Beta Amyloid Peptide Role in Animal Modeling Trial of Alzheimer's Disease

Emma Kamelia[a], Hadiyat Miko[b*], Marni Br Karo[c], Sinar Pertiwi[d], Cahyono Kaelan[e], Andi Asadul Islam[f], Mochammad Hatta[g]

[a,b]*Dental Health Study Program of Health Polytechnic Tasikmalaya, 46196, West Java, Indonesia*
[c]*Midwifery Program of Medistra Health Higher School, Jakarta 17114. Indonesia*
[d]*Midwifery Study Program of Health Polytechnic Tasikmalaya, 46196, West Java, Indonesia*
[e]*Department of Pathological Anatomy, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia*
[f,g]*Molecular Biology and Immunology Laboratory for Infection Diseases, Faculty of Medicine, Hasanuddin Makassar 90245, Indonesia*
[a]*Email: kamelia.emma@gmail.com*
[b]*Email: drgmiko@yahoo.com*
[c]*Email: marni_karo@yahoo.com*
[d]*Email: ateubdg@yahoo.co.id*
[e]*Email: cakaelan@yahoo.com*
[f]*Email : andiasadul@yahoo.co.id*
[g]*Emai: hattaram@indosat.net.id*

Abstract

The Objective of this paper is to investigate the profile changes of *Amyloid Beta (Aβ)* levels in the blood serum using *AmyloidASerum (SAA)Mouse ELISA Kit* and histopathological image of hippocampal tissue in mice injected with amyloid beta-protein *(Aβ)* fragment 1-42.

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* Corresponding author.

Wistar rats were randomly selected and were divided into two cages. First Cage contained three mice which were injected Aβ1-42 peptide in the intracerebroventricullar *(ICV)* area with each dose of 0.2 μg, 0.13 μg and 0.1 μg. While the second cage contained three mice which were injected Aβ1-42 peptide in *intraperitoneal (IP)* area with each dose of 0.2 μg, 0.13 μg and 0.1 μg. Observation was done in a month. *SAA mouse ELISA Kit* Test and histopathologic image were used to detect Aβ1-42 peptide levels and the accumulation of amyloid plaques layer formation on the extracellular (senile) and *neurofibrillary tangles* (NFT) in the intracellular. profile changes showed that the injection of 0.2 μg peptide Aβ1-42 in the *intracerebroventricullar(ICV)*area increased *Aβ* levels compared to other doses both in the *ICV* and *IP* areas. PA results showed hypoxia state on nerve cells followed by vacuoles shrinkage in a large number of cells at 0.2 μg injection dosage in the *ICV* area. The results presented showed the importance of the establishment of Aβ1-42 peptide injection doses in experimental animals to build a more complete understanding of the molecular mechanisms result of Aβ1-42 peptide exposure in injected mice in which the sign shown resemble Alzheimer's disease sign. Thus, various research efforts trials of the drug as well as natural ingredients intended for *AD* treatment can be growing.

Keywords: amyloid beta; intraperitoneal; intracerebroventricullar; alzheimer's disease.

1. Introduction

Alzheimer's Disease (AD) was first discovered by Alois Alzheimer in 1906, a Germany'*neurologist* who in one occasion when undergone an autopsy found set of characteristic lesions in the brain*, AD* in a prior diagnosis lead to elimination before the final death.Overall damage result in dementia that can lead to stroke, brain tumors and other degenerative diseases [1]. Alzheimer's disease (AD is a common cause of dementia in the elderly (the elderly), which 7% -10% happens in individuals over the age of 65 years old and 40% in individuals over 80 years old [2]. Alzheimer's Drug Discovery Foundation (ADDF) estimated that one in three people in age group of 80 years old will develop the disease. Even in 2025, the cases will reach 34 billion [3]. Alzheimer's Disease (AD) is a neurodegenerative disease that occurs progressively which characterized by cognitive decline and memory loss. Pathological characteristic changes of Alzheimer's Disease (AD) usually include differences in neuronal loss or apoptosis level, senile plaques formed by deposits of beta amyloid in extracellular and intracellular neurofiblillary tangles (NFT), from hiperfosforilasi microtubule of atau protein (Tau) group in the brain. With the rapid growth in the elderly population in the world, the incidence of the number of people with AD also increased from year to year [4]. Characteristic lesions in the brains of AD is characterized by the presence of extracellular neuritic plaques (senile) and intracellular neurofibrillary tangles section which spread throughout the area of the cerebral cortex, especially found in large quantities in the hippocampus. Neuritic plaques consist of central core form in an extracellular, with compact form, fibrous protein known as beta amyloid (Aβ) surrounding the dendritic and axonal nerve endings. Neurofibrillary tangles is an abnormal solid cluster, such as filament in pairs similar to helic form that accumulates in the cell body which can be affected by neurons [5]. Many etiologic factors which may cause the occurrence of AD such as a genetic mutation, a susceptible gene and environmental factors that support the accumulation and formation of insoluble Aβ and hyperphosphorylated tau. An animal model that shows AD characteristics has been developed using the injection of beta amyloid (Aβ) in the region of mice' ICV [6,7,8]. Various techniques have been developed to detect the characteristic of Alzheimer's disease in patients and animal models. Among them use ultramicroscopy

to see an ideal images for monitoring deposits β-amyloid plaque state in experimental animals' brains. Those become a basis for studying the relationship between tissue degeneration of neurons in the cerebral β-amyloidosis and for asessing Aβ against therapeutic targets. In another reference, cDNA microarray was used to look at changes in guine pig *cerebral cortex* gene expression profile after injected by beta protein fragment (Aβ) 25-35, others use water Mize Moris test (MWM) for measuring spatial memory and learning in Alzheimer's disease guine pig model [4]. In this study, we used the Amyloid A Serum (SAA) Mouse ELISA Kit parameters to measure the levels of beta-amyloid (Aβ) in the blood serum of experimental animals and used anatomic pathology examination(PA) to see the accumulation layer formation of *amyloid plaques* and *neurofibrillary tangles (NFT)*. Specific knowledge about the causes of neurodegenerative etiology is as important pace setter for the development of various kinds of drugs for the neuroprotective treatment. There for, it required a means of making animal Alzheimer's disease modeling to test various kinds of drugs both chemical and phytotheraphy.

2. Materials and Method

Experimental procedures carried out in Molecular Microbiology and Immunology Laboratory, Medical Faculty, HasanuddinUniversity, Makassar.

2.1. Drug

Amyloid1-42 beta peptide (Aβ1-42 peptide) was derived from Abcam [9]. Amyloid β (1-42) human peptide was dissolved at a concentration of 1 µg / ml 100% HFIP (1,1,1,3,3,3-hexafluoro-2-propanol). The solution was further incubated at room temperature for 1 hour and occasionally rotated sufficiently. Next, the solution was vibrated for 10 minutes in a water tube sonicator. The solution of peptide / HFIP then dried under nitrogen gas stream. 100% DMSO was used to mix the peptide. The solution was further incubated at room temperature for 12 minutes and occasionally been rotated. Finally the solution subsequently formed into small volumes and stored at temperature -80 ° C. To use the solution add 500-1000 mL D-PBS (depending on the final concentration used) into the peptide stock solution and incubation in room temperature to provide an opportunity for the peptide not to agglomerate. Researcher estimated plan in determining the dose calculation was as follows:

1 µg / ml = 1000 ug / ml = 1000 g / 1000 mL = 1 µg / ml

10 g = 10µl

1: 500 ≈ 1: 750 ≈ 1: 1000

[For 1: 500] ie; 10 mL + 490 mL of PBS → 10/500 x 10 µg = 0.2 µg / 10µl

[For 1: 750] ie; 10 mL + 740 mL of PBS → 10/750 x 10 µg = 0.13 µg / 10µl

[For 1: 1000], namely; 10 mL + 990 mL of PBS → 10/1000 x 10 µg = 0.1 µg / 10µl

2.2. Animal Model and Aβ1-42 peptide injection

Six wistar rats was adapted for 1 week in the laboratory (in a standardized cage) at room temperature (25 ° C ± 2 °), dark and light cycles (12/12 h), fed and watered according to *ad libitum* [10]. Selection of experimental animals done randomly, after the adaptation period was completed then we made experimental animal models of Alzheimer's Disease (AD) which were placed on stereotaxic apparatus. Later in the cage I Aβ1-42 peptide injection was slowly injected over 10 minutes using a 10 mL microsyringe on intracerebroventricular (ICV) with each dose of 0.2 µg, 0.13 µg and 0.1 µg. While in the cage II, Aβ1-42 peptide was injected in intraperitoneal (IP) with each dose of 0.2 µg, 0.13 µg and 0.1 µg, then observed for 21 days, during the time, we did weighing and blood sampling from a vein in the animal tail [11] amounted to 0.1 ml using a hemetocrate syringe [12] on days 0,1,7,14 and day 21 with the aim to see fluctuations in Beta amyloid exposure. Blood taken 5 times (days 0,1,7,14 and 21) were collected and centrifuged to obtain serum. Serum then stored in a sterile tube and in refrigerator -20 ° C in temperature [11]. Until the time of measurement was finished,we measured the levels of Aβ1-42 peptide using Amyloid A Serum (SAA) ELISA Kit Mouse Test, where as on day 21 when the last day of the blood drawn, the experimental animals ware sacrificed and hippocampal brain tissues were taken for PA examination, to see the accumulation of amyloid plaque formation in the extracellular part (senile) and neurofibrillary tangles (NFT) in the intracellular part.

2.3. Procedures for Aβ Levels

In this measurement method, amyloid A serum from the sample to be reacted with the antibody anti-serum amyloid A, which will be absorbed on the surface of the polystyrene microtiter in wells. After eliminating protein which was not attached to the antibody by washing mecanism, then amyloid A Serum Antibody were conjugated with the addition of horseradish peroxidase (HRP). The enzyme in labeled antibody formed a complex amyloid A bond, followed by the washing process, the amount of enzyme which binds to the complex was measured by adding a chromogenic substrate 3,3 ', 5,5'-tetramethylbenzidine (TMB). The quantity of enzyme bonding varies proportionally to the concentration of amyloid A serum in the samples tested. Absorbance at 450 nm was one measurement of amyloid A serum concentrations in the samples tested. The quantity of amyloid A serum in the samples tested could be inter-polarisated from standard curves which were constructed and corrected for diluted of samples [13].

Summary ab157723- Amyloid A Serum (SAA) ELISA Kit Mouse Measurement from 'abcam were as follows:

1) Eliminate exactly the amount of the antibody that coated well, balance the entire reagent at room temperature. Prepare all the reagents, samples and standards.
2) Add standard or sample in each well used, then incubation process at room temperature.
3) Asspirated and washed each well, then add HRP previously prepared and labeled second antibody detectorincubation process at room temperature.
4) Asspirated and washedeach well, then add the chromogen substrate solution into each well. Furthermore slowly log visible color development.

2.4. Sample Preparation

General Sample information. Measurements for the quantification amyloid Aserum in the sample, for each sample were diluted before use. For one determination stage, a dilution of 1 / 1,000 was appropriate for most samples of serum / plasma. For the absolute quantification sample results lies outside the standard curve, dilution was less than or greater than the existing sample. If the level of the sample was doubt, a serial dilution with one or two representative samples earlier then undergo *running* with a plate that had been recommended. To prepare the 1 / 1.000 dilution of the sample, transfer 5 mL of the sample to 495 mL of 1x diluents. Here give 1/100 dilution. Next, made 1/100 dilution of the sample by removing30 mL up to 270 mL of 1x diluents. We should have a 1 / 1,000 dilution of our sample, fully mixed at each of these stages.

2.5. Measurement procedure

Balance the entire material ingredients and prepare reagents at room temperature before use. It is recommended to measure all the standards, control and samples in duplicate.

1) Pipette 100 mL of each standard, including zero control in duplicate, into a well fitted.
2) Pipette 100 mL samples (in duplicate) into well fitted.
3) Incubate the microtiter plate at room temperature for 60 (60 ± 2) minutes. Close the plate during incubation.
4) Continue incubation, aspiration materials from wells
5) Fill each well with wash buffer dilution one time appropriately then aspirations. Repeat 3 times, to entirely do the washing for 4 times. If washing by hand, fill out the charging each well with wash buffer, subsequently reversed plate pour the contents into a cleaning container. Continue the process on the well carefully on absorbent paper which was intended to remove residual buffer. Repeat until 3 to 4 times entirely on whasing process.
6) Pipette 100 uL precisely conjugate 1X antibody enzyme into each well. Incubation at room temperature for 30 (30 ± 2) minutes. Protect the plate with dark cover during incubation process.
7) Wash blot on well
8) Pipette 100 uL of TMB substrate solution into each well
9) Incubation in the dark at room temperature for 10 minutes
10) Add 100 mL stop solution to each well
11) Absorbance determination (450 nm) in each well. Calibration plate reader to establish specifications

2.6.Calculation

Standard readings duplication equated to each standard, sample and blank control. Subtract blank control of the entire average results. Results of average standard that has been set as opposed to the concentration and draw the best smooth curve pierce point to establish a standard curve. Most software plate reader or graph can be set as value and the actual arch. Four parameters of the algorithm (4PL) is usually available. Other equations can be tested for accuracy (such as linear, semi-log, log / log, 4-logisticparameter). Taking into account the protein

concentration to something unknown and control samples from the standard curve that has been determined. Samples generate large signals from standard and in further dilution by 1X in incubation buffer and then re-analyzed, then doubling the concentration obtained from the appropriate dilution factor

2.7. PA inspection procedures

For PA inspection used staining hematoxyllin eosyn (HE) technique, in which the brain tissue embedded into fixative consentrate containing 4% paraformaldehyde for 24 hours, and then dipped in paraffin, were cut using a microtome with intervals of 5 mm along the coronal plane [14].

3. Results

Results Analysis Of Aβ1-42 Peptide Injection Using Amyloid A Serum (SAA) ELISA Kit MouseTests:

Effect of Aβ injection from the measurement Aβ1-42 peptide levels value using Amyloid A Serum (SAA) Mouse ELISA Kit Test, can be seen in the following table 1 below:

Table 1: Aβ1-42 peptide levels value

Injection Areas& Animal Model	Injection in The Intraperitoneal (IP) Area						Injection In The intracerebroventricullar (ICV) Area					
	1	2	3	4	5	6	7	8	9	10	11	12
SO (A)	0,766	0,783	0,704	0,682	0,721	0,742	0,744	0,746	0,729	0,736	0,779	0,786
S1 (B)	1,485	1,407	1,423	1,409	1,359	1,365	2,306	2,319	2,307	2,318	2,394	2,369
S2 (C)	1,682	1,669	1,606	1,602	1,667	1,669	2,471	2,443	2,464	2,455	2,589	2,578
S3 (D)	1,812	1,858	1,832	1,853	1,865	1,873	2,544	2,539	2,602	2,616	2,719	2,709
S4 (E)	2,011	2,037	2,068	2,079	2,072	2,052	2,727	2,824	2,771	2,787	2,804	2,808
Blank (F)	0,065	0,098	0,074	0,063	0,073	0,068	0,069	0,066	0,068	0,081	0,071	0,068
Blank (G)	0,068	0,063	0,061	0,077	0,064	0,063	0,069	0,071	0,063	0,076	0,066	0,065
Blank (H)	0,069	0,071	0,073	0,067	0,067	0,071	0,068	0,074	0,083	0,069	0,066	0,086

According to Table 1 results above showed the data before Aβ1-42 peptide injection on days 0, we do blood sampling to 0 (called S0) and continued injection of Aβ1-42 peptide. The next day was the day after the injection / 24 hours post-injection (called S1), continued day 7 (called S2) and day 14 (called S3), and the last on day 21 (called S4) which was the last week , While the code 1 up to 12 were codes description for experimental animals at varying doses given:

1 s / d 6: 1 & 2 (Yellow Code.Head = 0.2 µg))

3 & 4 (Yellow Code.The Back = 0.13 µg))

5 & 6 (Yellow Code.Tail= 0.1 µg))

7 s / d 12: 7 & 8 (Yellow Code.Head= 0.2 µg))

 9 & 10 (Yellow Code.The Back= 0.13 µg))

11 & 12 (Yellow Code.Tail= 0.1 µg))

Data results in Table 1 above were made in duplicate for each dose, then from the same data also were made mean / average calculation on each of these doses and the results can be seen in the following table 2 below.

Mean Result Of "β-Amiloid Levels" Using Elisa KIT

Table 2 : Mean / average Values of beta amyloid measurements in the area of ICV and IP

	Injection In Intraperitoneal (IP) Area			Injection intracerebroventricullar (ICV) Area		
	(1+2)/2	**(3+4)/2**	**(5+6)/2**	**(7+8)/2**	**(9+10)/2**	**(11+12)/2**
SO (A)	0,7745	0,693	0,7315	0,745	0,7325	0,7825
S1 (B)	1,446	1,416	1,362	2,3125	2,3125	2,3815
S2 (C)	1,6755	1,604	1,668	2,457	2,4595	2,5835
S3 (D)	1,835	1,8425	1,869	2,5415	2,609	2,714
S4 (E)	2,024	2,0735	2,062	2,7755	2,779	2,806

• From Table 2 above showed that the injection of the peptide in the area Aβ1-425 ICV at a dose = 0.2 µg seen an increase compared to the number value in the area of IP and ICV with different dosage. The final result obtained that injection of Aβ1-42 peptide in the area intracerebroventricular (ICV) at a dose of 0.2 µg appears to be rising significantly compared with a dose of 0.13 µg and 0.1 µg in both ICV area and in the area of IP . To see the Figure clearly can be seen in the following chart
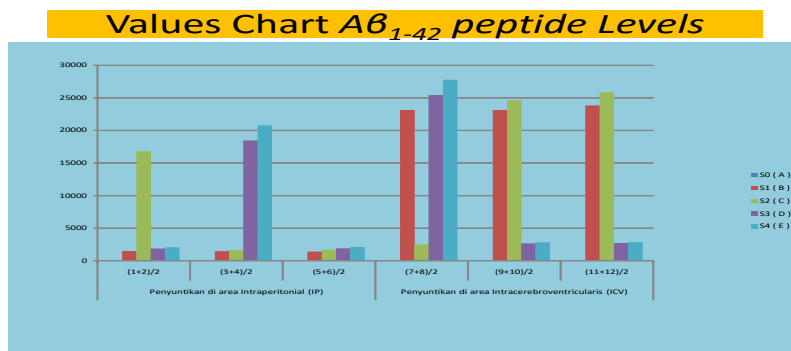
Figure 1: Values Chart *Aβ₁₋₄₂ peptide* Levels

The results of the analysis of histopathologic picture / PA in hippocampal brain tissue were as follows:
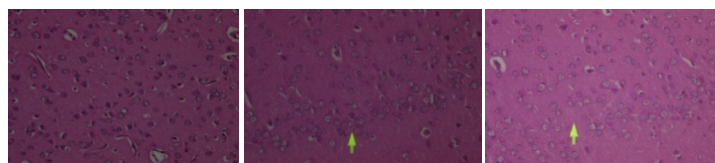

Figure 2a   Figure 2b   Figure 2c

Figure 2: analysis of histopathologic picture / PA in hippocampal brain tissue

In the figure 2a, 2b and 2c with dose of 0.1 µg, 0.13 µg, 0.2 µg ICV injection area looked their hypooxygenization (indicated by the white circle surrounding a nucleus) were increasing and were visible in Figure 2c, namely at a dose of 0.2 µg followed by the formation of beta amyloid plaques (marked with arrows) as well as the contraction of karyopiknosis cell.

4. Discussion

Research has been done showing that injection of Aβ1-42 peptide in the area intracerebroventricular (ICV) in the Wistar rat can induce pathological symptoms of Alzheimer's disease. The Significant Symptoms in patients with AD is the accumulation of Aβ-peptide in the brain, Amyloid Beta (Aβ) peptide is a toxic produced by endoproteolytic of amyloid precursor protein. Insoluble Beta amyloid is normally accumulated in the brain parenchyma and blood vessels of patients with AD [15]. Frautschy and his colleagues in their research said that *little amyloid* can be formed 30 days after injection into the cerebral ventricle Aβ1-40 [16]. Some theory and journal references said that typical characteristics of AD brain is characterized by the presence of lesions caused by extracellular neuritic plaques (senile) and intracellular neurofibrillary tangles part which spread throughout the area of the cerebral cortex, especially found in large quantities in the hippocampus [5]. The initial amyloid plaques can be detected at week 4 to week 6 in the area of the neocortex [17]. Staining technique using hematoxylin eosin (HE) was used to analyze the histopathological damage to the hippocampus area [18]. In addition to techniques HE, technique of staining may be used such as congo red, silver staining and choline acetyl transferase (CHAT), the results showed that the cognitive abilities of the experimental animals model suffered damage to the neurons and occurs karyopiknosis, and even further the formation of senile plaques (SP) and neurofibrillary tangles (NFT) in the cortex and hippocampus, choline acetyl transferase decreased then trigger the increased in Aβ. Overal,l these changes lasting more than 3 months [19]. So it can be tested for an extended time in the future study.

5. Conclusion

The results presented showed the importance of the establishment of Aβ1-42 peptide injection doses in experimental animals to build a more complete understanding of the molecular mechanisms result of Aβ1-42 peptide exposure in injected mice in which the sign shown resemble Alzheimer's disease sign. Thus, various research efforts trials of the drug as well as natural ingredients intended for *AD* treatment can be growing.

Acknowledgment

Conflicts of interest

The authors declare no conflict of interest.

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