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Characterization of Fe, Cu and Zn in organs of PDAPP transgenic mice by XRF spectrometry†

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A large body of evidence indicates that abnormalities in the levels of iron, copper and zinc and their metabolism are associated with neurodegenerative diseases. However, it is difficult to decide whether any observed changes of trace elements reflect the primary disease process or are secondary to a primary process or mechanism. In the present study, Fe, Cu and Zn in organs of transgenic mice which express the familial Alzheimer's disease (AD) gene and normal mice of the same species and ages were determined by X-ray fluorescence (XRF) spectrometry. The results show that Fe concentrations in a variety of organs and tissues were significantly increased whereas Zn concentrations decreased in the transgenic mice as compared with the 'normals'. The levels of Cu in transgenic mice were also altered. Data obtained in the present study suggest that expression of the familial AD gene in mice results in altered homeostasis of Fe, Cu and Zn in organs of the animals, which may in turn accelerate the process of neurodegeneration.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in older people and is characterized by memory loss, language deterioration, impaired visuospatial skills, poor judgment and indifferent attitude, but preserved motor function. AD is defined by brain pathology and its important aspects are neuritic plaques, neurofibrillary tangles, chronic inflammation, death of neurons and loss of connections between them. The pathogenesis of the origin of AD has not yet been accurately explained and the latest studies have proven AD to be a disease of multifactorial etiology.[1] In the past decade, the role of trace elements in the neuropathology of AD has attracted great attention.[2–6] It is reported that the homeostasis of several trace elements and their respective binding proteins is significantly altered in the AD brain.[7–9]

PDAPP transgenic mice represent a valuable model in studying AD pathogenesis and for testing novel therapeutic strategies. This transgene used a platelet-derived (PD) growth factor-B chain promoter to drive a human amyloid precursor protein (APP) minigene encoding alternatively spliced hAPP that contains the mutation V3F in position 717 that is associated with familial AD.[10]

Various XRF techniques are especially suited to studies of AD and play an important role. Murray *et al*.[11] investigated the elemental content of hippocampal slices from normal human brain and from brains of AD patients by XRF using both electron and proton beam microprobes. Tokutake *et al*.[12] analyzed the Al and Si contents of lipofuscin granules from autopsied brains of AD subjects and those without dementia by energy-dispersive x-ray spectrometry with a scanning electron microscope. Ishihara *et al*.[13] demonstrated the potential of synchrotron radiation x-ray fluorescence (SRXRF) spectroscopy for studying the relationship between trace elements and AD by describing the application of this method to the mapping and quantification of metallic elements within certain single neurons from brain tissues affected by AD. Collingwood *et al*.[8] found a disrupted brain–iron metabolism in human AD brain tissue sections using SRXRF. However, it is difficult to decide whether any observed changes of trace elements reflect the primary disease process or are secondary to a primary process or mechanism.

The aim of the present study was to clarify whether transgenosis can induce changes in Fe, Cu and Zn levels in the organs of PDAPP transgenic mice. XRF spectrometry was used for the determination of Fe, Cu and Zn in the study of transition metal levels in multiple organs of transgenic mice which express the familial AD gene.

EXPERIMENTAL

Sample preparation

Twelve PDAPP^{V717} transgenic mice of both sexes (M : F 1 : 1) aged 12 months and 12 normal mice of both sexes (M : F 1 : 1) of the same species and age were supplied by the Institute of Experimental Animals, Chinese Academy of Medical Sciences. They were fed a standard laboratory diet and given water *ad libitum* prior to use. Animals were killed

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under ether anesthesia and the brain, femur, heart, kidney, liver, lung, spleen and testis (male) were removed. The organs were rinsed immediately with deionized water and then freeze-dried. The dried samples were triturated and homogenized using an agate mortar.

For each sample, 30–50 mg of powder were accurately weighed and digested with 100 µl of 1:1 (v/v) nitric acid after ashing and 100 µl of a standard solution of YCl_3 (0.105 mg ml^{-1}) were added. Finally, 10 µl of the solution were pipetted on to a Mylar backing film and dried in a clean cabinet.

Equipment and measurements

XRF spectrometry was used to determine concentrations of Fe, Cu and Zn. On a Philips PW 1830 x-ray generator, the X-ray beam used for sample irradiation was generated by an x-ray tube working at a high voltage (50 kV) with an electron current of 15 mA. The cross-section of the beam at samples was about 4 mm and the observation angle to the exciting beam direction was 90°. In order to improve the detection limit of the x-ray peak, especially for Cu, the sides of the Si (Li) detector were wrapped in Al and Nd metal foil to reduce the background of Compton scattering. The intensities of Kα lines were measured for the elements of interest. Each spectrum was collected for 400 s. The spectra were analyzed using the AXIL program.

Certified reference materials (NIST CRM 1577a Bovine Liver and CRM 1566a Oyster) were simultaneously prepared in the same manner as the analytical control.

Statistical analyses were performed using SPSS 11.0 for Windows software (Microsoft). The differences in the mean values between two groups were tested by an independent sample *t*-test and between four groups by one-way analysis of variance (ANOVA) followed by the LSD *post hoc* test for multiple comparisons. Statements of significant differences are based on accepting $p < 0.05$.

RESULTS

XRF is a powerful and sensitive multielement technique for studying trace elemental concentrations in biological tissues and is advantageous when compared with other techniques. Table 1 gives the results obtained and the certified values for two certified reference materials. The results are in good agreement with the certified values, which means that the proposed method is suitable to be used in studies of transgenic mice.

The concentrations of Fe, Cu and Zn in brain, femur, heart, kidney, liver, lung, spleen and testis (male) of transgenic mice and normal mice are shown in Fig. 1. Significant sex-dependent elemental concentrations can be

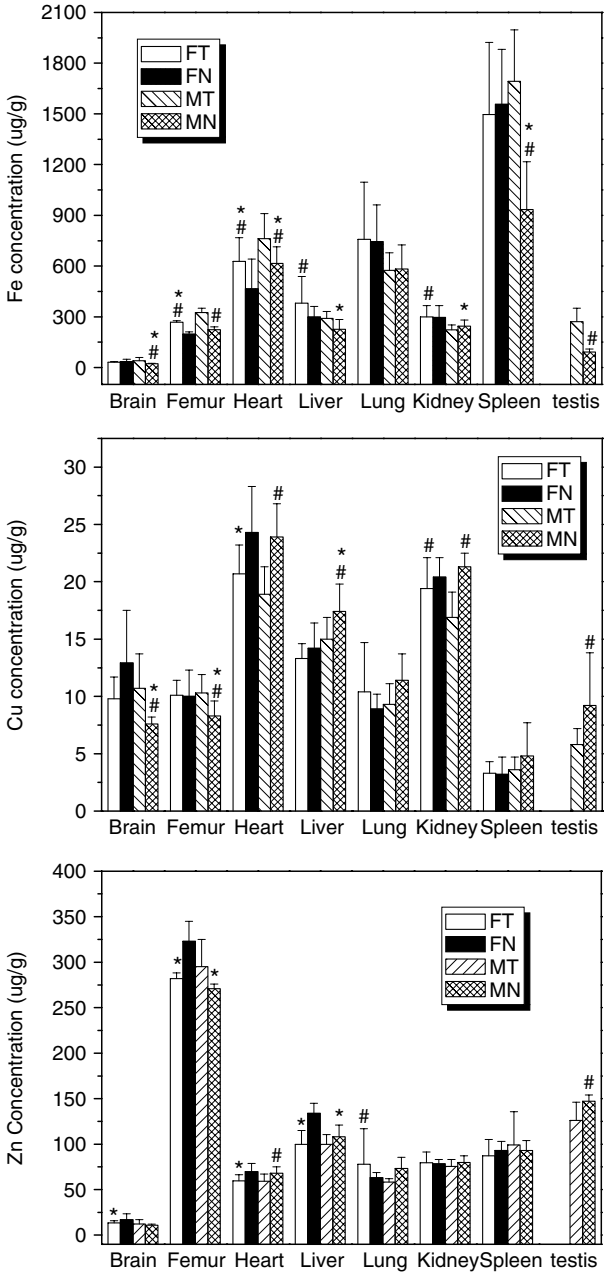

Figure 1. Fe, Cu and Zn concentrations in organs of transgenic and normal mice. FT, female transgenic mice; FN, female normal mice; MT, male transgenic mice; MN, male normal mice. $^{*}p < 0.05$ compared with normal male mice; $^{\#}p < 0.05$ compared with transgenic female mice.

Table 1. Comparison of Fe, Cu and Zn concentrations (µg · g^{-1}) measured in certified reference materials (mean ± standard deviation) and the certified values

	This work			Certified value		
Sample	Fe	Cu	Zn	Fe	Cu	Zn
NIST 1577a	175 ± 18	164 ± 11	127 ± 8	181 ± 19	153 ± 11	122 ± 5
NIST 1566a	527 ± 51	77.5 ± 5.6	983 ± 59	518 ± 29	77.7 ± 1.3	800[a]

[a] Non-certified value.

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observed in both the transgenic and normal mice. For normal mice, males showed a higher Fe content in heart and lower Fe concentrations in brain, kidney, liver and spleen compared with females. The Cu concentrations in liver were higher but in brain and femur were lower in males than in females. Females had higher Zn contents in brain, femur and liver than males.

For transgenic mice, males showed higher Fe concentrations in brain and heart and lower Fe concentrations in kidney and liver compared with females. The Cu contents in kidney and Zn contents in lung were higher in females.

For females, transgenic mice showed higher Fe contents in femur and heart and lower Zn contents in femur, heart and liver compared with normal mice. The Cu concentrations in heart were lower in transgenic mice than normal mice.

For males, transgenic mice showed higher Fe contents in brain, femur, heart, spleen and testis and lower Zn contents in heart and testis compared with normal mice. The Cu concentrations in brain and femur were higher in transgenic mice than normal mice.

DISCUSSION

The role of trace elements, notably Fe, Cu and Zn, in AD has been of interest in recent years. There is increasing evidence that Fe, Cu and Zn are closely related to AD by enhancing the formation of reactive oxygen species and toxic Aβ oligomers and facilitating the formation of the hallmark amyloid deposits in the AD brain.[14] In the present study, Fe, Cu and Zn in organs of transgenic mice which express the familial AD gene and normal mice of the same species and ages were determined.

Significant sex influences on trace element concentrations in organs and tissues of humans and animals have been reported. Rahil-Khazen *et al*.[15] determined trace element levels in human autopsy tissues by inductively coupled atomic emission spectrometry. They found that generally males had higher concentrations of trace elements in the different tissue samples than females, with the exception of Mn in the brain front lobe and heart and Sr in the liver. Sturaro *et al*.[16] reported that the contents of Zn, Cu, Ni, Mn and Pb were higher in female than in male hair. In a study performed on urban populations of moles, female moles showed higher Zn concentrations in spleen, gonads and skin, higher Cd concentrations in femur and stomach and lower Cu concentrations in kidneys compared with males.[16] The observed significant sex-dependent organ and tissue contents could be ascribed to sex-specific physiological regulatory mechanisms. For example, a second Zn-binding protein besides metallothionein has been assumed, which only occurs in the female shrew *Sorex araneus*.[17]

This study shows that Fe concentrations in a variety of organs and tissues were significantly increased whereas Zn concentrations decreased in transgenic mice of both sexes as compared with 'normals', which were age matched. Iron, which is of considerable importance for normal neurological function, is highly regulated in all organ systems. Excessive tissue accumulation of Fe enhances the production of the highly reactive and toxic hydroxyl radical, thus stimulating oxidative damage.[2,3,5] There is a well-established link between iron overload in the brain and pathology associated with neurodegeneration in a variety of disorders including AD.[8] This association was first discovered in AD tissue by Goodman.[7] In addition to abnormally high concentrations of iron in autopsy brain tissue, Fe has also been shown to accumulate at sites of brain pathology such as senile plaques. Zn is an essential element and is required for growth and normal development, and there are over 200 identified zinc-containing enzymes. Moreover, it is a constituent of the powerful antioxidant Cu/Zn superoxide dismutase (Cu/Zn SOD), which catalyzes the dismutation of free radical $O_2^{\bullet}$ to H_2O_2, therefore reducing the risk of formation of the hydroxyl radical $HO^{\bullet}$, which is the most reactive species. Decreased Zn levels in tissues may result in reduction of Cu/Zn SOD activity and subsequently accelerate the process of cell aging and death via oxidative damage.

There is increasing interest in the role of Cu in the neuropathology of neurodegenerative disorders.[4] In the present study, it was found that transgenic mice of both sexes showed lower Cu contents in heart. For males, the Cu concentrations in brain and femur were higher whereas in kidney they were lower in transgenic mice than normal mice. Cu is an essential element for life and the function of numerous enzymes is basic to neurobiology. Decreased Cu levels in tissues may also result in a reduction in Cu/Zn SOD activity. However, free or incorrectly bound Cu^{2+} can catalyze the generation of the most damaging radicals, such as hydroxyl radical, giving a chemical modification of the protein, alternations in protein structure and solubility and oxidative damage to surrounding tissue.[4] Further studies on the status of the antioxidative system and lipid peroxidation in tissues of transgenic and normal mice should be carried out to evaluate the role of Fe, Cu and Zinc in oxidative damage.

CONCLUSIONS

In most studies on the relationship between transition metals and AD, only metal levels in the brain were discussed. The present study has demonstrated that expression of familial AD gene in mice results in disturbed homeostasis of Fe, Cu and Zn both in brain and in other organs to different extents. This is secondary to a primary mechanism but may in turn accelerate the process of neurodegeneration.

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