ORIGINAL ARTICLE

Correlation amyloid in brain, kidney, and CSF of castrated guinea pigs

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Abstract

OBJECTIVES: The association between low testosterone levels and Alzheimer's disease (AD) amyloid β-peptide (Aβ) metabolism was investigated in brain and kidney of guinea pigs.
METHODS: The expression of Aβ peptide in the brain and kidney was assessed by using the immunohistochemistry method.
RESULTS: No expression of Aβ was seen in both groups of animals. This negative staining was found until the fourth week following castration. The formation of Aβ in guinea pigs is perhaps not a short duration process and may undergo different metabolic pathway compare to humans.
CONCLUSION: castration was not associated with the formation of Aβ in the brain and kidneys during a 1-month period and might require a longer period of time.

1. INTRODUCTION

Beta-amyloid (Aβ) is a peptide of 40 to 42 amino acids which can accumulate as extracellular amyloid plaques in the brain and cause nerve fiber damage leading to neurodegeneration. The formation of Aβ is thought to play a major role in the neuropathology of patients with Alzheimer's disease (AD) (Checler, 1995 & Yanker, 1996). $A\beta_{42}$ is predominantly found in the amyloid plaques of AD brains and may be the initial step in amyloid plaque formation. These amyloidogenic proteins are insoluble in water and have a high β-sheet secondary structure that is associated with a tendency to aggregate or polymerize.

Men with low testosterone levels may be at an increased risk for AD (Hogervorst *et al*., 2003; Moffat *et al*., 2004; Rosario *et al*., 2004). Studies in humans have shown that lower androgen levels are associated with increased plasma Aβ in older men with memory loss or dementia (Gillet *et al*., 2004). In addition, therapy for prostate cancer which depletes endogenous testosterone also resulted in elevated plasma levels of Aβ (Gandy *et al*., 2001; Almeida, 2004). Moreover, earlier studies in cell culture and animal models (Gouras *et al*., 2000, Ramsden *et al*., 2003; Rosario *et al*., 2006) have shown that testosterone may regulate the level of Aβ.

Studies on the formation of Aβ plaques and the neuropathology of AD in humans are difficult since the diagnosis of AD requires postmortem confirmation. Therefore, many animal models have been developed to mimic these biological conditions in humans. Guinea pigs are non-transgenic animals which produce Aβ peptide that is

identical to human Aβ in their amino acid sequences and are therefore ideal for the assessment of changes of physiological levels of Aβ as a result of hormone deprivation (Beck *et al*., 1997).

This study aimed to investigate the association between low testosterone levels and the metabolism of Aβ peptide in the brain of non-transgenic guinea pigs. This objective was tested using castrated guinea pigs to mimic the decrease in testosterone levels seen in most older men (Kaufman *et al*., 2005).

2. METHODS

The current study was carried out in the Mochtar Riady Institute for Nanotechnology in Indonesia between April and July 2008. Ethical approval for the experiment was given by the local ethical committees for animal research.

Adult male guinea pigs [6–7 weeks old, body weight (BW) 500 gram] were caged in an animal house with light:dark cycles of at a ratio of 1:1 for about a week in order for them to adapt to the environment prior to experimentation. They were fed a soy-free diet (in order to eliminate any effects due to phytoestrogen intake) and drink *ad libitum*. The diet consisted of vegetables (lettuce and carrots) as fiber souce, milk as casein source, bran as protein source, wheatflour and corn flour as carbohydrate sources, and vitamin supplements.

Guinea pigs were either left intact (uncastrated) or were surgically castrated to mimic the loss of testosterone seen with age andropause in most men (Kaufman *et al*., 2005). For castration, all guinea pigs were anaesthetised with a combination of Atropine (0.05 mg/kg BW subcutaneous), Diazepam (5 mg/kg BW Intraperitoneal) and Hypnorm (1 ml/kg BW intramuscular). The anaesthetics were administered to result in 45 minutes of deep anaesthesia.

2.1. Expression of Aβ in the brain and kidney

In this phase of the study, castrated animals were sacrificed at week-1, 2, 3, and 4. Uncastrated animals were sacrified at week 1. Expression of Aβ was tested using the immunohistochemistry method discussed below.

The left cerebral hemisphere and a kidney from the guinea pigs were fixed and embedded in paraffin wax, and then cut into 10 μm sections with a microtome. The sections were then treated with 0.01M sodium citrate buffer at pH 6.0 for 20–30 minutes in the microwave for antigen retrieval. Blocking of endogenous peroxidase was done by treating the sections with 3% hydrogen peroxide for 10 minutes and incubating with TBST for 60 minutes at room temperature. The sections then were incubated with a mouse monoclonal antibody against β-amyloid (DE2B4, Cat. No.# sc-58508, Santa Cruz Technology Inc., USA) as the primary antibody (1:500 dilution in TBST) overnight at 4°C. After washing with TBST, the sections were incubated with secondary horse-radish peroxidase (HRP)-conjugated anti-mouse antibody (DAKO-LSAB-2 System HRP kit, DAKO Corporation, Glostrup, Denmark) without dilution in TBST for 60 minutes at room temperature. This was followed by applying diaminobenzidine (DAB) substrate (Pierce, Rockford, IL, USA) at a 1:10 dilution in peroxidase buffer and incubation at room temperature until the desired intensity of sufficient staining was obtained (usually within 2–5 minutes). Sections were counterstained with hematoxylin to visualize tissue morphology and then mounted with permount medium. A section from human bladder which has been known to contain beta amyloid was included to serve as positive control.

3. RESULTS

Twenty-four guinea pigs were used for this phase of study, consisting of 12 castrated and 12 uncastrated animals. Three animals in the castrated group were sacrificed each week for immunohistochemistry evaluation (see methods section). There was no positive staining of Aβ in either group. Expression of Aβ was not detected up to four weeks after castration **(Figure 1)**.

Previous experiments had shown that castration produced significant decrease of plasma testosterone level. On the other hand, castration also results in significant increase of CSF Aβ and plasma Aβ **(Table 1)**.

4. DISCUSSION

Despite the increased level of Aβ peptides in CSF reflecting increased levels of Aβ in the brain, our present study failed to show any expression of Aβ peptides or plaques in brain or kidney during the study period. The absence of staining is unlikely to be technical. Antibodies selected for histochemical staining were derived from mouse antibodies against human Aβ and were shown to successfully bind Aβ from cerebrospinal fluid from guinea pigs using the ELISA method (Wahjoepramono *et al*., 2008). On the other hand, the risk of death of guinea pigs during and/or after castration was a major limiting factor in this study. Therefore, a high number of animals were included at the beginning of study to avoid loss of power at the end of the study. There were 12 castrated and 3 uncastrated guinea pigs alive at the end of the study, but in none of the castrated animals could Aβ staining be detected.

Our previous study has shown that serum testosterone levels were significantly reduced following castration compared to controls. In addition, castrated animals had significantly increased Aβ levels in cerebrospinal fluid (CSF) on day-18 and day-36, which was more than twice the level of CSF Aβ of controls. The concentration of Aβ in plasma tended to increase only after day-36 following castration, suggesting that it was rapidly cleared from the body (Wahjoepramono *et al*., 2008). It could be the case that the formation of plaques

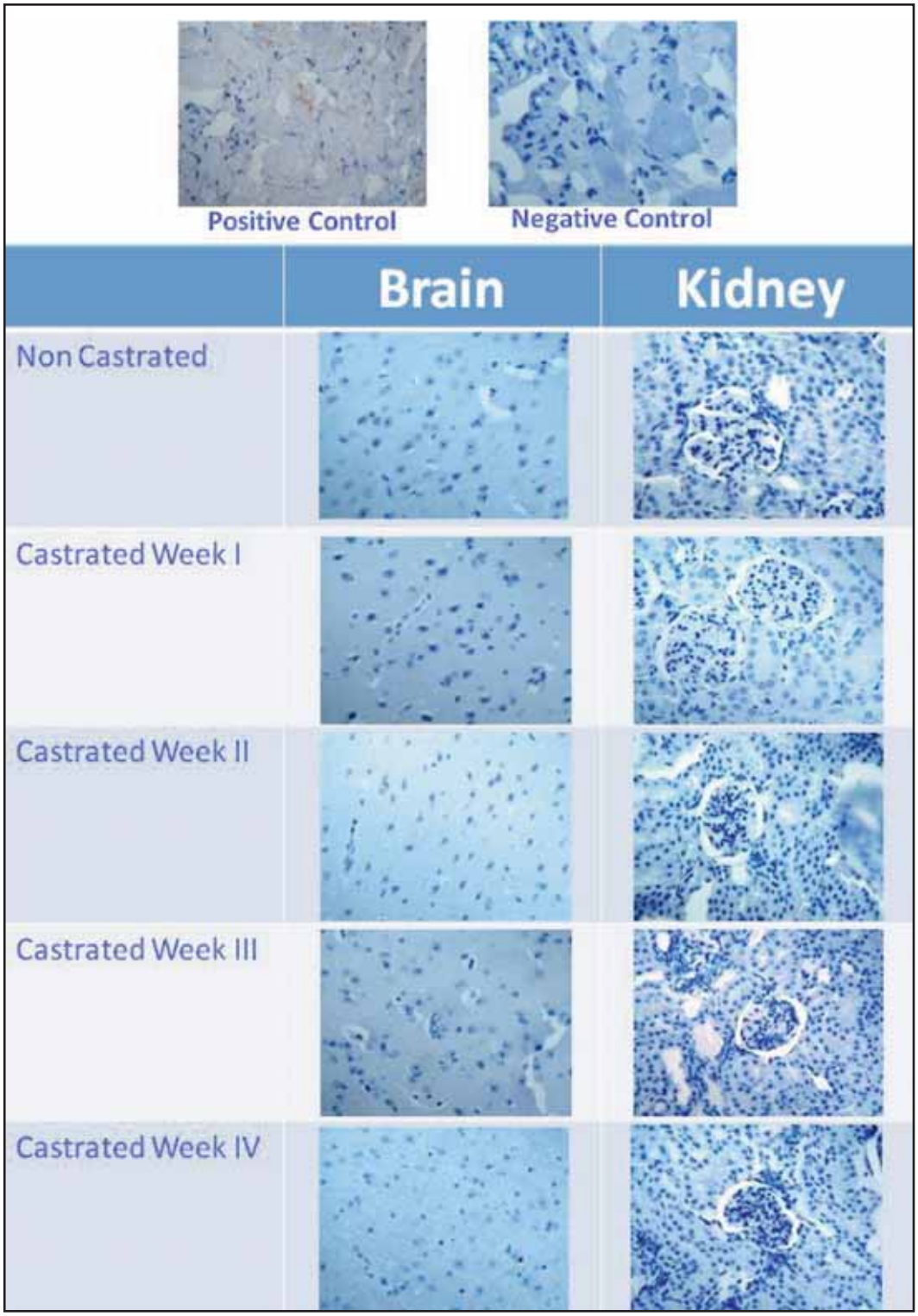

Figure 1. Negative expression of β-amyloid in the brain and kidney of uncastrated and castrated guinea pigs overtime by immunohistochemistry.

in the brain requires more time, which was not tested in this current experiment.

In contrast, in an aggressive model of transgenic mouse expressing double mutations (*APP* and *PS1* genes), Aβ deposits appear as early as 2.5 months of age (Blanchard *et al.*, 2003). Most current animal studies of AD have been performed using transgenic mice. These animals are genetically engineered to harbor mutations in genes essentials for AD development, i.e. the Aβ precursor proteins (*APP*), presenilin-1 (*PS1*), and tau*P301L*. Triple transgenic mice have also been developed and have been used to study the timing of amyloid deposition (Oddo *et al.*, 2003). However, mutations on these genes are rarely seen in humans and

Table 1. Mean differences of serum testosterone and amyloid-β levels between controls and castrated animals at 36 days following castration.

Group	Control	Castrated	*p* value (ANOVA)
Mean serum testosterone level (nmol/L)	28.7 + 29.83	2.74 + 0.94	< 0.001
Mean CSF Aβ level (pgl/mL)	118.0 + 73.77	265.8 + 114.67	<0.001
Mean plasma Aβ level (ngl/mL)	47.2 + 19.47	64.1 + 13.27	0.026

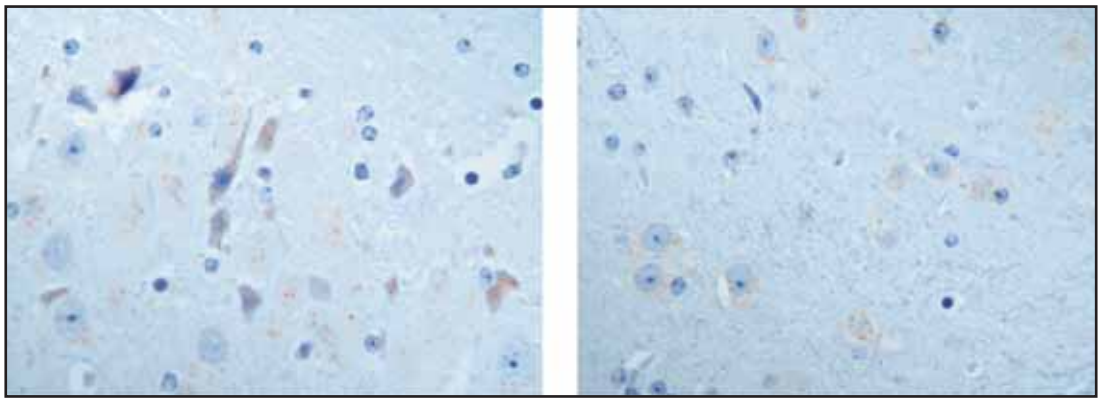

Figure 2. Positive Aβ immunostaining in temporal lobe (left) and occipital lobe (right) of a guinea pig at 10 weeks after castration (x400).

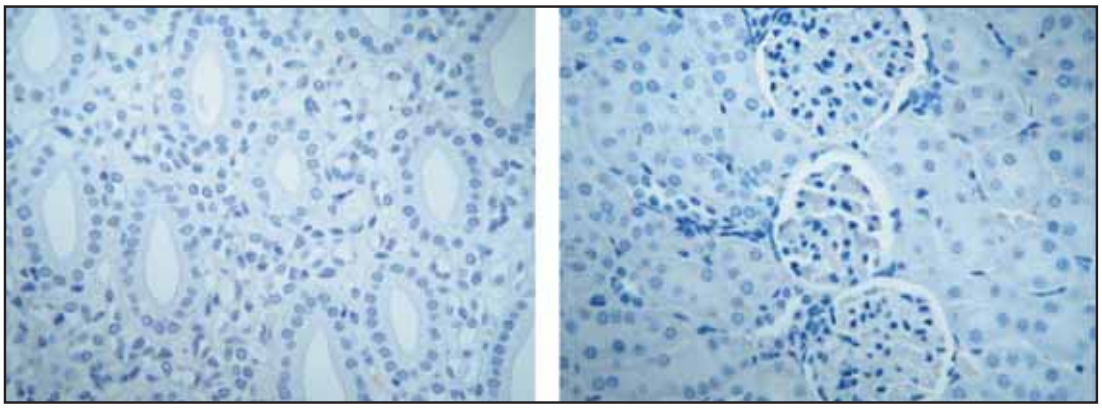

Figure 3. Negative Aβ immunostaining in the kidney tubuli (left) and glomeruli (right) of a guinea pig at 10 weeks after castration (x400).

therefore may not reflect the situation of true metabolism of Aβ in humans. The amino acid sequence of Aβ in rodents differs from that of humans by three amino acid substitutions.

Other species (hamster, guinea pig, and rabbit) have Aβ peptides identical to humans and appear well suited for experimental studies. Furthermore, 80–90% of Aβ peptides in the guinea pig CSF are of the 1-40 species, which are similar to the majority of Aβ species in human (Beck *et al*., 2003). Studies using castrated guinea pigs are limited. Previous experimental studies have used female guinea pigs subjected to ovariectomy to mimic postmenopausal women. An increase of total Aβ levels was observed in these animals, which could be attenuated by treatment with 17-β estradiol (Pentaceska *et al*., 2000).

Incidentally, there was one live guinea pig at 10 weeks after castration, which was not included in the experimental group. Immunohistochemical staining showed a positive Aβ expression in temporal and occipital lobes **(Figure 2)** but negative in the kidney tubuli and glomeruli. **(Figure 3)**. These results supported that the formpation of Aβ plaque in the brain may require more time after the increase of CSF Aβ. On the other hand, increased Aβ plasma did not pruduce Aβ deposits in the kidney.

5. CONCLUSION

In conclusion, castration of guinea pigs was not associated with the formation of Aβ plaques in the brain and kidneys during a 1-month period of observation. It probably requires a longer period of time for such

expression to occur. Further studies are needed to elucidate the role of androgens in the formation of Aβ plaques and the pathology of Alzheimer's disease.

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