



**WORLD JOURNAL OF PHARMACOLOGICAL  
RESEARCH AND TECHNOLOGY**

**STEREOTACTIC SURGERY THROUGH BETA-AMYLOID<sub>1-42</sub>: A VALID  
EXPERIMENTAL MODEL FOR COGNITIVE CHANGES IN RODENTS**

**Felipe Carmo de Moura<sup>1\*</sup>, Conceicao da Silva Martins<sup>2</sup>, Paula Matias Soares<sup>1,2</sup>, Gerly  
Anne de Castro Brito<sup>2</sup>**

<sup>1</sup>Superior Institute of Biomedical Science, Ceara State University, Fortaleza, Ceara, Brazil

<sup>2</sup>Postgraduate Program in Morpho functional Sciences, Faculty of Medicine, Morphology  
Department, Ceara Federal University, Fortaleza, CE, Brazil

**ABSTRACT**

Neurodegenerative diseases are a progressive neurological disorders class with signs and symptoms caused by Central Nervous System (CNS) deterioration cells. Alzheimer's disease (AD) is pathology without accurate knowledge of its etiology and beta-amyloid peptide (A $\beta$ ) presence in CNS is the main structural feature. Memory and learning cognitive loss are associated with AD and appear as disease first signs. Thus researches aimed at new therapeutic approaches are primarily applied in animal models and require a similar model for their treatments. Several models are presented, but through a thorough literature search, we found model induced by stereotaxic surgery with A $\beta$  injection presents a viable way to reproduce cognitive losses in a short time period. Through Morris Water Maze behavioral test, it is possible verify such losses related to memory and learning after seven days from surgery. It is therefore concluded that A $\beta_{1-42}$  injections in stereotaxic surgery are a valid experimental model to induce changes similar to AD.

**Keywords:** Alzheimer's disease,  $\beta$ -amyloid<sub>1-42</sub>, Morris Water Maze.

Received 15 January 2016, Accepted 25 January 2016

## INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder commonly associated with brain beta-amyloid peptide (A $\beta$ ) accumulation, being one of the earliest disease pathological features, followed by neurofibrillary tangles associated with Tau protein hyperphosphorylation [1-4]. A $\beta$  is a peptide consisting about 39-43 amino acids produced by Amyloid Precursor Protein (APP) cleavage in amyloidogenic pathway by  $\beta$ -secretase e  $\gamma$ -secretase cleavage and it is found in soluble form in brain extracellular space [5-7]. At disease onset, individuals with this disorder have short-term memory impairments, but maintain alert state, sensory and motor functions preserved, progressing to total cognitive functions loss [8-10].

AD is the most common dementia cause and it is associated with an approximately annual cost of US\$ 226 billion in 2015 [11]. Currently, it is estimated that approximately 24 million people are afflicted by some dementia form and the forecast by 2050 is this number will increase around four times due to increased population longevity [1,9,12,13].

Diagnosis combines clinical and subjective patterns, and definitions as "possible", "probable" and "definitive AD" are terms used in clinical practice. These settings are established by The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) since its creation in 1984 [14]. However, disease accurate diagnosis occurs only with histological brain regions analysis, which is observed amyloid plaques accumulation and neurofibrillary Tau protein tangles [1,15].

Unfortunately AD current treatments, although they have their benefits, also have many side effects and results are quite heterogeneous, where some patients respond as expected on a single substance, while others do not show the same response [16-19]. Many patients also receive medication for psychiatric and behavioral symptoms (such agitation, anxiety, depression, etc.), but in a short period to prevent further cognitive losses caused by side effects of these ones [18,20].

Thus, new therapeutic approaches have been proposed for disease treatment, since Selective Serotonin Reuptake Inhibitor (SSRIs), antiepileptic, antihypertensive and anti-inflammatory drugs, plant derivatives, among other substances, as well as combination of one and more treatments [18,21,22]. Due to physiological complexity, animal models have been used in

research on new drugs for various disease treatments, especially in CNS, showed positive results when compared to research in cell culture [23,24].

Animal model similar to AD occurs by A $\beta$  intracerebral injection through stereotaxic surgery, however other studies have genetically modified animal models or processes in cell culture. Thus, in an attempt to better targeting for cognitive function changes, the purpose of our research is to explicit experimental model through A $\beta$ <sub>1-42</sub> intrahippocampal or intraventricular injection as an affordable model to verify cognition specific alterations.

## EXPERIMENTAL PROCEDURE

A $\beta$ <sub>1-42</sub> peptide (Sigma-Aldrich) remains stored lyophilized at -80°C. A $\beta$ <sub>1-42</sub> aggregation is generally prepared by saline or Fetal Bovine Serum (FBS) at pH 7.5 and solution undergoes vigorous stirring (800 rpm) at 23°C for 36 h for hatching. Next, preparation is centrifuged for 10 min at 15000 rpm for full aggregation. Aggregate suspension may then be stored at -80°C [25].

Animals are submitted to stereotaxic surgery after anesthesia which usually occurs with xylazine (10mg/kg, intraperitoneally, ip) and ketamine (80mg/kg, ip). Surgical procedure consists in animal fixing apparatus by ear bars and clamps snout and after cleanliness a small incision is made on middle line with a scalpel, using hooks to keep area opened to allow bregma and lambda visualization. Locating coordinates for hippocampus and ventricle are determined by an atlas usually ranging from zero point which is located in bregma according to rodent type: rat or mouse. Injection is applied for 15 minutes with a Hamilton microsyringe (26-gauge), with an infusion pump assistance for application of 5  $\mu$ l each hippocampus or less in third ventricle (2  $\mu$ l), with permanence syringe in region for Five minutes to complete infusion [26]. Animals remain in isolated cages after surgery for 07-14 days [25,27].

### Open Field Test

A method widely used to analyze rodents emotional behavior is Open Field Test. Animals behavior is analyzed by spontaneous locomotor activity, rearing, grooming and fecal boli [28,29]. Results in Open Field Test discard animal motor incapacity, generally used as an initial screening to perform after Morris Water Maze. Animal is placed in a transparent acrylic box with 60 x 60 x 60 cm dimensions (to rats) and during one minute above mentioned parameters are not considered for animal new environment adaptation. Over next five minutes these parameters are recorded [30,31].

### **Morris Water Maze (MWM)**

MWM was developed by Richard Morris as a method to evaluate the spatial learning and memory [32]. MWM use to learning and memory assessment has been employed as the main test in animal models [33]. This test is based on a water tank divided into four quadrants labeled with different symbols to animal location. A submerged platform is placed in one of quadrants as an escape means for animal to climb without needed swimming. Training sessions are conducted daily for four to six days and then it is realized the probe test in platform absence. Each training day consists to release the animal in front to each quadrant symbol in water for a minute until animal find platform, or otherwise conduct animal with a metal Rod to remaining platform it for 15 seconds. At the last day, probe test, animal remains time in quadrant which was platform is measured [34]. Test has been considered to have a strong relationship with hippocampal synaptic plasticity [33,34]. Thus, this method is based on animals escape latency calculated by time taken to animals to find the hidden platform [33-35].

### **Cognitive Loss Evidenced Through MWM**

The two most important  $A\beta_{1-42}$  species (fibrillar  $A\beta_{1-42}$  and  $A\beta_{1-42}$  oligomers) play a key role in disease pathogenesis. In a comparative study between them to explore effects on cognitive function of hippocampal stereotactic surgery in rats, it was observed that animals that received  $A\beta_{1-42}$  oligomers showed greater loss on memory and learning compared to animals that received fibrillar one. Thus, it can be concluded that aggregation procedure described above enhances damaging effects caused by  $A\beta_{1-42}$  injection [36].

In a recent study on tamoxifen use and its relationship in animals' memory induced by  $A\beta_{1-42}$ , it was shown through MWM ten days after surgery, control group showed significant results when compared to experimental one, considering animal's time spent in quadrant that had platform during training period. Tests carried out a day before surgery were similar between groups, demonstrating that  $A\beta_{1-42}$  induction was able to promote cognitive losses in surgery group [37].

Testosterone using effects and its relation to rat cognitive performance was studied in experimental animal model induced by  $A\beta_{1-42}$  through bi-hippocampal injection on five days protocol of training performed daily on consecutive days; it is possible to suggest that surgery was able to impair memory retention by MWM [38].

In a study with rats to examine leptin effects on memory and learning, it was observed by MWM that animals induced with Alzheimer's model by  $A\beta_{1-42}$  injection showed significant cognitive losses in control group after ten surgery days [39].

An article about lycopene supplementation effects in rats also showed that stereotactic surgery with  $A\beta_{1-42}$  application was able to promote significant loss of spatial memory in animals subjected to induction process. Results were followed by biochemical tests that show high levels of reactive oxygen species, indicating association between cognitive memory losses caused by  $A\beta_{1-42}$  with oxidative stress [40].

*Alpinia oxyphylla* extract has been used to treat various disorders and inflammatory disease in traditional Chinese medicine. A study conducted on its anti-inflammatory properties in Alzheimer model induced by  $A\beta_{1-42}$  in mice has shown that cognitive losses were observed after 14 days procedure, subsequently associated with structural changes in hippocampus region through histological analysis [41].

*Semen Ziziphi Spinosae* has been used in Chinese medicine and in other oriental countries as a sedative substance and studies has demonstrated its anti-inflammatory and antioxidant effects promoted interest in checking effects on Alzheimer model induced by  $A\beta_{1-42}$ . Thus, large quantities analysis of  $A\beta_{1-42}$  observed post-mortem was associated with stereotactic surgery as well as losses related to learning and memory observed in MWM [42].

Further studies showed similar findings related to cognitive memory and learning impairment via MWM, thereby demonstrating that induction model stereotaxic with  $A\beta_{1-42}$  injection, either intrahippocampal or intraventricular, are valid for experimental induction model Alzheimer's [43-47].

## CONCLUSION

Cognitive tests are extremely important to assess animal spatial learning and memory. MWM has been presented in literature as the most reliable test model used for neurodegenerative disease such AD. Thus, this study presents  $A\beta_{1-42}$  induction model in several studies that attesting the procedure used effectiveness for testing new therapeutic approaches seeking to slow or even stop cognitive losses caused by AD. Thus it is concluded that  $A\beta_{1-42}$  injections in stereotaxic surgery are a valid experimental model enabling different substances use in an induce changes model very similar to AD.

## ACKNOWLEDGMENT

Scientific and Technological Development National Brazilian Council (CNPq), Higher Education Personnel Improvement Coordination (CAPES), Cearense Foundation for Support of Scientific and Technological Development (FUNCAP), Post-graduate Program in Morphofunctional Sciences (PPGCM), Center for Studies in Microscopy and Image Processing (NEMPI), and Post-graduate Program in Physiological Sciences (PPGCF) of Biomedical Sciences Higher Institute (ISCB).

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\*Correspondence Author Felipe Carmo de Moura, Ceara State University, Superior Institute of Biomedical Science, Fortaleza, Ceara, Brazil