RESEARCH PAPER

Acta Neurobiol Exp 2018, 78: 51–59
DOI: 10.21307/ane-2018-004

Long-term behavioral, histological, biochemical and hematological evaluations of amyloid beta-induced Alzheimer's disease in rat

Raheleh Kheirbakhsh[1], Mahnaz Haddadi[2], Ahad Muhammadnejad[1], Alireza Abdollahi[3], Farshad Shahi[4], Behzad Amanpour-Gharaei[5,6], Azadeh Abrahim-Habibi[7], Tahereh Barati[1] and Saeid Amanpour[1]*

[1] Cancer Biology Research Center, Cancer research Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran, [2] Vali-e-Asr Reproductive Health research center, Tehran University of Medical Sciences, Tehran, Iran, [3] Dept. of Pathology, School of Medicine, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran, [4] Islamic Azad University, Tehran Medical Branch, Tehran, Iran, [5] Neuroimaging and Analysis Group, Research Center for Molecular and Cellular imaging, Tehran University of Medical Sciences, Tehran, Iran, [6] International Graduate School of Neuroscience, Ruhr University, Bochum, Germany, [7] Biosensor Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran,
** E-mail: Amanpour_s@tums.ac.ir*

Alzheimer's disease (AD) is a mental impairment and neural degeneration which causes progressive loss of memory and cognitive functions. This age-dependent illness is associated with extracellular amyloid plaques accumulation and twisted neurofibrillary tangles. Amyloid plaques are experimentally generated in animal models in order to investigate the disease process. In this study, we followed a rat model of AD for over a year. Wistar rats were divided randomly into two groups as control group (surgery without injection Aβ), and experimental group (two-sided intrahippocampal amyloid-beta injection into hippocampus). From each group, three animals were investigated 42 days after injection, and the remaining four animals were studied after one year. All animals were tested for learning abilities and memory. Finally, samples from blood, brain, heart, kidney, liver, colon and spleen were examined. In the experimental group, the size of amyloid plaques were increased significantly after one year and learning abilities and memory were concomitantly decreased. Onsets of various other conditions such as liver and kidney disorders, diabetes, and metabolic syndrome were observed, which indicates that the animals may be prone to cardiovascular disorders and ischemia.

Key words: Alzheimer's disease, amyloid beta, animal model, long-term evaluation

INTRODUCTION

Irreversible loss of memory and cognitive functions are associated with Alzheimer's disease (Duleu et al. 2010). The symptoms begin with short-term memory loss and evolve toward time and space confusion, depression, speech disorders (aphasia), withdrawal, mood change, mental problems, and respiratory disorders (Alzheimer's Association 2010, Rainon et al. 2016). Abnormal formation and accumulation of Aβ peptides and tau proteins in the brain are two important neuropathological features of Alzheimer's disease (AD), with microscopic features such as neuritic plaques and intracellular neurofibrillary tangles, respectively (Davies et al. 2009). These deposits formation is associated with damage to the brain blood vessels and synapses, lack of neurotransmitters, death of neurons, and loss of memory and learning problems (Frozza et al. 2009). In addition, AD reduces the brain blood flow speed (Hamel et al. 2004,

Received 9 February 2017, accepted 29 January 2018

Iadecola et al. 2004), which is due to Aβ deposit on the vessels tunica, which reduces their elasticity (Petrella et al. 2003). In animal studies, intra-hippocampal injection of Aβ has resulted in memory loss, learning problems, and cholinergic system dysfunction (Lu et al. 2009). Since 1993, numerous studies have been performed on animals in which AD was induced with beta-amyloids of different lengths and other compounds such as streptozotocin, ibotenic acid and insulin amyloid fibrils (Cleary et al. 1995, Kheirbakhsh et al. 2015, Limón et al. 2012, Shi et al. 2011, Yaghmaei et al. 2013).

Aβ is generated by the action of beta- and gamma-secretase enzymes on the amyloid precursor protein (APP). It has an oxidative activity which increases abnormal active oxygen and nitrogen, while decreasing endogen antioxidants, and causing neurons degeneration (Yan et al. 2014).

Reactive oxygen species and oxidative stress are known mediators in neurodegenerative disorders affecting the brain function, and cause lipids and proteins oxidation and cellular damage in the central nervous system. On the other hand, insulin signal pathway is important for regulating feeding behaviors and cognitive functions (Jhoo et al. 2004). Decrease of insulin levels in the brain and central nervous fluids and impairments in the insulin receptors function have been reported in AD patients (Hoyer et al. 2000). Increase in the blood sugar level, which cause oxidative stress and lipid peroxidation in some brain regions including hippocampus, is a result of disturbance in neuronal insulin signals. These facts demonstrate that there is a close relationship between AD and diabetes (Lupien et al. 2003, Sima et al. 2005).

Amyloid plaques contain other proteins in addition to Aβ, such as Apolipoprotein E (APOE) which is encoded by another AD developing gene (Duleu et al. 2010). Apolipoprotein E4 is the main genetic risk factor of AD and cause high accumulation of amyloids in the brain before AD symptoms appearance (Polvikoski et al. 1995).

In this study, we compared the amyloid plaques density in the beginning of AD, and one year after intrahippocampal injection of Aβ in animal model. Our main aim was to observe an AD model for a relatively long period in order to assess its characteristics over the long run. To this end, hematologic and blood biochemistry parameters, brain and vital organs histopathology, and memory loss were assessed during one year.

METHODS

Animals and experimental groups

The study was carried out in accordance with the European Communities Council Directive of 22 September, 2010 (2010/63/EU) for care of laboratory animals and after approval of the Local Ethic Committee of Tehran University of Medical Sciences.

Fourteen male Wistar rats (6 months old at the time of surgery, 250–300 g, from Pasteur Institute of Iran) were used in this study. Animals were housed in a pathogen- and temperature-controlled (22±2) room with a constant 12-h light/dark cycle where they had access to food and water *ad libitum*.

Animals were divided into control and Aβ-treated group with 7 animals in each group. Forty two days after surgery, 3 animals from each group were chosen randomly to examine the disorder occurrence. The remaining were tested for histology, hematology, and biochemistry characteristics after one year.

Surgery and amyloid beta injection

To 100 µg Aβ1-42 (purchased from Sigma, St Louis, MO, USA), 100 µl PBS was added and incubated for seven days at 37°C. The resulting amyloid fibrils, which are neurotoxic (Goryacheva et al. 2010) were injected bilaterally in CA1 region of rats hippocampus. Two µl of Aβ1-42 peptide was injected slowly with Hamilton syringe (Hamilton, USA) at each side with the help of a stereotaxic apparatus (SR-6N Narishig, Japan) (Calabrese et al. 2000), and at coordinates indicated in the Paxinos atlas (Paxinos et al. 2007). The used coordinates were: anterior-posterior (AP)=–4.8 mm, medial-lateral (ML)=±3.5 mm, and dorsal-ventral (DV)=–4 mm. Rats were first anesthetized by injection of ketamine (50 mg/kg, Alfasan) and xylazin (5 mg/kg, Woerden-Holland). In the control group, empty syringe was used. Animals were then given 5–7 days recovery time.

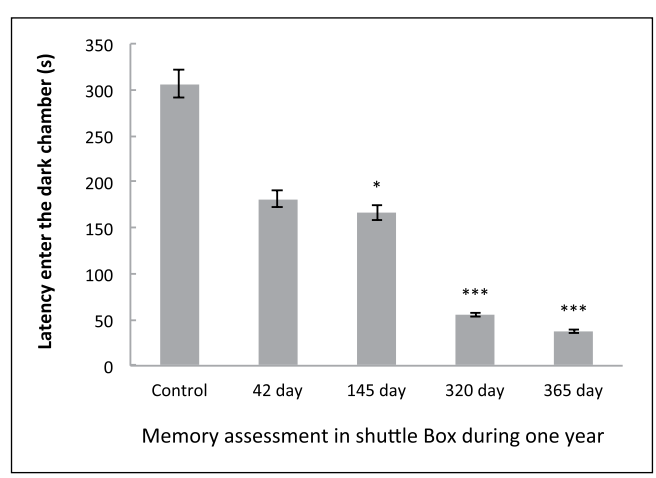

Fig. 1. Comparing delay times to enter the dark compartment after 5 min in the test day in the initial stages of AD and during a year post AD induction. *** (P<0.001) * (P<0.05) (The Control group was evaluated after 42 days).

Passive avoidance apparatus (shuttle box)

The shuttle box consisted of a light and a dark compartment of the same size (20×20×30 cm each), separated by a retractable door (7×9 cm). Behavioral measurements were carried out at 42, 145, 320, 365 days and one year after surgery. In each measurement, animals were trained one day and tested the next day (Everss et al. 1998). In training step, each animal was put in the lightened section of the box for 5 seconds. The door was opened after what the animal could enter the dark section. After entrance the door was closed immediately, and the animal was allowed to explore the dark part for 10 s. The procedure was repeated after 30 s, but the animal received electrical stimulation from the dark compartment floor for 5 s. The procedure was repeated with 2 min intervals until the animal showed a delay before entering the dark part. That delay was considered as a sign of successful learning. On the test day, electrical stimulation was not applied. Animals were positioned in the lightened section, the door was opened after 5 s, and the delay duration before entering the dark section was recorded as a criterion for memorizing. The maximum allowed delay was 300 s (Urban et al. 1995).

Histology and hematological study

365 days after Aβ injection, remaining animals were anesthetized by intraperitoneal injection of ketamine and xylazine (60 and 20 mg/kg body weight, respectively). Cardiac puncture was then performed to make a terminal blood collection for hematologic and biochemistry measurements. Vital organs such as brains, hearts, kidneys, livers, colons, and spleens were excised and kept in a solution of 10% formalin. Six µm thick sections were prepared from the samples. Brain tissues were stained using Thioflavin S (Sigma and St Louis, MO, USA) for fluorescence microscopy while other tissues were stained with haematoxylin and eosin for routine light microscopy evaluations.

Data analysis

Results were expressed as mean ±S.E.M. In order to make multiple comparisons between groups, ANOVA method followed by Tukey's *post hoc* test were used, with $p<0.05$ taken as statistically significant. In the case of insulin levels, Independent Samples t-Test was used.

RESULTS

Evaluation of the shuttle box test data

Comparison of the animals delay times to enter the dark chamber showed that the longer the disease persisted, the more impairment of learning and memory was observed. Delay time's data corresponding to days 365 and 320 following induction of AD were different

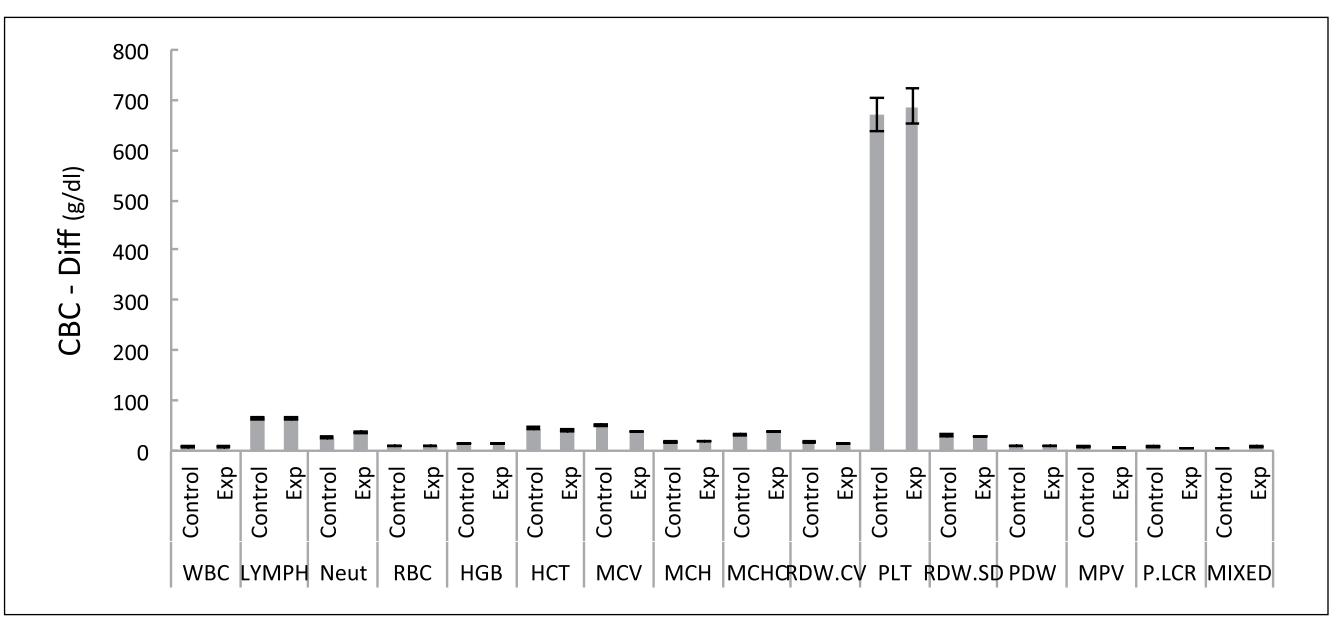

Fig. 2. Hematology parameters of the animals, one year after AD induction. For statistical analysis, Anova method followed by Tukey's *post hoc* test was used. WBC: White blood cell, LYMPH: Lymphocyte, Neut: Percentage of neutrophils, RBC: red blood cell, HGB: Hemoglobin, HCT: Compact RBC volume, MCV: The average blood cell volume, MCH: Mean weight of hemoglobin in a red blood cell, MCHC: Mean concentration of hemoglobin in red blood cells, RDW-CV: The range of red blood cell size changes, P-LCR: The ratio of large platelets, PLT: Platelet, PDW: Scale of platelet volume, MPV: Average platelet volume.

at the most significance level (p<0.001) in comparison with day 42. Data of day 365 after AD induction also demonstrated significant change in comparison to the control group (p<0.05) (Fig. 1). In other words, retention time of short-term memory is decreased during one year, which is indicative of the upward progression of the disease.

Hematology and biochemistry results

Evaluation of hematology factors and complete blood count with differential (CBC-Diff) after one year did not show any significant difference in the AD rats (Fig. 2). However, evaluation of the biochemical factors of serum indicated marked changes. According to the statistical analysis, triglyceride (TG), hepatic enzyme, aspartate aminotransferase (AST or OT1), and glucose (Glue) level demonstrated highly significant change compared to the control group (p<0.001). Alkaline phosphatase (ALP1) and alanine aminotransferase (ALT) also indicated marked differences compared to the control group (Fig. 3). Further, insulin hormone increased compared to the control group (Fig. 4).

Histology results

Comparison of the brain tissues of animals one year post AD induction with the day 42 tissues demonstrated increase in size of the amyloid plaques (Fig. 5). It should be mentioned that higher brightness of the plaques states the increased concentration of beta amyloid, and is indicative of neural pathways disturbance and exacerbation of the disease (Fig. 5). Assessment of other tissues illustrated notable disorders. In liver and kidney of animals, amorphous deposits resulting from amyloid bodies were observed (Fig. 6). In these tissues, inflammation and changes in blood vessels are observed: monocular inflammatory cells are found between hepatocytes (Fig. 6A-1), and abnormal congested blood vessels are detected (Fig. 6A-2).

DISCUSSION

The present study results demonstrated that Aβ injection in the CA1 regions of both brain's hemispheres in Wistar rats causes memory loss and amyloid plaques formation which are exacerbated over time. Aβ-treated group (experimental group) showed significant difference in learning and memory capabilities, as demonstrated by decreased entrance delay in shuttle-box test with respect to control group, both in the early days after injection and later. In addition, histological analysis confirmed the presence of amyloid plaques which is an important characteristic of Alzheimer's disease (Selkoe et al. 2016).

In 1991, Aβ deposits were hypothesized to be the main cause of AD (Hardy and Allsop 1991). The 4 kDa beta-amyloid peptide with 42–43 amino acids is derived from APP (Hardy and Allsop 1991). APP is expressed in

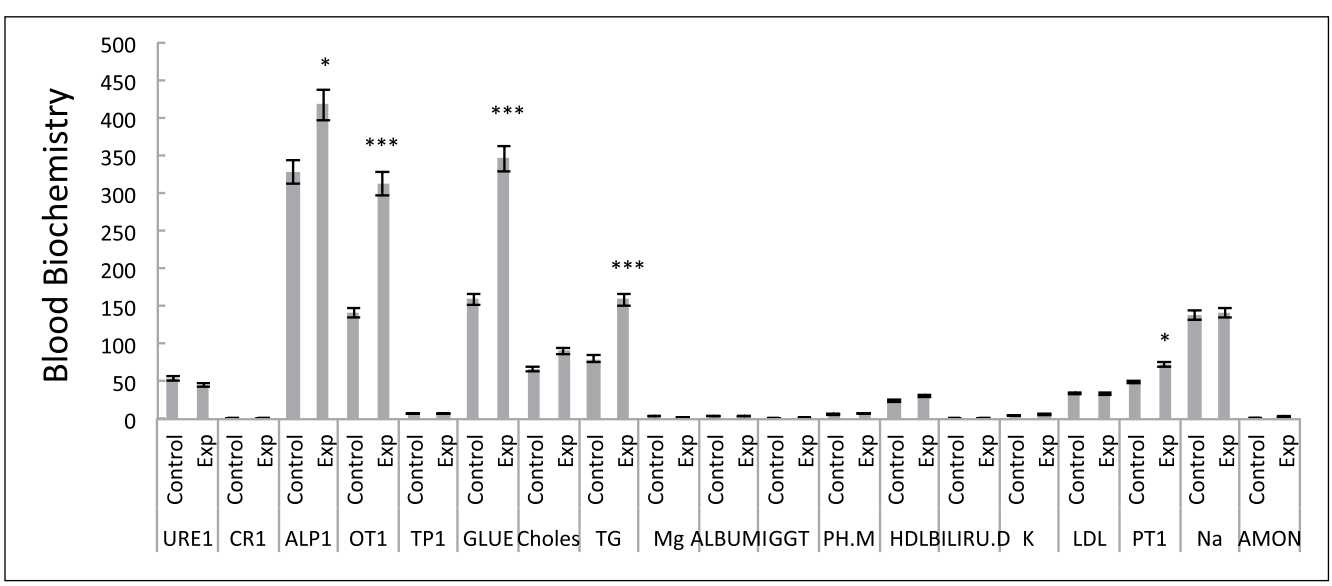

Fig. 3. Biochemical evaluation of the animals, one year after AD induction. For statistical analysis, Anova method followed by Tukey's *post hoc* test was used. URE1: Urea, CR1: Creatinine, ALP1: Alkaline phosphatase, OT1(AST): Aspartate aminotransferase, PT1(ALT): Alaninetransaminase, Glue: Glucose, Choles: Cholesterol, TG: Triglyceride, Mg: Magnesio, AlBumio: Albomin, GGT: Gamma-Glutamyl Transferase, TP1: Total protein, BIlRu.D: Bilirubina Direct, K: Potassium, Amon: Ammonia, HDL and LDL: High and Low-density lipoprotein.

nervous system cells and involved in cytoskeleton, extracellular matrix, and cellular connectivity (Gandy et al. 2005). However, Aβ protein deposits cause inflammation, oxidative reactions, and neurodegeneration in the brain cortex and hippocampus (Stanga et al. 2016). Aβ activates microglia and astrocytes, causing reactive oxygen generation and nitric oxide production resulting into neurons death (Vassar et al. 1999). Aβ 1-42 injection into brain gives rise to noticeable memory impairment (Delgado et al. 2005), and is accompanied by hippocampal neurodegenerative alterations (Ceccom et al. 2012). In fact, memory impairment may start from hippocampus which is involved in learning (Choi et al. 2011, Ting et al. 2007). Overall, oxidative reactions, inflammation, and neurodegeneration in the hippocampus and the cortex are considered to cause AD symptoms.

Accumulation of amyloids increases the distance between neurons and interferes with connections (Frozza et al. 2009), which could be related with our observa-

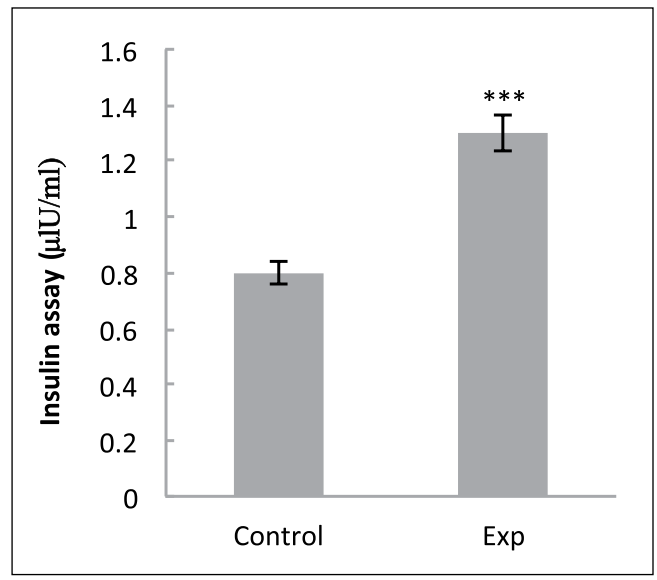

Fig. 4. Evaluating blood insulin of the AD animals, one year post AD induction. For statistical analysis, Independent Samples t-Test was used.

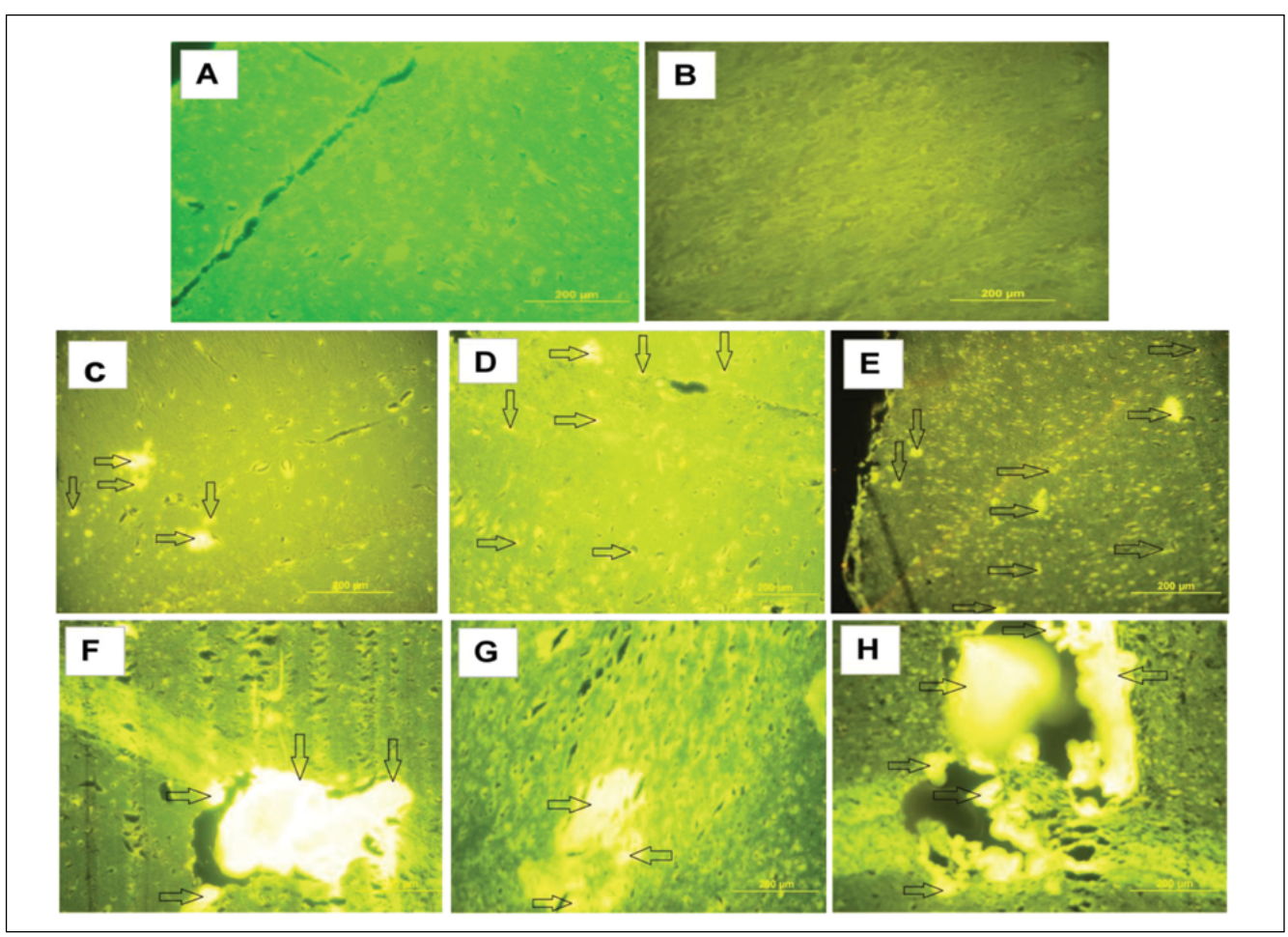

Fig. 5. Comparing brain section of control group, AD animals following 42 days and 365 days post AD induction. Amyloid plaques are indicated by arrows. A and B: control groups. C, D and E: the animal brain tissue 42 days post AD induction and F, G and H, brain tissues 365 days post AD induction. Hippocampus area is shown with×40 magnification e.

tions in both behavioral experiment, and histological tests performed one year after Aβ injection. Testing memory at days 120 and 365 shows a marked memory impairment compared with previous conducted tests; this downtrend of the graph columns is indicative of increasing memory deterioration associated with AD. Moreover, comparison of the images C, D, and E (42 days post AD induction) with images F, G and H (365 days post AD induction) illustrates considerable differences in the size of amyloid plaques and show increased neuronal necrosis (Fig. 5).

Besides direct damages in the brain, evaluation of blood and other tissues 365 days post AD induction demonstrated the occurrence of peripheral complications. Glucose, TG, insulin and OT1 levels of the AD group clearly shows a significant change in comparison with the control group. The liver enzymes ALP1 and PT1 have also significantly changed which is suggestive of other tissues degeneration alongside with brain damage.

Various conditions such as diabetes, mid-life obesity and vascular events are considered to increase the risk of AD occurrence (Fitzpatrick et al. 2009, Janson et al. 2004). Hence, factors such as elevated blood glucose levels or dyslipidemia may be considered as causative parameters in the disease. On the other hand, brain function damage may also lead to complications in other organs. The idea that AD may actually be a systemic disease has been discussed at length in a recent review, where the authors point out the "chicken or egg" problem when dealing with AD and the "whole body changes" that occur (Morris et al. 2014). Our results seem to be more in accordance with a causal effect of AD itself, which may somehow spread from the brain toward other organs. More exactly, what we see is a subsequent peripheral effect of amyloid plaques initially injected in the brain.

Further studies are needed to characterize the agents or pathways that are involved in this process. Based on previous studies, the role of oxidative stress is extremely important in AD (Tönnies and Trushina 2017).

In healthy condition, there is an equilibrium between active oxygen-nitrogen species and anti-oxygenation defense. When this equilibrated state is disturbed, oxidative stress occurs which impairs biological macromolecules such as extra- and intra-cellular proteins, and cell membrane phospholipids. Oxidative stress is involved in many pathologies such as cardiovascular diseases, dia-

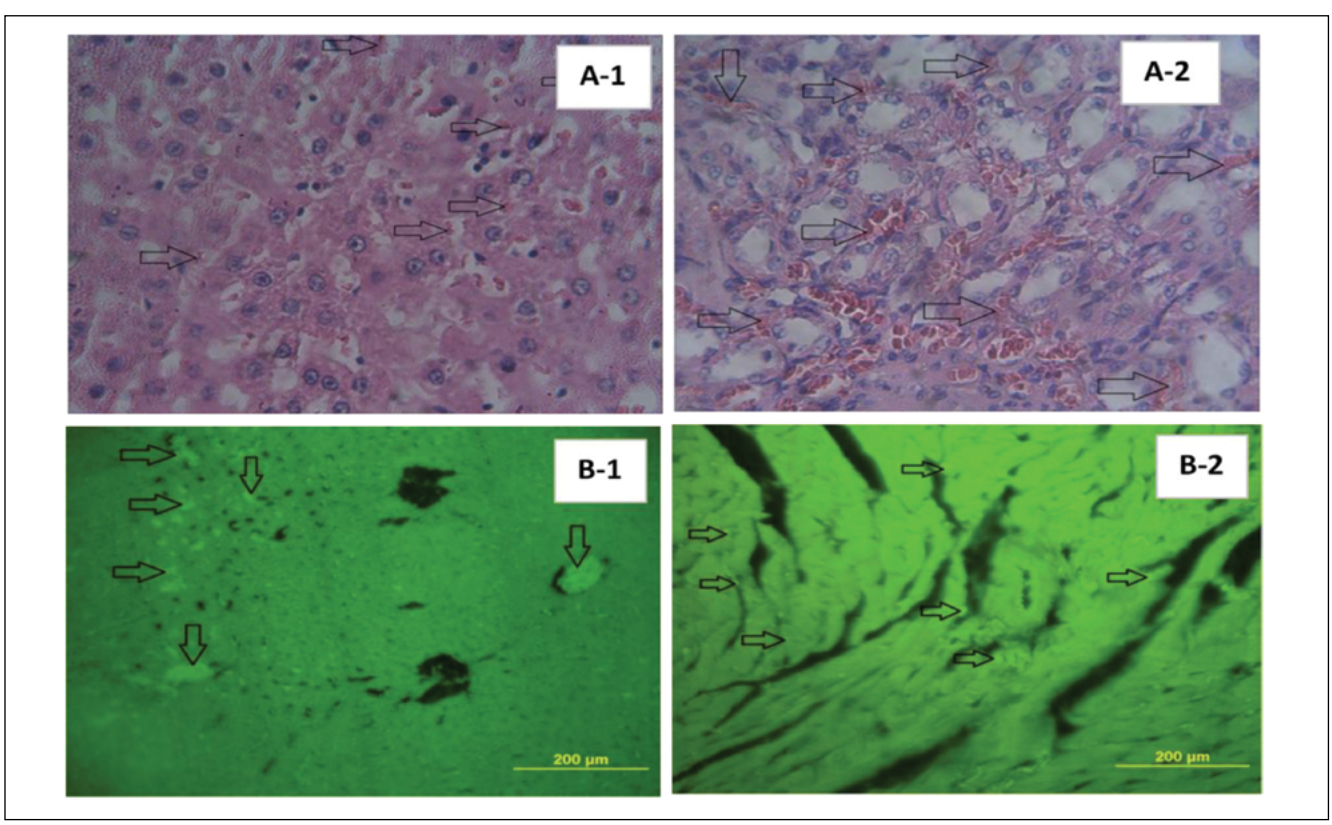

Fig. 6. Comparison of animals liver (1) and kidney (2) tissues with control group one year after Aβ injection. (A) optical microscopy; tip of the arrows indicates necrotic lesions in the tissue. (A-1) see monocular inflammatory cells, (A-2) see congested blood vessels. (B) Fluorescence microscopy, arrows tips indicate amyloid deposits in the tissue.

betes, AD, and Parkinson (Afshari et al. 2007, Duleu et al. 2010). Lipid peroxidation, which is caused by free radicals and changes the chemical characteristics, structure, and function of cell membranes plays also a role in neurodegenerative diseases (Kota et al. 2008).

Amyloid angiopathy may also occur in AD, resulting into brain blood circulation decrease (Hamel et al. 2004, Iadecola et al. 2004). Morphologic lesions related to beta amyloid accumulation in the brain vessels lead to decreased vasodilatation (Petrella et al. 2003). Damage to the brain vessels is followed by impairment of intracellular oxidative metabolism and consequent reduction in glucose, neurotransmitters, calcium and phosphate (Sultana et al. 2006). Ischemia and vascular obstruction result into a ten-fold increase of free radical production in the extracellular space of the hippocampal system and the basal ganglia (Magenta et al. 2013). The produced free radicals damage neuronal membrane and cause brain edema due to unsaturated fatty acids peroxidation in the membrane phospholipids; ultimately, neuronal death happens (Fujita et al. 2013).

In addition, a significant drop in brain glucose usage has also been reported in AD. This abnormality occurs due to dysfunction of insulin neuronal signals. Decreased insulin concentration and functional perturbation of insulin receptors were observed in postmortem examination of the brain and cerebrospinal fluid of AD patients (O'Connell et al. 2013). Even though glucose transportation into the brain is not insulin dependent, insulin passage through blood-brain barrier is linked to insulin receptors. Insulin receptors and insulin-like factors are present in neurons and astrocytes, and hippocampus and hypothalamus which play main roles in learning and memory present the highest insulin receptor density in the brain (Hoyer et al. 2000). In insulin-resistant individuals, the cells do not respond in the normal way to insulin and glucose cannot easily enter the cells. As a consequence, more and more insulin has to be secreted to assist glucose transportation into cells, resulting into insulinemia. Finally, when the body is no longer able to produce enough insulin to maintain normal glucose levels, diabetic state occurs (Baker et al. 2011).

In the present study, increased amounts of both glucose and insulin in the blood are indicative of insulin resistance in the brain cells. Glucose is the main energy source of the brain and its normal metabolism is necessary for accurate brain function and preserving cell energy as ATP molecules. A decrease in glucose levels leads to problems in cognitive functions and affects the process of amyloid precursor proteins production which results into higher accumulation of beta amyloid plaques (Rafacho et al. 2014).

Alongside with cholesterol, TGs constitute the plasma lipids. Elevated levels of blood TG, even in the absence of other risk factors, are directly linked with higher probability of cardiovascular pathologies, nephropathies and pancreatitis (Wenk et al. 1989). Increased blood TG is known to be one of main causes of metabolic syndrome. Other key pathologic factors include increased insulin level in the blood (Clearfield et al. 2014, Nordestgaard and Varbo 2014), hypertension, glucose tolerance disorder, abdominal obesity (Yamaoka et al. 2014) and increased blood lipids. Besides two main health problems, namely diabetes and cardiovascular diseases, metabolic syndrome has numerous other consequences such as liver steatosis, cirrhosis, chronic kidney failure, albuminuria, hyperuricemia and gout, polycystic ovary syndrome, sleep apnea, cognitive disorder and dementia (Dietrich et al. 2014, Parapid et al. 2014).

Considerable increases were observed in the liver enzymes of the animal model of AD. The most sensitive and commonly used diagnostic liver enzymes are aminotransferases AST, OT1, and ALT (PT1) which are released into blood circulation upon liver damage. Consequently, elevation of these enzymes is indicative of widespread death of hepatocytes (hepatic necrosis)which is also observed in many inflammatory diseases (Choi et al. 2007, Raurich et al. 2015). High levels of aminotransferases have been reported in 90% of ischemic or toxic hepatic damages (Giboney 2005). A study indicated that in 98% of alcoholic hepatitis patients, blood AST level increased 6–7 times compared to normal levels (Dufour et al. 2000). Moreover, patients with 10-fold increase in their enzyme levels have been diagnosed with acute extrahepatic bile duct obstruction (Karim et al. 2015). Increased ALT and AST levels have been observed in 79% of hepatic cirrhosis (Ferenci et al. 2003).

ALP1 is another enzyme which has increased in the AD model compared with healthy rats. It is a nonspecific enzyme with a widespread production in all tissues. High level of ALP1 is observed in kidney tubules and urologic disorders. Its levels rises in bone growth, malignancies, fracture repair, hepatic diseases, heart failure, over-activity of parathyroid gland and many other diseases (Lucyk et al. 2015). Studies have shown that high rates of ALP1 play an important role in urologic disorders and prostate malignancies (Kamali et al. 2010). In patients with prostate malignancy, high serum level of ALP1 is attributed to intensified bone metabolism which is indicative of metastasis to the bones (Lehmann et al. 2004). High ALP1 rate has been reported as a sensitive indicator of tubular damages in patients with inflammatory bowel disease (IBD) who suffer also from kidney failure (Krones et al. 2015). Moreover, studies have demonstrated that hepatic damage resulting from medicines consumption can emerge

with a cholestatic pattern or pathologic increase in ALP1 (Ghaznavi et al. 2005). Further, pathologic ALP1 levels can also be related to metastatic lymphoma and liver cancers (Oorts et al. 2016, Pratt and Kaplan 2000).

In conclusion, one year after Aβ injection in the CA1 regions of both hemispheres in Wistar rats, memory loss and Alzheimer's plaques formation were seen. Other progressive pathologic events and disorders occurred in parallel with increased learning and memory impairments in liver and kidney of test animals. Based on the current results, AD state may be the cause of these peripheral disorders, which would be of importance in AD patients' condition.

REFERENCES

Afshari AT, Shirpoor A, Farshid A, Saadatian R, Rasmi Y, Saboory E (2007) The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food Chemistry 101: 148–153.

Alzheimer's Association. (2010) 2010 Alzheimer's disease facts and figures. Alzheimers Dement 6: 158–194.

Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S (2011) Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. Arch Neurol 68: 51–57.

Calabrese V, Bates TE, Stella AMG (2000) NO synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: the role of oxidant/antioxidant balance. Neurochem Res 25: 1315–1341.

Ceccom J, Coslédan F, Halley H, Francès B, Lassalle JM, Meunier B (2012) Copper chelator induced efficient episodic memory recovery in a non-transgenic Alzheimer's mouse model. PLoS One 7: e43105.

Choi HK, Ford ES, Li C, Curhan G (2007) Prevalence of the metabolic syndrome in patients with gout: the Third National Health and Nutrition Examination Survey. Arthritis Rheum 57: 109–115.

Choi JG, Moon M, Kim HG, Mook-Jung I, Chung SY, Kang TH, Kim SY, Lee EH, Oh MS (2011) Gami-Chunghyuldan ameliorates memory impairment and neurodegeneration induced by intrahippocampal Aβ 1–42 oligomer injection. Neurobiol Learn Mem 96: 306–314.

Clearfield M, Pearce M, Nibbe Y, Crotty D, Wagner A (2014) The "New Deadly Quartet" for Cardiovascular Disease in the 21st Century: Obesity, Metabolic Syndrome, Inflammation and Climate Change: How Does Statin Therapy Fit into this Equation? Curr Atheroscler Rep 16: 1–11.

Cleary J, Hittner J, Semotuk M, Mantyh P, O'Hare E (1995) Beta-amyloid (1–40) effects on behavior and memory. Brain Res 682: 69–74.

Davies P, Koppel J (2009) Mechanism-based treatments for Alzheimer's disease. Dialogues Clin Neurosci 11: 159.

Delgado JY, O'Dell TJ (2005) Long-term potentiation persists in an occult state following mGluR-dependent depotentiation. Int J Neuropharmacol 48: 936–48.

Dietrich P, Hellerbrand C (2014) Non-alcoholic fatty liver disease, obesity and the metabolic syndrome. Best Pract Res Clin Gastroenterol 28: 637–653.

Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB (2000) Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. Clin Chem 46: 2027–2049.

Duleu S, Mangas A, Sevin F, Veyret B, Bessede A, Geffard M (2010) Circulating antibodies to IDO/THO pathway metabolites in Alzheimer's disease. Int J Alzheimers Dis: 501541.

Everss E, Parra A (1998) Inhibitory avoidance with a two-way shuttle-box. Psicothema 10: 387–391.

Ferenci P, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F (2003) Diagnosis and phenotypic classification of Wilson disease. Liver Int 23: 139–142.

Fitzpatrick AL, Kuller LH, Lopez OL, Diehr P, O'Meara ES, Longstreth WT Jr, Luchsinger JA (2009) Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Arch Neurol 66: 336–342.

Frozza RL, Horn AP, Hoppe JB, Simão F, Gerhardt D, Comiran RA, Salbego CG (2009) A comparative study of β-amyloid peptides Aβ1-42 and Aβ25-35 toxicity in organotypic hippocampal slice cultures. Neurochem Res 34: 295–303.

Fujita K1, Yoshimoto N, Kato T, Imada H, Matsumoto G, Inakuma T, Nagata Y, Miyachi E (2013) Lycopene inhibits ischemia/reperfusion-induced neuronal apoptosis in gerbil hippocampal tissue. Neurochem Res 38: 461–469.

Gandy S (2005) The role of cerebral amyloid β accumulation in common forms of Alzheimer disease. J Clin Invest 115: 1121–1129.

Ghaznavi R, Faghihi M, Kadkhodaee M, Shams S, Khastar H (2005) Effects of nitric oxide on gentamicin toxicity in isolated perfused rat kidneys. J Nephrol 18: 548.

Giboney PT (2005) Mildly elevated liver transaminase levels in the asymptomatic patient. Am Fam Physician 71: 1105–1110.

Goryacheva AV, Kruglov SV, Pshennikova MG, Smirin BV, Malyshev IY, Barskov IV, Manukhina EB (2010) Adaptation to intermittent hypoxia restricts nitric oxide overproduction and prevents beta-amyloid toxicity in rat brain. Nitric Oxide 23: 289–299.

Hamel E (2004) Cholinergic modulation of the cortical microvascular bed. Prog Brain Res 145: 171–178.

Hardy J, Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci 12: 383–388.

Hoyer S, Lee SK, Löffler T, Schliebs R (2000) Inhibition of the neuronal insulin receptor An in vivo model for sporadic Alzheimer disease? Ann NY Acad Sci 920: 256–258.

Iadecola C (2004) Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat Rev Neurosci 5: 347–360.

Janson J, Laedtke T, Parisi JE, O'Brien P, Petersen RC, Butler PC (2004) Increased risk of type 2 diabetes in Alzheimer disease. Diabetes 53: 474–481.

Jhoo JH, Kim HC, Nabeshima T, Yamada K, Shin EJ, Jhoo WK, Woo JI (2004) β-Amyloid (1-42)-induced learning and memory deficits in mice: involvement of oxidative burdens in the hippocampus and cerebral cortex. Behav Brain Res 155: 185–196.

Kamali K, Fereshtehnejad SM, Rahmanian M, Gohardehi G, Shafiei Sabet, Kamali N (2010) Evaluation of the Association between Serum Level of Alkaline Phosphatase, White Blood Cells and Tumor Grade in Transitional Cell Carcinoma (TCC) of the Ureter. RJMS 17: 43–51.

Karim SMF, Rahman MR, Shermin S, Sultana R (2015) Correlation between Aminotransferase Ratio (AST/ALT) and Other Biochemical Parameters in Chronic Liver Disease of Viral Origin. Delta Med Col J 3: 13–17.

Kheirbakhsh R, Chinisaz M, Khodayari S, Amanpour S, Dehpour AR, Muhammadnejad A, Ebrahim-Habibi A (2015) Injection of insulin amyloid fibrils in the hippocampus of male Wistar rats: report on memory impairment and formation of amyloid plaques. Neurol Sci 36: 1411–1416.

Kota N, Krishna P, Polasa K (2008) Alterations in antioxidant status of rats following intake of ginger through diet. Food Chem 106: 991–996.

Krones E, Erwa W, Trauner M, Fickert P (2015) Serum alkaline phosphatase levels accurately reflect cholestasis in mice. Hepatology, 62: 981–983.

Lehmann J, Retz M, Nürnberg N, Schnöckel U, Raffenberg U, Krams M, Stöckle M (2004) The superior prognostic value of humoral factors compared with molecular proliferation markers in renal cell carcinoma. Cancer 101: 1552–1562.

Limón D, Díaz A, Hernandez M, Fernandez-G JM, Torres-Martínez AC, Pérez-Severiano F, Rendón-Huerta EP, Montaño LF, Guevara J (2012) Neuroprotective effect of the aminoestrogen prolame against impair-

ment of learning and memory skills in rats injected with amyloid-β-25–35 into the hippocampus. Eur J Pharmacol 685: 74–80.

Lu P, Mamiya T, Lu LL, Mouri A, Zou LB, Nagai T, Nabeshima T (2009) Silibinin prevents amyloid β peptide-induced memory impairment and oxidative stress in mice. Br J Pharmacol 157: 1270–1277.

Lucyk SN, Hoffman RS, Nelson LS, Fuentes M, Tavangarian K (2015) Challenges with AST/ALT ratio in acetaminophen poisoning. Clin Toxicol, 53: 786–786.

Lupien SB, Bluhm EJ, Ishii DN (2003) Systemic insulin-like growth factor-I administration prevents cognitive impairment in diabetic rats, and brain IGF regulates learning/memory in normal adult rats. J Neurosci Res 74: 512–523.

Magenta A, Greco S, Gaetano C, Martelli F (2013) Oxidative stress and microRNAs in vascular diseases. Int J Mol Sci 14: 17319–17346.

Morris JK, Honea RA, Vidoni ED, Swerdlow RH, Burns JM (2014) Is Alzheimer's disease a systemic disease? Biochim Biophys Acta 1842: 1340–1349.

Nordestgaard BG, Varbo A (2014) Triglycerides and cardiovascular disease. Lancet 384: 626–635.

O'Connell KM, Littleton-Kearney MT (2013) The role of free radicals in traumatic brain injury. Biol Res Nurs 15: 253–263.

Oorts M, Baz A, Bachellier P, Heyd B, Zacharias T, Annaert P, Richert L (2016) Drug-induced cholestasis risk assessment in sandwich-cultured human hepatocytes. Toxicol in Vitro 34: 179–186.

Parapid B, Ostojic MC, Lalic NM, Micic D, Damjanovic S, Bubanja D, Milic NM (2014) Risk factors clustering within the metabolic syndrome: a pattern or by chance. Hellenic J Cardiol 55: 92–100.

Paxinos G, Franklin KB (2004) The mouse brain in stereotaxic coordinates. Gulf Professional Publishing.

Petrella JR, Coleman RE, Doraiswamy PM (2003) Neuroimaging and Early Diagnosis of Alzheimer Disease: A Look to the Future 1. Radiology 226: 315–336.

Polvikoski T, Sulkava R, Haltia M, Kainulai K, Vuorio A, Verkkoniemi A, Kontula K (1995) Apolipoprotein E, dementia, and cortical deposition of β-amyloid protein. N Engl J Med 333: 1242–1248.

Pratt DS, Kaplan MM (2000) Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 342: 1266–1271.

Rafacho A, Ortsäter H, Nadal A, Quesada I (2014) Glucocorticoid treatment and endocrine pancreas function: implications for glucose homeostasis, insulin resistance and diabetes. J Endocrinol 223: R49-R62.

Rainon TM, Cifuentes D, Kubis N, Lévy B (2016) Risque cardiovasculaire et maladie d'Alzheimer: rôle de l'hypertension artérielle. De l'épidémiologie aux modèles animaux. Sang Thromb Vaiss 26: 12–18.

Raurich JM, Llompart-Pou JA, Rodríguez-Yago M, Ferreruela M, Royo C, Ayestarán I (2015) Role of Elevated Aminotransferases in ICU Patients with Rhabdomyolysis. Am Surg 81: 1209–1215.

Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med 8: 595–608.

Shi X, Lu X, Zhan L, Liu L, Sun M, Gong X, Chen J (2011) Rat hippocampal proteomic alterations following intrahippocampal injection of amyloid beta peptide (1–40). Neurosci Lett 500: 87–91.

Sima AA, Li ZG (2005) The effect of C-peptide on cognitive dysfunction and hippocampal apoptosis in type 1 diabetic rats. Diabetes 54: 1497–1505.

Stanga S, Zanou N, Audouard E, Tasiaux B, Contino S, Vandermeulen G, Dewachter I (2016) APP-dependent glial cell line-derived neurotrophic factor gene expression drives neuromuscular junction formation. FASEB J 30: 1696–1711.

Sultana R, Perluigi M, Butterfield DA (2006) Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of neurodegeneration from redox proteomics. Antioxid Redox Signal 8: 2021–2037.

Ting KK, Brew BJ, Guillemin GJ (2007) Effect of quinolinic acid on gene expression in human astrocytes: implications for Alzheimer's disease. In: International Congress Series (Vol. 1304, pp. 384–388).

Tönnies E, Trushina E (2017) Oxidative stress, synaptic dysfunction, and Alzheimer's disease. J Alzheimers Dis 57: 1105–1121.

Urban IJA, Ontskul A, Croiset G, Cheng Y, De Wied D (1995) A long-lasting increase and decrease in synaptic excitability in the rat lateral septum are associated with high and low shuttle box performance, respectively. Behav Brain Res 68: 173–183.

Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Luo Y (1999) β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286: 735–741.

Wenk GL (1989) An hypothesis on the role of glucose in the mechanism of action of cognitive enhancers. Psychopharmacol 99: 431–438.

Yaghmaei P, Kheirbakhsh R, Dezfulian M, Haeri-Rohani A, Larijani B, Ebrahim-Habibi A (2013) Indole and trans-chalcone attenuate amyloid β plaque accumulation in male Wistar rat: in vivo effectiveness of two anti-amyloid scaffolds. Arch Ital Biol 151: 106–113.

Yamaoka M, Maeda N, Takayama Y, Sekimoto R, Tsushima Y, Matsuda K, Funahashi T (2014) Adipose hypothermia in obesity and its association with period homolog 1, insulin sensitivity, and inflammation in fat. PloS One 9: e112813.

Yan R, Vassar R (2014) Targeting the β secretase BACE1 for Alzheimer's disease therapy. Lancet Neurol 13: 319–329.