NEUROPROTECTIVE COMPOSITION EFFECTIVE FOR AMELIORATING MENTAL DECLINE: VOLUME MR IMAGE IN VIVO

G. Li[1], X-F. Yang[1], Q. C. Chan[1], G. J. Gu[1], and E. S. Yang[1]

[1]Jockey Club MRI Centre, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HK, HKSAR, China, People's Republic of

Introduction:

In the last several years there has been an increasing interest in the possible role of oxidative stress in Alzheimer's disease (AD). Antioxidants are believed to be important in health maintenance through the modulation of oxidative processes in the body. Oxidative damage with the unregulated production of reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals has been implicated in a growing number of clinical disorders such as atherosclerosis, stroke, Parkinson's disease and AD. Mechanisms responsible for the ROS-mediated injury to cells and tissues mainly include lipid peroxidation, oxidative DNA damage, and protein oxidation, but there is also evidence that ROS can induce the process of cell death. Indeed, unbalance in the endogenous antioxidant system can modulate cellular proliferation, either in a positive or a negative way, respectively leading to stimulation in cell proliferation at low levels of peroxides or to apoptotic/necrotic cell death at higher concentrations. Based on this background, it is clear that investigating compounds able to counteract this oxidative damage may have a relevant clinical impact for MCI and AD.

Materials and methods:

One hundred and twelve mice (28 APP/PS1, 28 APP, 28 PS1 and 28 wild types) were studied. Half of each genotype group (n=14 per group) was treated with CoQ10 2400 mg/kg/day, Acetyl-L-Carnitine 1000 mg/kg/day, D-ribose 800 mg/kg/day, lecithin 1200 mg/kg/day, vitamin E 800 I.U/kg/day and Grape Seed 300 mg/kg/day and the other half with placebo for 60 days.

MRI was performed twice on a Philips Achiva 3 T whole body MRI at the Jockey Club MRI Centre, Hong Kong with a transmit/receive wrist coil before and after treatment. To optimize contrast between grey and white matter for morphometric measures, we used a high-resolution proton density weighted (PDW) 3-dimensional (3D) turbo spin echo (TSE) sequence. Transverse brain images were taken with repetition time (TR) = 200 ms, echo time (TE) = 26 ms, flip angle = 90 degrees, water fat shift (WFS)/bandwidth (BW) = 3.029 pix/143 Hz, field-of-view (FOV) = 10 × 10 cm, matrix size = 400 × 400 producing an in-plane voxel size of 0.25 mm X 0.25 mm, TSE factor = 3, the thickness of the slices in the through-plane direction = 0.5 mm without slice gap, and number of signal averages (NSA) = 12. The hemispheric and hippocampal volumes were delineated with manual region of interest (ROI) tracing using the image processing software Analyze 6.0 (AnalyzeDirect USA). For the calculation of hemispheric and hippocampal volume, ROIs were manually drawn on whole hemisphere (HM) and whole hippocampus (H) of the PDW-3D-TSE images using Analyze (Figure 1). In order to correct for normal size differences between animals, volume results were expressed as a ratio relative to the intracranial volume. The intracranial volume was determined by tracing the margins of the skull's inner table (Figure 1). This normalizes hippocampal and hemispheric volume for inter animal differences in brain size.

Plaques were quantified by manual counting. Circular regions of interest (ROIs) 0.75 mm in diameter were placed electronically over the cortex, six ROIs per section. This was repeated on five coronal sections evenly spaced throughout the cortex for a total 60 ROIs per image volume. The number of plaques in each ROI was counted manually. The mean number of plaques per ROI was summed for each type of image (Congo Red, antibodies Aß 40 and 42) (Figure 2). Plaque counts were repeated four times on successive days to assess precision of the method. The SD over four repeated measures at each ROI was averaged across all plaque-containing ROIs in each animal. The diameters of plaques were measured using Axiovision image analysis software (Zeiss , Thornwood, NY).

Results:

In placebo group (Figure 3A), the relative hippocampal volume was significantly decreased after 60 days in APP/PS1 mice ($P<0.005$) and in APP mice ($P<0.01$) with the comparison with before the formula treatment. As for formula treated group (Figure 3B), no significantly hippocampal atrophy was observed in all four mouse groups. The result indicates that the formula preserves hippocampal volume in APP/PS1 double transgenic mice and APP single transgenic mice. The relative hemispheric volume was significantly decreased after 60 days placebo treatment with the comparison of before the placebo treatment in APP/PS1 mice ($P<0.001$); APP mice ($P<0.005$) and PS1 mice ($P<0.05$) (Figure 4A). The hemispheric volume was not significantly decreased after 60 days formula treatment in all four mice groups (Figure 4B). The result indicates that the formula preserves hemispheric volume in APP/PS1; APP and PS1 mice. Figure 2 shows samples of plaques in APP/PS1 double transgenic mice. The number of plaques in both hippocampus and hemisphere was decreased significantly after 60 days of formula treatment in APP/PS1 double transgenic mice using the images stained with antibodies Aß 40 (Figure 5A); Aß 42 (Figure 5B) and Congo Red (Figure5C) compared with the placebo treatment. The result indicates that the formula can reduced the number of plaques in the aging brain.

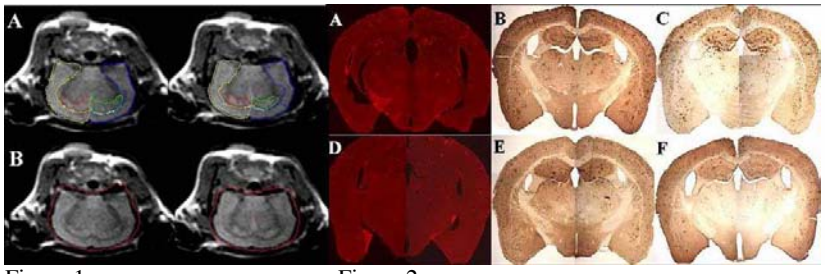

Figure 1.

Figure 2

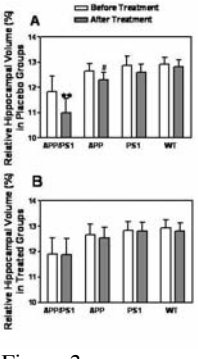

Figure 3

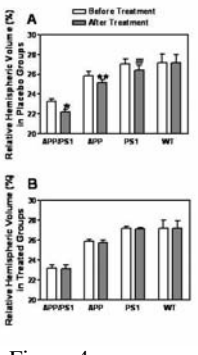

Figure 4

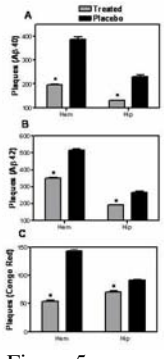

Figure 5

Conclusion: APP / PS1, APP, PS1 and wild type mice treated with the formula exhibited significantly less atrophy in hemisphere and hippocampus than those receiving placebo. APP/PS1 and APP mice treated with the formula exhibited significantly less number of plaques in the brain. The neuro-protective effect of the formula on hemispheric volume, hippocampal volume and the number of plaques in the mouse brain were related to genotype; greater in APP/PS1 and APP than PS1 mice and least in wild type mice. The formula appears to have a neuron-protective effect on hemisphere, hippocampus and number of plaques in aged mice. Our result indicated that the formula may have therapeutic potential in the prevention and treatment of MCI and AD. It has an anti-aging and memory retention function.

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