運動對阿茲海默氏症貝它澱粉樣蛋白在腦中代謝及清除之研究 期中進度報告

計畫類別：整合型計畫
計畫編號：NSC92-2321-B-006-003-
執行期間：92年08月01日至93年07月31日
執行單位：國立成功大學解剖學科

計畫主持人：郭余民

報告類型：精簡報告
處理方式：本計畫可公開查詢

中華民國93年5月28日
中文摘要
阿茲海默氏症（Alzheimer’s disease）是一種神經細胞死亡、退化所造成的疾病，其主要病理特徵之一是神經細胞外與腦血管壁貝它澱粉樣蛋白(\(\beta\)-amyloid)纖維狀聚集沉積。貝它澱粉樣蛋白聚集會引發許多病理現象，因此，被認為是引發阿茲海默氏症的關鍵因子。然而大多數阿茲海默氏症患者的腦中貝它澱粉樣蛋白生成並無明顯上升，顯示貝它澱粉樣蛋白在腦中之累積與代謝、清除發生異常有關。我們推測若能有效的從腦血管清除貝它澱粉樣蛋白，將可抑制或延遲阿氏症之發生。本實驗以小鼠作為研究貝它澱粉樣蛋白在腦中代謝、清除之動物模型，研究運動是否會增加貝它澱粉樣蛋白在腦中之清除。我們把貝它澱粉樣蛋白注入運動4週後小鼠右側之海馬廻，並於24小時後犧牲動物。研究結果顯示，運動4週並無改變貝它澱粉樣蛋白在各腦區的量與分布。此外，運動4週後，小鼠大腦皮質貝它澱粉樣蛋白前驅蛋白、貝它澱粉樣蛋白分解酶-neprilysin、以及在血管的結合蛋白-RAGE 的表現量都沒有顯著改變；而另一與貝它澱粉樣蛋白從血管清除有關之結合蛋白-LRP 的表現量，似乎有增加的趨勢。而且，LRP 在貝它澱粉樣蛋白注入側之表現量，有高於對側的趨勢。組織免疫染色結果顯示：微小膠細胞無明顯活化現象、貝它澱粉樣蛋白並無與血管共存的現象。因考慮到運動對腦中貝它澱粉樣蛋白之清除也許需要更長的時間，因此，我們加長運動的時間至12週。
初步的研究結果顯示，運動12週並沒有改變貝它澱粉樣蛋白在各腦區的量與分布。而且，大腦皮質與海馬廻中 RAGE 和 SOD 的表現量都沒有顯著改變。但是，貝它澱粉樣蛋白注入側，大腦皮質之 RAGE 似乎有增加的趨勢；而 SOD 則有下降的現象，然而都未達顯著差異。我們的研究顯示在正常、健康的狀態下，運動並無增加腦中貝它澱粉樣蛋白之代謝及清除。因此，本子計劃下一年度將先把小鼠腦血流降低，或以 LPS 引發小鼠發炎反應後，再研究運動對的腦中貝它澱粉樣蛋白代謝及清除之影響。

英文摘要
One of the neuropathology of Alzheimer’s disease (AD) is the deposition of fibrillar \(\beta\)-amyloid (A\(\beta\)) peptide extracellularly and surrounding the wall of cerebrovasculature. The abnormal A\(\beta\) aggregates in brain could induce abundant pathologies, hence A\(\beta\) is considered as the major culprit in the pathogenesis of AD. Recent studies indicated that the production of A\(\beta\) was not increased in most AD patients implying that either metabolism or clearance of the A\(\beta\) peptide in brain is disturbed. Evidence suggested that pathological changes in cerebral vessels could potentially impede the clearance of A\(\beta\). Therefore, this study was designed to explore the effects of chronic physical exercise on the metabolism and clearance of brain A\(\beta\) peptides. A\(\beta\) peptide was injected into the right hippocampus of male C57BL/6 mice, that were either received 4-week exercise training or no training (control), and sacrifice the animals 24h afterward. Our analyses indicated that 4-week exercise did not alter the distribution and levels of A\(\beta\) in each brain regions. The expression levels of cortical amyloid-precursor protein, A\(\beta\) degradation enzyme-neprilysin, and vascular A\(\beta\) binding/transpotor molecule-RAGE remained the same between the exercise and control groups. Interestingly, the injection of A\(\beta\) showed a trend in elevating the cortical LRP levels, which was more pronounce in the exercise group. Activation of microglia or the localization of A\(\beta\) in blood vessel were not evident by immunohistochemistry. We then extent the exercise training period to 12 weeks and the mice were killed 48 hr after the end of training. Our preliminary results showed that
the distribution and levels of Aβ in each brain regions were not affect by 12 weeks of exercise training. Furthermore, the levels of RAGE and SOD in cortex and hippocampus were no difference between control and exercise group. Although did not reach significant level, the Aβ-injection ipsilateral side seemed to have higher RAGE and lower SOD levels in the 12-week exercise group than those of control animals. Taken together, these results suggest that exercise does not enhance the brain Aβ clearance efficacy under normal and health condition. In the following year, the effects of exercise on the clearance of brain Aβ will be investigated in the hypoperfused and/or LPS-challenged mice.

**缘由與目的**

The elderly population is increasing quickly in Taiwan. As the population ages, the odds of acquiring senile dementia rise as well. The most prevalent senile dementia in Taiwan is Alzheimer’s disease (AD) (Liu et al., 1995). The preeminent pathologic feature of AD brains is the enigmatic death of large quantities of neurons from cortex, hippocampus, and some subcortical nuclei, with proliferation of adjacent glial cells (Coleman and Flood, 1987). One of the most prominent neuro-pathological lesions is the accumulation of fibrillar β-amyloid (Aβ) peptide extracellularly and surrounding the wall of cerebrovasculature.

The Aβ peptides are derived from the larger amyloid precursor protein (APP). Except for the few familial AD cases, the production of Aβ was not increased in most sporadic type of AD patients implying that either metabolism or clearance of the Aβ peptide in AD brain is disturbed (Selkoe, 2001). New evidences revealed that the accumulation of Aβ during the course of AD may result from the lack of Aβ clearance due to resistance to proteolytic degradation or defective transportation or from both (reviewed in Selkoe, 2001). Since the cerebrovascular pathways representing one of the major Aβ drainage pathways in brain, any pathological changes in cerebral vessels could potentially impede the clearance of Aβ. In other words, maintaining the cerebral vessels in healthier status could bring another beneficial on lowering the chance of affecting AD, on top of cerebrovascular accident.

Over the past decade, a number of studies on humans have shown the benefits of exercise on brain health and function (reviewed in Cotman and Berchtold, 2002). Some reports indicate that physical activity is associated with lower risks of cognitive impairment, AD, and dementia of any type, and that physical inactivity may be a risk factor for the AD (Friedland et al., 2001; Laurin et al., 2004). It is also noticed that regular exercise alleviates negative mood states, such as depression and anxiety (Byrne and Byrne, 1993), although its underlying mechanisms remain obscured.

In animal studies, physical exercise not only can improve blood vessel relaxation and reduce thrombosis, but also enhance angiogenesis (Huang et al., 2000; Koenig and Ernst, 2000; Gustafsson et al., 1999). Furthermore, voluntary wheel running increases neurogenesis and long-term potentiation in the dentate gyrus, and enhances spatial learning performance (Fordyce and Wehner, 1993; van Praag et al., 1999a; van Praag et al., 1999b). Several studies suggest that exercise leads to changes in the expression of a number of genes that are involved in synaptic function and plasticity. For instance, exercise increases the brain-derived neurotrophic factor (BDNF) gene expression in hippocampus and caudal neocortex (Neeper et al., 1998; Oliff et al., 1998), increases the expression of basic fibroblast growth factor in the hippocampus, cerebellum, and cerebral cortex of rats (Gomez-Pinilla et al., 1997;
Gomez-Pinilla et al., 1998), and increases the phosphorylation of a transcriptional regulator, cyclic AMP response element-binding protein (Shen et al., 2001). In addition, combined antidepressant treatment and physical activity have an additive, potentiating effect on BDNF mRNA expression in the rat hippocampus (Russo-Neustadt et al., 1999; Russo-Neustadt et al., 2000). These results imply that exercise has beneficial effects on the brain function by regulating downstream anatomical and functional changes that support brain plasticity.

Therefore, this study was designed to explore the effects of chronic physical exercise on the metabolism and clearance of brain Aβ peptides. We hypothesize that increase physical exercise may maintain the healthy state of cerebral vessels, which in turn contribute to the clearance of Aβ peptides, and thus lowering the chance of acquiring AD.

結果與討論

Eighteen male C57BL/6 mice (10 weeks old) were used in the first study. They were divided into two groups (n=9 for each group): the control and the exercise groups. The animals in the exercise group received running training, whereas the controls did not receive the exercise program. After one-week familiarization, animals in the exercise group ran on a leveled treadmill at the speed of 12 m/min for 60 min/d, 5 d/wk, 4 weeks in total. One day after the training, two groups received intra-hippocampal Aβ injections (2 µg/0.4 µl), and the animals were sacrificed 24 hours later. The brains of five animals in each group were dissected into cortex, hippocampus, cerebellum, and sub-cortical regions. The brain tissues were homogenized in homogenizing buffer (H buffer) containing guarnidine-HCl in 50 mM Tris/HCl, pH 8.0 with protease inhibitor cocktails. The homogenates were spun at 100,000 x g for 1h at 4°C, and the protein concentrations of the supernatants were determined. The protein concentration adjusted supernatants were either subjected to ELISA for Aβ quantification, or western blotting for protein quantification. The other four mice received transcardial perfusion with ice-cold saline followed by 4% paraformaldehyde.

Our ELISA analyses indicated that no differences in the brain regional distribution and Aβ levels were observed between the control and 4-week exercise groups. Approximately 80% of the injected Aβ remained in the ipsilateral hippocampus, whereas the rest mainly resided in the ipsilateral cortical regions. The total Aβ levels in the contralateral hemisphere was lower than 2%. Western blotting analysis revealed that exercise did not alter the expression of cortical APP, Aβ degradation enzyme-neprilysin, and the vascular Aβ binding/transpotor molecule-RAGE. Neither did the injection of Aβ change the expression of these proteins. Interestingly, the injection of Aβ showed a trend in elevating the cortical LRP levels. Such effect was more pronounce in the exercise group.

The paraformaldehyde fixed, free-floating sections (30 µm) were be treated with 1% bovine serum albumin (BSA) in PBS for 30 minutes at RT before incubation with primary antibodies (6E10: Aβ; CD11B: microglia; anti-GFAP: astrocyte; anti-VE-cadherin: vessel) diluted in 1% BSA in PBS. After washing, sections were stained with an immunoperoxidase method and visualized by the avidin-biotin-peroxidase (ABC, Vector Laboratories, Burlingame, CA)/DAB method. Our results revealed no activation of microglia, astroglia or the localization of Aβ in blood vessel in both the control and exercise groups.

Considering longer time may be required for the exercise to become effective in the
clearance of brain Aβ, we then extent the exercise training period to 12 weeks. Eighteen male C57BL/6 mice (20-week-old) were used in the second study. After one-week familiarization, animals in the exercise group ran on a leveled treadmill at the speed of 12 m/min for 60 min/d, 5 d/wk, 12 weeks in total. One day after the training, two groups received intra-hippocampal Aβ injections (100µg/0.2µl), and the animals were sacrificed 48 hours later. The brains of five animals in each group were dissected into cortex, hippocampus, cerebellum, and sub-cortical regions. The brain tissues were homogenized and the supernatnats were prepared afterwards. The other four mice received transcardial perfusion with ice-cold saline followed by 4% paraformaldehyde as described previously.

Our preliminary analyses indicated that no differences in the brain regional distribution and Aβ levels were observed between the control and 12-week exercise groups. Majority of the injected Aβ remained in the ipsilateral hippocampus. Western blotting analysis revealed that 12-week exercise did not change the expression levels of RAGE and SOD in cortex and hippocampus. Although did not reach significant level, the Aβ-injection ipsilateral side seemed to have higher RAGE and lower SOD levels in the 12-week exercise group than those of control animals.

Taken together, these results suggest that chronic exercise does not enhance the brain Aβ clearance efficacy under normal and health condition. Because the injection of Aβ was perform at the end of exercise, it is possible that exercise-exerted effects are not potent enough to last for more than 48 hours. Our study in Sub-project 1 also indicated that the exercise-induced hippocampal up-regulation of BDNF was acute and transient, that were last less than 48 hours. Therefore, in the following year, the effects of exercise on the clearance of brain Aβ will be investigated during the exercise training period. Furthermore, it is also possible that only when the animals encounter insults or certain stresses, can the beneficial effects of exercise be noticed. Therefore, the effects of exercise on Aβ clearance in brain will be investigated in the hypoperfused and/or LPS-challenged mice.

參考文獻

Huang TY, Chu TF, Chen HI, Jen CJ. (2000) FASEB J 14, 797-804.