.1016/j.molmet.2015.07.008.
14. McClean, P.L.; Parthsarathy, V., Faivre, E., Holscher, C., 2011. The diabetes drug liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. J. Neurosci. 31, 6587–6594. DOI: https://doi.org/10.1523/JNEUROSCI.0529-11.2011.
15. McClean, P.L., Holscher, C., 2014. Liraglutide can reverse memory impairment, synaptic loss and reduce plaque load in aged APP/PS1 mice, a model of Alzheimer's disease. Neuropharmacology, 76, 57–67. https://doi.org/10.1016/j.neuropharm.2013.08.005.
16. Parthsarathy, V., Holscher, C., 2013. Chronic treatment with the GLP1 analogue liraglutide increases cell proliferation and differentiation into neurons in an AD mouse model. PLoS ONE. 8 (3), e58784. ? https://doi.org/10.1371/journal.pone.0058784.

17. Ali, A. A., Ahmed, H. I., and Abu-Elfotuh, K. 2016, Modeling Stages Mimic Alzheimer's Disease Induced by Different Doses of Aluminum in Rats: Focus on Progression of the Disease in Response to Time. J Alzheimers Parkinsonism Dementia. 1(1), 1-11. Google scholar.
18. Solmaz, V., Cınar, B. P., Yiğittürk, G., Çavuşoğlu, T., Taşkıran, D., Erbaş, O., 2015. Exenatide reduces TNF-α expression and improves hippocampal neuron numbers and memory in streptozotocin treated rats. European journal of pharmacology. 765, 482-487. https://doi.org/10.1016/j.ejphar.2015.09.024.
19. Minnesota, U., 2009. Euthanasia Guideline. URL ¡http://www.ahc.umn.edu/rar/euthanasia.html¿
20. Morris, R.G.M., 1981. Spatial Localization Does Not Require the Presence of Local Cues. Learning and Motivation. *12* (2), 239-260. https://doi.org/10.1016/0023-9690(81)90020-5.
21. Prashar, Y., gill, D., perween, A., 2014. Protective effect of lagenaria siceraria in reversing ALCL3 induced learning and memory deficits in experimental animal model. International journal of recent advances in pharmaceutical research. 4(4), 87-104. ISSN: 2230-9306.
22. Trinder, P., 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6, 24-25.
23. Zhu, B., Yang, C., Ding, L. C., Liu, N., 2014. 3-methyladenine, an autophagic inhibitor, attenuates therapeutic effects of sirolimus on scopolamine-induced cognitive dysfunction in a rat model. International journal of clinical and experimental medicine. 7(10), 3327-3332. PMID: 25419365
24. Ma, Y. Q., Wu, D. K., Liu, J. K., 2013. mTOR and tau phosphorylated proteins in the hippocampal tissue of rats with type 2 diabetes and Alzheimer's disease. Molecular medicine reports, 7(2), 623-627. https://doi.org/10.3892/mmr.2012.1186.
25. Bancroft, J. D., Gamble, M., 2002. Theory and Practice of Histological Technique. 5th edn., New York. Phladelphia. Sydney. 163 (200), 679-699.
26. Ajith Kumar, S. P., Kumaresan, D., Nithya, S., Muralidharan, P., 2018. Therapeutic effects of L-arginine on aluminum chloride-induced dementia of Alzheimer's type in mice. Drug Invention Today, 10(9), 1708-1711. Google scholar.
27. Singh, S., Singh, R., Kushwah, A. S., Gupta, G., 2014. Neuroprotective role of antioxidant and pyranocarboxylic acid derivative against AlCl3 induced Alzheimer's disease in rats. Journal of Coastal Life Medicine. 2(7), 571-578. doi:10.12980/JCLM.2.2014J58.
28. Prakash, A., Kumar, A., 2009. Effect of NAcetyl Cysteine against Aluminium induced Cognitive Dysfunction and Oxidative Damage in Rats. Basic & clinical pharmacology & toxicology. 105(2), 98-104. https://doi.org/10.1111/j.1742-7843.2009.00404.
29. Thippeswamy, A.H., Rafiq, M. Viswantha, G.L.S., 2013. Evaluation of Bacopa monniera for its Synergistic Activity with Rivastigmine in Reversing Aluminum- Induced Memory Loss and Learning Deficit in Rats. J Acupunct Meridian Stud. 6(4):208-213. https://doi.org/10.1016/j.jams.2013.02.004.
30. Rather, M. A., Thenmozhi, A. J., Manivasagam, T., Bharathi, M. D., Essa, M. M., Guillemin, G. J., 2018. Neuroprotective role of Asiatic acid in ALCL3 induced rat model of Alzheimer's disease. Front Biosci. 10, 262-275. Google Scholar.
31. Foster, H. D., 2000. How aluminum causes Alzheimer's disease: the implications for prevention and treatment of Foster's multiple antagonist hypothesis. Journal of Orthomolecular Medicine. 15(1), 21-51. Google Scholar.
32. Tripathi, S., Mahdi, A.A., Hasan, M., Mitra, K., Mahdi, F., 2011. Protective potential of Bacopa monniera (Brahmi) extract on aluminum induced cerebellar toxicity and associated neuromuscular status in aged rats. Cell Mol Biol. 57(1), 3-15. PMID: 21366957.
33. Sharma, S., Mells, J. E., Fu, P. P., Saxena, N. K., Anania, F. A., 2011. GLP-1 analogs reduce hepatocyte steatosis and improve survival by enhancing the unfolded protein response and promoting macroautophagy. PloS one. 6(9), 25269. https://doi.org/10.1371/journal.pone.0025269.
34. Taïr, K., Kharoubi, O., Taïr, O. A., Hellal, N., Benyettou, I., Aoues, A., 2016. Aluminium-induced acute neurotoxicity in rats: Treatment with aqueous extract of Arthrophytum (Hammada scoparia). Journal of Acute Disease. 5(6), 470-482. https://doi.org/10.1016/j.joad.2016.08.028.
35. Alsayed, A.M., Abdel-Rahman Al-Rashidy A.H., Ahmed Abd El-Mawla A.M., 2011. Role of propolis in improving male rat fertility affected with aluminum chloride cytotoxicity. Spatula DD. 1(4),189-198.

DOI 10.5455/spatula.20111122065544.

36. Belaïd-Nouira, Y., Bakhta, H., Haouas, Z., Flehi-Slim, I., Neffati, F., Najjar, M. F., Cheikh, H. B., 2013. Fenugreek seeds, a hepatoprotector forage crop against chronic AlCl 3 toxicity. BMC veterinary research. 9(1), 22. https://doi.org/10.1186/1746-6148-9-22.
37. HM, O., Hassan, K. A., Kh, A. E. S. EA A., 2003. Aluminium toxicity in rats: The role of tannic acid as antioxidant. Ass. Univ. Bull. Environ. Res. 6(2), 1-14. Google Scholar.
38. Kalaiselvi, A., Reddy, G. A., Ramalingam, V., 2015. Ameliorating effect of ginger extract (Zingiber officinale Roscoe) on liver marker enzymes, lipid profile in aluminium chloride induced male rats. Int. J. Pharm. Sci. Drug Res. 7(1), 52-58. Google Scholar.
39. Metwally, F.M., Mazhar, M.S., Ibrahim, K.H.S., 2002. Serum essential metals changes among pot room worker in aluminum industry. Kaasr El Aini Med. J. 8(2):129-140.
40. Hu, C., Yang, J., He, Q., Luo, Y., Chen, Z., Yang, L., Yang, Y., 2018. CysLTR1 blockage ameliorates liver injury caused by aluminum-overload via PI3K/AKT/mTOR-mediated autophagy activation in vivo and in vitro. Molecular pharmaceutics, 15(5), 1996-2006. https://doi.org/10.1021/acs.molpharmaceut.8b00121.
41. Spilman, P., Podlutskaya, N., Hart, M. J., Debnath, J., Gorostiza, O., Bredesen, D., Galvan, V., 2010. Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-β levels in a mouse model of Alzheimer's disease. PloS one, 5(4), 9979. https://doi.org/10.1371/journal.pone.0009979.
42. Peel, A.L., Bredesen, D.E., 2003. Activation of the cell stress kinase PKR in Alzheimer's disease and human amyloid precursor protein transgenic mice. Neurobiology of disease.14:52–62. https://doi.org/10.1016/S0969-9961(03)00086-X.
43. Griffin, R.J., Moloney, A., Kelliher, M., Johnston, J.A., Ravid. R., Dockery, P., O'Connor, R., O'Neill, C., 2005. Activation of Akt/PKB, increased phosphorylation of Akt substrates and loss altered distribution of Akt and PTEN are features of Alzheimer's disease pathology. J Neurochem. 93:105–117. https://doi.org/10.1111/j.1471-4159.2004.02949.x.
44. Pei, J.J., Björkdahl, C., Zhang, H., Zhou, X., Winblad, B., 2008. p70 S6 kinase and tau in Alzheimer's disease. J Alzheimers Dis. 14:385–392. Doi: 10.3233/JAD-2008-14405
45. Caccamo, A., Magrí ,A., Medina, D.X., Wisely, E.V., López-Aranda, M.F., Silva, A.J., Oddo, S., 2013. mTOR regulates tau phosphorylation and degradation: implications for Alzheimer's disease and other tauopathies. Aging cell. 12:370–380. https://doi.org/10.1111/acel.12057.
46. Keeling, E. , Lotery, A.J., David, A. Tumbarello, and Ratnayaka, J.A., 2018. Impaired Cargo Clearance in the Retinal Pigment Epithelium (RPE) Underlies Irreversible Blinding Diseases. Cells. 7(2), 1- 16. https://doi.org/10.3390/cells7020016.
47. Pickford, F., Masliah, E., Britschgi, M., Lucin, K., Narasimhan, R., Jaeger, P. A., Wyss-Coray, T., 2008. The autophagy-related protein beclin 1 shows reduced expression in early Alzheimer disease and regulates amyloid β accumulation in mice. The Journal of clinical investigation. 118(6), 2190-2219. https://doi.org/10.1172/JCI33585.
48. Liang X.H., Jackson S., Seaman M., Brown K., Kempkes B., Hibshoosh H., Levine B., 1999. Induction of autopohagy and inhibition of tumorigenesis by Beclin 1. Nature. 402, 672-76. https://doi.org/10.1038/45257.
49. Lee, J. A., Gao, F. B., 2008. Regulation of Aβ pathology by beclin 1: a protective role for autophagy?. The Journal of clinical investigation. 118(6), 2015-2018. 10.1172/JCI35662.
50. Said, M. M., Gehan, A., 2015. The possible neurotherapeutic effects of mesenchymal stem cells on AlCl3- induced Alzheimer's disease in adult albino rats. Journal of Pharmacy and Biological Sciences. 10 (5), 33-40. DOI: 10.9790/3008-10513340.
51. Thenmozhi, A. J., Raja, T. R. W., Janakiraman, U., Manivasagam, T., 2015. Neuroprotective effect of hesperidin on ALCL3 induced Alzheimer's disease in Wistar rats. Neurochemical research. 40(4), 767-776. https://doi.org/10.1007/s11064-015-1525-1.
52. Cataldo, A.M., Hamilton, D.J., Nixon, R.A., 1994. Lysosomal abnormalities in degenerating neurons link neuronal compromise to senile plaque development in Alzheimer disease. Brain Res. 640:68–80. https://doi.org/10.1016/0006-8993(94)91858-9.

53. Nixon, R.A., Wegiel, J., Kumar, A., Yu, W.H., Peterhoff, C., Cataldo, A., Cuervo, A.M., 2005. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol. 64:113–122. https://doi.org/10.1093/jnen/64.2.113.
54. Nixon, R. A., Yang, D. S., 2011. Autophagy failure in Alzheimer's disease—locating the primary defect. Neurobiology of disease, 43(1), 38-45. https://doi.org/10.1016/j.nbd.2011.01.021.
55. Steele, J. W., Fan, E., Kelahmetoglu, Y., Tian, Y., Bustos, V., 2013. Modulation of autophagy as a therapeutic target for Alzheimer's disease. Postdoc journal: a journal of postdoctoral research and postdoctoral affairs, 1(2), 21-34. PMID: 28286801
56. Benzing, W.C.; Mufson, E.J.; Armstrong, D.M., 1993. Alzheimer's disease-like dystrophic neurites characteristically associated with senile plaques are not found within other neurodegenerative disease unless amyloid β-protein deposition is present. Brain Res. 606, 10–18. https://doi.org/10.1016/0006-8993(93)91563-8.
57. Nixon, R.A.; Yang, D.S., 2008. Lee, J.H. Neurodegenerative lysosomal disorders: A continuum from development to late age. Autophagy . 4, 590–599. https://doi.org/10.4161/auto.6259.
58. Eskelinen, E. L., Saftig, P., 2009. Autophagy: a lysosomal degradation pathway with a central role in health and disease. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1793(4), 664-673.*https://doi.org/10.1016/j.bbamcr.2008.07.014*.
59. Martinez, M., Cuervo, A. M., 2007. Autophagy and neurodegeneration: when the cleaning crew goes on strike. The Lancet Neurology, 6(4), 352-361. https://doi.org/10.1016/S1474-4422(07)70076-5.
60. Perry, T., Lahiri, D. K., Sambamurti, K., Chen, D., Mattson, M. P., Egan, J. M., Greig, N. H., 2003. Glucagon-like peptide-1 decreases endogenous amyloid-β peptide (Aβ) levels and protects hippocampal neurons from death induced by Aβ and iron. Journal of neuroscience research. 72(5), 603-612. https://doi.org/10.1002/jnr.10611.
61. Chen, S., Liu, A. R., An, F. M., Yao, W. B., Gao, X. D., 2012. Amelioration of neurodegenerative changes in cellular and rat models of diabetes-related Alzheimer's disease by exendin-4. Age. 34(5), 1211-1224. https://doi.org/10.1007/s11357-011-9303-8.
62. Perry, T. A., Greig, N. H., 2004. A new Alzheimer's disease interventive strategy: GLP-1. Current drug targets. 5(6), 565-571. https://doi.org/10.2174/1389450043345245.
63. Barnett, A., 2007. Exenatide. Expert Opinion on Pharmacotherapy.*8* (15), 2593-2608. https://doi.org/10.1517/14656566.8.15.2593.
64. Zhan, J., Wang, Y. J., Wang, Y., Tang, Z. Y., Tan, P., Huang, W., Liu, Y. S., 2015. The protective effect of GLP-1 analogue in arterial calcification through attenuating osteoblastic differentiation of human VSMCs. International journal of cardiology. 189, 188-193. https://doi.org/10.1016/j.ijcard.2015.04.086
65. Chen, J., Wang, Z., Mao, Y., Zheng, Z., Chen, Y., Khor, S., Liu, Y., 2017. Liraglutide activates autophagy via GLP-1R to improve functional recovery after spinal cord injury. Oncotarget, 8(49), 85949 –85968. 10.18632/oncotarget.20791.
66. Sharma, D.R., Wani, W.Y., Sunkaria, A., Kandimalla, R.J., Verma, D., Cameotra, S.S., 2013. Quercetin protects against chronic aluminum induced oxidative stress and ensuing biochemical, cholinergic, and neurobehavioral impairments in rats. Neurotox Res. 23(4), 336-57. https://doi.org/10.1007/s12640-012-9351-6.
67. Bugyei-Twum, A., Switzer, J., Singh, K. K., Connelly, K. A., 2017. The GLP-1 Receptor Agonist Liraglutide Improves Autophagy Impairment in the Diabetic Heart. Arteriosclerosis, Thrombosis, and Vascular Biology. 37(1), 368-368. Google Scholar.
68. Cui, Y., He, Y., Cao, W., Gao, J., Jiang, L., 2018. The Multivesicular Body and Autophagosome Pathways in Plants. Frontiers in Plant Science. 9:1837. 10.3389/fpls.2018.01837.
69. Caleb, V., Kyle, S., Dennis, M., Darcy, W., Jerome, C., Sidhartha, D.R., Siyaram, A.P., 2019. Bird's-Eye View of the Multiple Biochemical Mechanisms that Propel Pathology of Alzheimer's Disease: Recent Advances and Mechanistic Perspectives on How to Halt the Disease Progression Targeting Multiple Pathways, Journal of Alzheimer's Disease, 69, 3, 631-649. 10.3233/JAD-181230.*List of figure legends:*

Figure 1:

Effects of exenatide treatment on hippocampal mTOR [A] and beclin-1 gene experessions [B]. At $p < 0.05$ *, # indicate significant changes versus control normal , AD groups respectively (One-way ANOVA followed by Tukey post hoc test).**Figure 2:**Effects of exenatide treatment on the number of degenerated hippocampal neuronal cells . At $p < 0.05$ *, # indicate significant changes versus control normal , AD groups respectively (One-way ANOVA followed by Tukey post hoc test).

Figure 3:

Photomicrograph of a paraffin section of the hippocampus (H&E X400). A. control group showing the molecular (M), the pyramidal (P) and the polymorphic (PO) layers of CA. The pyramidal layer displays 4-5 layers neurons with vesicular nuclei. The neurons were arranged closely, had a circular shape Note the astrocytes (arrow head) and blood capillaries (arrow). **B.** Alzheimer model group showing numerous irregular destructed and shrunken pyramidal cells with condensed nuclei (arrows).**C.** Exenatide group showing few shrunken pyramidal cells (arrow) compared to aluminum model group and increase number of normal closely arranged cells (H&E X400).

Figure 4:

A photomicrograph of ultrathin EM section of the hippocampus: A. control rat showing nerve cell and neurites. The cytoplasm displays many mitochondria (arrow), ER (crossed arrow) and few lysosomes (curved arrow). Myelinated axons (thick arrow). Note the absence of autophagic vacuoles in either nerve cells or neurites (asterisk).**B.** AD model group .showing a nerve cell exhibiting many autophagic vacuoles (arrow). **C.** AD model group illustrating myelinated axons. The axoplasm shows many autophagic vacuoles (asterisk). The myelin display areas of protrusion (arrow), breakdown (crossed arrow) and blurring (thick arrow). **D.**Exenatide group showing a nerve cell note the preservation of mitochondria (arrow) and ER (crossed arrow). Few small dense lysosomes (thick arrow) and lipofuscin inclusion (curved arrow) can be seen. Note the intact myelin (arrow heads). The neurite shows small dense lysosomes (thick arrows). B. The cytoplasm in addition showed autophagolysosomes (arrow).

Hosted file

`List of tables.docx` available at https://authorea.com/users/328638/articles/455809-exenatide-stimulates-hippocampal-autophagic-activity-in-alzeheimer-rat-model

Hosted file

`List of figures.docx` available at https://authorea.com/users/328638/articles/455809-exenatide-stimulates-hippocampal-autophagic-activity-in-alzeheimer-rat-model