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Evaluation of Caspase-3 mRNA Gene Expression Activity in Amyloid Beta-induced Alzheimer's Disease Rats

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Caspase-3 plays a role in cell death in experimental models of several acute and chronic neurodegenerative disorders, caspase-3 activation has been proposed as an early neurodegenerative event in the progression of Alzheimer disease (AD). Alzheimer disease (AD) is a progressive neurodegenerative disease of the central nervous system characterized by neuropathological deposition of amyloid beta (Aβ) peptides, with the function as an extracellular signal molecule for caspase-3 activation in AD. Amyloid beta (Aβ) peptide is widely believed to play a central and etiological role in Alzheimer disease (AD). The overproduction of Aβ in the brain is a primary cause of AD and various research activities are conducted for inhibition of Aβ generation has become a hot topic in AD research. Therefore, in this study tried to make AD modeling through induction using Aβ, it is expected to increase the activity of caspase-3 mRNA which is the early sign of Alzheimer's disease. Hence, the aim of this study was to evaluate caspase-3 mRNA levels in the wistar rat induced of Aβ. Twenty wistar rats were randomized into five groups K0: Without AD induction, K1: AD induction, K2: AD induction, K3: AD induction and K4: AD induction. Alzheimer's disease (AD) induction was performed by $A\beta_{1-42}$ (0.2 μg) injection at the intracerebroventricullary area. The mRNA Caspase-3 level measurements were performed by RT-PCR. The data caspase-3 mRNA gene expression was statistically analyzed by one-way ANOVA followed by Tukey's test. Paired t-test analysis showed no significant differences of caspase-3 mRNA level before induction among 5 groups ($p>0.05$). At 6 weeks post-induction, there was significant increased caspase mRNA in all groups except K0 ($p<0.05$). Increased caspase-3 mRNA gene expression in amyloid beta-induced AD rats in all groups except K0 ($p<0.05$).

Key words: Alzheimer's disease (AD), intracerebroventricullary (ICV), caspase-3, amyloid beta (Aβ), wistar rat

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INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that was characterized by senile plaques, neurofibrillary tangles and neuronal loss[1,2]. Alzheimer's disease was a leading cause of dementia in the aging population[3]. Patients with AD experience symptoms including cognitive alterations, memory loss and behavioral changes[4,5]. Pathologically, a frequent characteristic of these diseases was the accumulation and aggregation of abnormal or misfolded proteins, as with amyloid-β (Aβ)[6,7]. The disease manifests usually after the age of 65 years. The frequency of AD in this age group was 1-3% and doubles every 5 years, exceeding 40% in 85 years old group. It was estimated that the number of patients suffering from AD was 21-24 million and this number will double in next 15 years. The disease occurs approximately two times more often in female than male[8]. Alzheimer's disease (AD) was estimated to have cost the world $604 billion in 2010 alone[9]. These costs are staggering, particularly in light of predictions that the worldwide number of AD cases, currently estimated at 36 million, will triple by 2050[9]. A common feature of AD is the accumulation of β-amyloid (Aβ), 39- to 43-amino acid peptides derived from the amyloid precursor protein (APP). The β-amyloid (Aβ) peptides aggregate to form fibrillar deposits that are the principal component of senile plaques [2]. In AD it has been hypothesised that β-amyloid spreads in prion-like manner giving different pathological stages of β-amyloid deposition[10]. The importance of Aβ in the pathogenesis of AD was suggested by several findings. Notably, mutations in APP or presenilin, two proteins that are implicated in familial forms of AD, lead to an increase in the amyloidogenic form of Aβ[2]. Increased Aβ level was consistently observed in animal models of AD, it has been shown that a single dose $A\beta_{1-42}$ injection into the wistar rat could induce increased of Aβ level[11]. The neurodegenerative process in AD have recently been shown to be accompanied by alterations in neurogenesis[12], to understand neurodegenerative events it is worth understanding the state of neurogenesis. Neurogenesis is the process of generating new nerve cell, including neurons, astrocytes, glia and others. Neuroplasticity refers to the ability of the brain and the central nervous system to adapt to environmental changes, response to injury and to acquire novel information by modifying neural connectivity and function[13]. The pathologic brain features described in 1907 by Dr Alois Alzheimer-namely senile plaques and neurofibrillary tangles-still serve as the hallmarks for final diagnosis of the alzheimer's disease. Landmark research since the 1980s has established that plaques are composed mainly of extracellular deposits of β-amyloid peptides, most of which contain 38-43 amino acids[14]. Several factors contribute to the progression of the Alzheimer's disease including amyloid beta (Aβ) accumulation, neurofibrillary tangle formation, cholinergic deficit, oxidative stress, neuroinflammation and apoptosis[15]. Apoptosis is a cell death program that is central to cellular and tissue homeostasis and is involved in many physiological and pathological processes[16]. Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents[17]. Apoptosis is characterized morphologically by a series of events that include cytoplasmic shrinkage, chromatin condensation, nuclear and cellular fragmentation and the formation of apoptotic bodies. The role of apoptosis in normal physiology is as significant as that of its counterpart, mitosis. It demonstrates a complementary but opposite role to mitosis and cell proliferation in the regulation of various cell populations. It was estimated that to maintain homeostasis in the adult human body, around 10 billion cells are made each day just to balance those dying by apoptosis[18]. The number can increase significantly when there was increased apoptosis during normal development and aging or during disease[19]. There are two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway. However, there was now evidence that the two pathways are linked and that molecules in one pathway can influence the other[19]. The intrinsic apoptotic signaling is most often induced by intracellular damage that leads to mitochondrial release of cytochrome c and the activation of intracellular cysteine proteases called caspases[20]. Extrinsic apoptotic signaling was initiated by stimulation of plasma membrane death receptors that initiate apoptosis by activation of caspase-8 and subsequent apoptotic signaling can proceed through the mitochondrial pathway or independently of mitochondria by caspase-8-mediated direct activation of caspase-3[21]. Although, caspase are the main players involved in apoptosis, there are other molecules involved in the progression of the apoptotic cascade that are relevant to AD. The neuronal death in AD may result directly and/or indirectly from the triggering insults caused by Aβ toxicity, glutamate excitotoxicity, long-lasting oxidative stress, DNA damage and elevation of intracellular calcium levels. Thus, the mode of cell death in AD remains a matter of controversy and it was possible that both apoptotic and non-apoptotic cell death coexist in the brains of affected patients[16]. The evidence that Aβ accumulation was a determining factor in AD makes it important to determine the mechanism by which Aβ induces neuronal cell death. Recent studies have shown that in AD brains and in cultures of neurons exposed to Aβ, the dying cells display the characteristics of apoptosis[22-24]. β-amyloid first accumulates in the basal temporal and orbitofrontal neocortex, then spreads throughout the neocortex and then to the hippocampi, amygdala, diencephalon and basal ganglia. Then finally, in severe cases, this spreads to the mesencephalon, lower brainstem and cerebellar cortex[10,25], how this pathological spread interacts with clinical phenotypic networks remains to be elucidated. Neuronal death in a variety of neurodegenerative diseases, including Alzheimer's disease

(AD), has been associated with deregulated caspase activation[26]. Caspases are a family of cysteine proteases that cleave their substrates after aspartic residues. They are usually synthesized as inactive zymogens that are proteolytically cleaved into subunits at the onset of apoptosis and function as active caspases after reconstitution to molecular heterodimers[27]. Caspases are divided into long prodomain caspases (caspases-2, -8, -9 and -10), which are initiators of apoptosis and short prodomain caspases (caspases-3, -6, -7 and -14), which are generally termed the effectors of apoptosis. However, some caspases, including caspase-3 (Casp3) and caspase-6 (Casp6), appear to function as both initiators and effectors. Aberrant activation of caspases has been implicated in several neurodegenerative diseases, such as AD, HD, various ataxias and amyotrophic lateral sclerosis[28]. Apoptotic injury during Alzheimer's disease may require caspase-mediated pathways. A strong body of evidence supports the premise that caspase activation is involved in the pathological process of Alzheimer's disease. The elevation of caspase genes including caspases 1, 2, 3, 5, 6, 7, 8 and 9 has been observed in human postmortem brains from Alzheimer's disease patients[27]. However, several lines of evidence suggest that the role of caspases in AD may involve more than just action as cellular executioners driven by upstream disease processes. Caspase-mediated cleavage of β-amyloid precursor protein (APP) has been reported[29], as has caspase activation by amyloid-β peptide[30]. In one murine AD model, caspase activation associated with disease onset occurred earlier than the induction of neuronal apoptosis[31]. Similarly, caspase activation has been noted before the development of neurofibrillary tangles of Tau in the brain of tau transgenic mice[32]. In the brains of Alzheimer's patients, single neurons with DNA fragmentation have been shown to contain cytoplasmic immunoreactivity for active caspase 3, implying that apoptotic injury results during Alzheimer's disease. Caspase-3 acts as a caspase executor in the apoptosis process[33]. In addition, activation of caspase 3 was found to occur in the parahippocampal gyrus in brains from patients with mild forms of Alzheimer's disease. Caspase 3 immunoreactivity was also co-localized with paired helical filaments in neurons, suggesting that caspase 3 activation may contribute to the formation of neurofibrillary tangles[27]. Increased levels caspase-3 and Aβ were found in neurodegenerative diseases, including Alzheimer's Disease (AD)[26]. Previous studies found that expression levels of Bcl-2 family proteins, such as Bax, Bak, Bad, Bcl-2, Bim, Bcl-w and Bcl-x are altered in the vulnerable neurons in AD[34]. That in cortical neurons exposed to Aβ, activated c-Jun N-terminal kinase (JNK) is required for the phosphorylation and activation of the c-Jun transcription factor, which in turn stimulates the transcription of several key target genes, including the death inducer Fas ligand. The binding of Fas ligand to its receptor Fas then induces a cascade of events that lead to caspase activation and ultimately cell death. By analyzing the effects of mutations in each of the components of the JNK-c-Jun-Fas ligand-Fas pathway, we demonstrate that this pathway plays a critical role in mediating Aβ-induced death of cultured neurons. The possibility that the JNK pathway may also contribute to Aβ-dependent death in AD patients[35].

In this study, caspase-3 mRNA gene expression in an AD rat model induced by injection of Aβ for 6 weeks was evaluated. The purpose of this study was to evaluate the effect of the caspase-3 mRNA gene expression in the wistar rats model of Alzheimer's disease, with the hypothesis that $A\beta_{1-42}$ of 0.2 µg injected in ICV for 6 weeks can increase the expression of the caspase-3 mRNA gene in wistar rats.

MATERIALS AND METHODS

Experimental procedures were carried out in the Molecular Microbiology and Immunology Laboratory, Faculty of Medicine Hasanuddin University Makassar Indonesia. This research was an experimental study *in vivo* pre- and post-design that was conducted in the period from February to July, 2016. The experiment has been approved by the Medical and Health Research Ethics Committee Faculty of Medicine Hasanuddin University Makassar Indonesia (Number: 391/H4.8.4.5.31/PP36-KOMTIK/2016).

Animals

Animal Preparation: Twenty wistar rats (2.5-3 months, 150-250 g) were selected from central animal house (Faculty of Medicine Hasanuddin University Makassar Indonesia) for this study. Animals were randomly divided into 5 groups (= 4 in each group): The control (K0) and treatment groups (K1, K2, K3 and K4). They were kept in the animal house (The Molecular Microbiology and Immunology Laboratory, Faculty of Medicine Hasanuddin University Makassar Indonesia) for 1 week for proper acclimatization before starting the experiment under controlled condition of illumination (12 h light/12 h darkness) and temperature 23±2°C. They were housed under ideal laboratory conditions, maintained on standard pellet diet and water *ad libitum* throughout the experimental period. All procedures were in accordance with the internationally accepted guideline for experimental animal use and care of laboratory animals of the Molecular Microbiology and Immunology laboratory, Faculty of Medicine Hasanuddin University Makassar.

Experimental design: In this study, total number of 20 animals were divided into following 5 groups having 4 rats in each group: K0 = No induction AD model, K1 = AD induction model, K2 = AD induction model, K3 = AD induction model and K4 = AD induction model. Caspase-3 mRNA expression was assessed before AD induction and 6 weeks after AD induction.

Animal model: In this study advance preliminary research was done to develop an animal model of AD. To develop an AD model, author used a dose of $A\beta_{1-42}$ of 0.2 µg injected in ICV and observed the mice for 6 weeks, Aβ levels were measured using the SAA Mouse Elisa Kit. The AD animal model was generated using $A\beta_{1-42}$ peptide from Abcam, code number.Ab120959 (Cambridge, MA, USA)[11], is a reference modeling AD protocol. The formation of amyloid plaques was detected at weeks 4-6 post-injection of Aβ in the area of the neocortex[36].

Blood collection: Blood samples were collected at two time points: day 0 before Aβ injection (baseline) and last was the day 42 (week: 6, changes). Blood was taken from the tail vein using a 0.1 mL hematocrit needle. The samples were centrifuged and was kept in a sterile tube at -20°C until analyses.

RT-PCR: Extraction of total RNA qualitative RT- PCR using the method of Boom[37], with stages: DNA extraction, PCR mixing and dilution. In the extraction of DNA, the materials used are diatoms, L6 (lysis buffer), L2 (washing buffer) and TE (elution buffer), Diatoms after the vortex and then inserted as much as 40 µ into the sample containing L6 then shaken for 15 sec and in the rotator for 15 sec and centrifuge 13,000 rpm for 15 sec, then wash with L2 (1 mL) to 2 times, L2 (1 mL) to 2 times, ethanol (1 mL) to 2 times and the last wash acetone (500 µ) as much as 1 times, then enter the heater block for 20 sec, add TRIS EDTA (TE) 100 µL, incubation for 10 min, centrifuge 13.000 rpm for 3 min, take the supernatant and place it into a mini tube and cover and keep it at -20°C. The reverse transcriptase polymerase chain reaction (RT-PCR) was carried out to determine the levels of caspase-3 mRNA gene expression. The RT-PCR was performed with the following primers, caspase-3 forward: 5'-AGCTTCTTCAGAGGCGA CTA-3' and reverse: 5'-GGACACAATACACGGGATCT-3'[38] and glycerol-dehyde-3-Phosphate dehydrogenase (GAPDH) forward: 5'-CTCAAGATTGTCAGCAATGC-3' and reverse: 5'-CAGGATGCCCTTTAGTGGGC-3'[39], reactions were performed using the One-Step RT-PCR kit (Macrogen, Korea), GADPH was used as an internal control. Primers for RT-PCR were designed using Oligo software (Bio-Rad CFX Manager). All samples in both the treatment and control in homogenized and a total RNA sample in the presented in the form of curves. Reactions were visualized by agarose gel electrophoresis analysis. Rate threshold cycle in the calculation of the standard software (Applied Biosystems). The level of expression extraction according to the instructions of the protocol (Ambion, Austin, TX). Making PCR mix in all using solution are shown in Table 1, for caspase-3 multiplied by 80 due to triplicate, for one run: K0 (12 tube)+K1 (12 tubes) = 24 tubes, triplicate made into 72 tubes, rounded to 80, so on for K2, K3, K4. Then take 5.2 µL caspase-3 into each well (each well requires a 5.2 µL PCR mix). Also distribute GAPDH (as standard) into the wells, Each well needs a 5.2 µL PCR mix. Here fill the samples in the wells each containing the PCR mix, caspase-3 and GAPDH, wait for 1.5 h. Then turn on the PCR tool and make a layout, then input the sample that has been idle for 1.5 h into the wells, each enter as much as 5.2 µL, neither with the standard (GAPDH). Next was dilution: 20 µ RNA free water inserted into new tube (tubes 1, 2, 3, 4, 5 and 6), then the DNA template of all samples take as much as 20 µ and diluted with 20 µ which had been diluted and so on up to the 6 tube. Then enter 5 µ to the wells that have been made earlier, close and insert into the PCR machine, perform an initial denaturation stage of 96°C for 3 min, next 95°C for 30 sec and next annealing 55°C for 30 sec with volume contents 5+5 = 10 µ for 40 times.

Table 1: Stages of PCR mix manufacturing

RT-PCR	Caspase-3 (×80)	GAPDH (×30)
Master mix = 2.5 µL	200 µL	75 µL
Free water = 2.5 µL	200 µL	75 µL
Primer Caspase-3 F= 0.1 µL	8 µL	3 µL
Primer Caspase-3 R = 0.1 µL	8 µL	3 µL
Template = 5 µL		

Figure 1 shows schematic of the research process. Twenty wistar rats were divided into 5 groups: K0 = No induction AD model, K1 = AD induction model, K2 = AD induction model, K3 = AD induction model and K4=AD induction model. Caspase-3 mRNA expression was assessed before AD induction and 6 weeks after AD induction.

Statistical analysis: The data caspase-3 mRNA gene expression were statistically analyzed and the significance calculated using one-way ANOVA (Bonferroni) followed by Tukey's test. All numerical values were expressed as Mean±SD and the value of $p<0.05$ was considered as statistically significant.

RESULTS

A total of 20 wistar rats mean age 2.5-3 months, with 150-250 mg kg^{-1} body weight were included in the analysis. The mean, standard deviation and range of study variables presented in Table 2. The animals are grouped into groups induced by Aβ (K1, K2, K3 and K4) and those not induced by Aβ (K0), then continuous variables between the groups are compared. In Table 2 it shown that the mean level of caspase-3 mRNA expression was significantly different $p<0.05$. Caspase-3 mRNA expression was assessed before Aβ induction and 6 weeks after Aβ induction. In this study, we examined the effects evaluation of caspase-3 mRNA gene expression in single doses of Aβ injection. Amyloid beta (Aβ) used in this study: peptide from Abcam, code number.Ab120959 (Cambridge, MA, USA). The levels of caspase-3 mRNA at various time points throughout measurements were performed by RT-PCR and the analysis was evaluated. The caspase-3 mRNA expression levels in all observation groups are shown in Table 2.

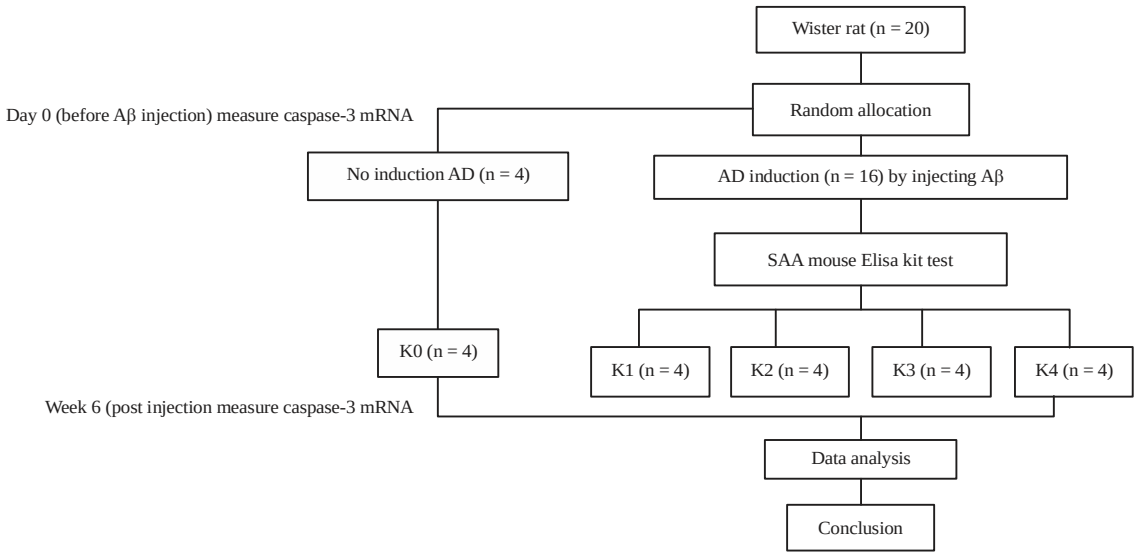

Fig. 1: Outline research process

Table 2: Caspase-3 mRNA expression in each group

	Observation time			
Variable	Groups	Before induction (baseline week 0)	6 weeks after induction (weeks 6)	0-6 p*
Caspase-3 mRNA	K0 (n = 4)	7.68 (0.25)	7.66 (0.23)	0.900
expression mean (SD)	K1 (n = 4)	7.65 (0.07)	12.69 (0.16)	<0.001
	K2 (n = 4)	7.54 (0.38)	12.49 (0.07)	<0.001
	K3 (n = 4)	7.63 (0.07)	12.73 (0.15)	<0.001
	K4 (n = 4)	7.68 (0.10)	12.53 (0.04)	<0.001

*p<0.05

Single-dose induction effect of 0.2 µg Aβ given by injection in the ICV region of wistar rat: The caspase-3 mRNA level at various time points increased in the AB-induced group compared with the non-induced Aβ, Aβ induction is given for 6 weeks (Table 2).

Table 2 show that caspase-3 mRNA expression before Aβ induction had no significant differences (p>0.05) between the 5 groups. After 6 weeks post Aβ induction there were significant increased (p<0.05) in all groups except K0 group. The mean increased level of caspase-3 mRNA expression in each group were (5.04) in K, (4.95) in K2, (5.10) in K3 and (4.86) in K4. There were no significant differences in Caspase-3 mRNA expression increased (p>0.05) between induction groups.

Paired t-test and one-way ANOVA (Bonferroni) showed changes in caspase-3 mRNA expression after 6 weeks post-induction Aβ, no significant differences in caspase-3 mRNA expression among all 5 groups before induction were observed (p>0.05). At 6 weeks post-induction Aβ, there was a significant increase (p<0.05) in caspase-3 mRNA expression in all groups except the K0 group. There were no significant differences in the increase in caspase-3 mRNA expression among induction groups (p>0.05). At 6 weeks after induction Aβ, it was detected increased caspase-3 mRNA expression in the induction Aβ groups, while no changes occurred in the non-induction Aβ group (K0).

DISCUSSION

Alzheimer disease (AD) is a progressive neurodegenerative disorder and the most common cause of senile dementia and is characterized by progressive dementia accompanied by personality changes, psychosis and language problems. Neuropathology is characterized mainly by extracellular senile plaques, consisting primarily of β-amyloid (Aβ) and intracellular neurofibrillary tangles, with hyperphosphorylated microtubule associated protein tau as a main constituent[40]. The accumulation of Aβ in the brains of AD patients has been implicated as a cause of the neuronal loss that occurs in Alzheimer's disease. However, the mechanisms by which Aβ induces neuronal death are not well understood. In the present study, showed the evaluation of caspase-3 mRNA expression of Aβ-induced AD rats. This study results showed that detected an increase of caspase-3 mRNA expression at 6 weeks after Aβ induction, which was similar to the result presented by Cetin *et al*.[41]. Their research found high caspase-3 activity levels in hypocampus, temporal and parietal cortexs in aged

mice injected with $A\beta_{1-42}$[41]. Amyloid β (Aβ) has been proposed as the main factor in the AD pathophysiology mechanism and the major component of senile plaques, was considered to play a central role in neuronal cell death (apoptosis), has received the most attention[43]. This has been demonstrated in neurons of human and rodent brains. In patients suffering from neurodegenerative disorders such as AD, there was a prominent increase in the neuronal RNA damage when compared to normal aging people[44]. The formation of extracellular plaques is described by the amyloid cascade (also Aβ-protein) theory of plaque causing pathology[6,45]. Plaques which are arise when the amyloid precursor protein (β-APP) was cleave by the beta-amyloid cleaving enzyme (BACE) to result the Aβ-42 type aggregations[46,47], because of its high lipid content and high oxygen consumption, the brain is particularly susceptible to oxidative stress. Several mechanisms have been proposed to explain Aβ neurotoxicity, production of reactive oxygen species such as hydrogen peroxide, nitric oxide, superoxide, highly reactive hydroxyl radicals and nitric oxide (NO), excitotoxicity with intracellular calcium accumulation, decreased membrane fluidity, energy depletion, alteration of the cytoskeleton, inflammatory processes and alteration of metal homoestasis[48-50]. All of these events converge into similar pathways of synaptic disruption, necrosis or apoptosis, leading to progressive loss of specific neuronal cell populations. The accumulation of Aβ in the brains of AD patients has been implicated as a cause of the neuronal loss that occurs in Alzheimer's disease, however, the mechanisms by which Aβ induces neuronal death are not well understood[51]. Amyloid β (Aβ) peptide contributes to the neurodegeneration in Alzheimer's disease (AD) and operates through activation of an apoptotic pathway. Apoptotic signal is driven by a family of cysteine proteases called caspases. The β-amyloid precursor protein (APP) is directly and efficiently cleaved by caspases during apoptosis, resulting in elevated β-amyloid peptide formation[52]. Changed expression of proteins linked to apoptosis, such as Bad, Bcl-2, Bax, p53 and Fas was reported in AD brains[53,54]. Caspases are a family of intracellular cysteine-aspartic proteases that are not only essential for triggering programmed cell death, but have also been shown to play key roles in non-apoptotic pathways, such as differentiation and proliferation of diverse cell types, axon guidance and synaptic activity and plasticity[28]. It is known that apoptosis with the participation of caspases e.g., caspase-3 may be involved in pathogenesis of AD[55,56]. Another results suggested that Aβ could function as an extracellular signal molecule for caspase-3 activation in AD[57]. These observations indicate the presence of a pathway from Aβ, as an *in vivo* trigger signal, to neuronal cell death through APP and caspase-3. It is also likely that other pathways mediated by APP derived fragments[58]. Play roles in Aβ -induced toxicity themselves and/or together with APP. Whether Aβ activates caspase-3 directly or via APP and/ or other proteins, our findings suggest that caspase-3 is a key molecule in Aβ-induced neuronal loss. Although *in vivo* model suggests that a relation between apoptosis and Aβ may exist, whether apoptosis occurs in AD brain remains unclear[59]. These results suggest that Aβ could function as an extracellular signal molecule for caspase-3 activation in AD. The yet unknown relationship between a soluble Aβ oligomer and amyloid burden as a sequestering reservoir remains to be studied. However, it is likely that Aβ derived from APP functions as a key suicide molecule through caspase-3 in AD and that genetic factors such as mutations of APP, presenilin-1, or presenilin-2 enhance this suicide cycle[57] and Aβ induces the activation of c-Jun in a JNK-dependent manner. JNK3 appears to promote apoptosis by phosphorylating and activating the transcription factor c-Jun.

CONCLUSION

The study concluded that although the molecular mechanism of damage to neurons in AD is not fully understood, but the beta-amyloid (Aβ) peptide has a biochemical toxicological effect in contributing to the onset of neurodegeneration in Alzheimer's disease (AD), it was seen after post-injection induction of Aβ in intracerebroventricullary (ICV) area for 6 weeks, i.e., the increment of caspase-3 mRNA significantly in all groups except K0 which was not induced. It is likely to operate through the activation of the apoptotic pathway. It is possible that Aβ activates caspase-3 directly or via APP and/ or other proteins, that Aβ could function as an extracellular signal molecule for caspase-3 activation in AD. Amyloid β (Aβ) derived from APP functions as a key suicide molecule through caspase-3 in AD. The evidence that Aβ induces the activation of c-Jun in a JNK-dependent manner. The c-Jun N-terminal kinase-3 (JNK3) appears to promote apoptosis by phosphorylating and activating the transcription factor c-Jun. However, it is suggested that more research on the effects of AB should be undertaken in AD using different experimental models.

SIGNIFICANCE STATEMENTS

This study discover the role of the expression activity of the Caspase-3 mRNA gene in Aβ-induced Alzheimer's disease (AD) rats, where it is known that caspase-3 acts as a caspase executor in the apoptosis process. Increased level caspase-3 and Aβ were found in neurodegenerative disease including AD. That can beneficial for developing animal modeling AD, so as to facilitate the researchers for the exploration of material and drug discovery trials for AD. This study will help the researcher to uncover the critical areas of the occurrence of AD earlier, that many researchers were not able to explore. Thus a new theory on induction of Aβ given to wistar rats by injection in the ICV area for 6 weeks, may increase the expression activity of the caspase-3 mRNA gene.

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