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Trace element analysis of hairs in patients with dementia

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The trace elements of scalp hair samples from $\geq$60-year-old dementia patients and normal persons have been studied by X-ray absorption near-edge spectroscopy (XANES) in fluorescent mode and wavelength-dispersive X-ray fluorescence spectrometry. Comparisons of hair trace element levels of age-matched dementia patients and normal persons revealed significantly elevated amounts of calcium, chlorine and phosphorus in dementia patients relative to normal persons. The results of XANES measurements identify the chemical forms of deposited calcium and phosphorus in the hair samples of both dementia patients and normal persons to be calcium chloride ($CaCl_2$) and phosphate (PO_4^{3-}), respectively. The amount of sulfur in hairs of dementia patients was found to be not significantly different from that in normal persons. The sulfur *K*-edge XANES spectra, however, show significantly higher accumulations of sulfur in the sulfate (SO_4^{2-}) form in hairs of Alzheimer's disease and Parkinson's disease dementia patients. This study presents the possible roles of calcium, chlorine, phosphorus and sulfur in the etiology of dementia in elderly patients.

Keywords: X-ray absorption spectroscopy (XAS); wavelength-dispersive X-ray fluorescence spectrometry (WDXRFS); X-ray absorption near-edge spectroscopy (XANES); dementia.

1. Introduction

Dementia is a syndrome characterized by loss of memory and degradation of other cognitive skills caused by disease and trauma (Prince, 2007). The memory loss and degradation of cognitive skills may occur gradually or acutely. Dementia may be classified based on clinical, genetic or neuropathological causes. The most common cause of dementia is Alzheimer's disease (Gottfries, 1995). Other major causes of dementia are vascular diseases, psychiatric illnesses and neurodegenerative diseases. Neurodegenerative dementia includes fronto-temporal dementia and Lewy body dementia (Gottfries, 1995). In 2000 there were approximately 18–25 million people worldwide suffering from dementia and the number is expected to increase to 32–40 million in 2020 (Prince, 2007). Understanding different types of dementia, as well as the causes of cognitive skill degradation and memory loss, can help doctors diagnose the disease early and give proper care to patients. The etiology of the disease is still not fully clear. Exposure to toxicants or oxidant species, as well as diet and age, may act as agents in the development of the disease. Imbalance of trace elements in the human body is a possible indicative factor of dementia. Previous reports show evidence of metals as an indicative factor in dementia caused by Parkinson's and Alzheimer's diseases (Barnham & Bush, 2008). The trace elements in dementia patients are reported to be present in cerebrospinal fluid (Hershey *et al.*, 1983), brain tissue (Sunde *et al.*, 1997; Miller *et al.*, 2006) and blood (Squitti *et al.*, 2006).

Since 1992, scalp hair has increasingly been used to access human systemic levels of elements instead of blood and other types of sample. Human hair is known to normally contain approximately 30 species of trace elements in the range 2500–10000 p.p.m. (Dutcher & Rothman, 1951; Pautard, 1963). Comparing with other types of clinical specimens, hair samples have some advantages over blood, serum or urine samples. It is easier and safer to collect, carry and store, and the analysis procedures are less expensive. Unlike blood, serum and urine, hair provides historical information on the concentration of trace elements in the body as well as the nutritional condition over a long period of time. Furthermore, trace elements are often more concentrated in hair than in body fluids. Hair analysis also provides information on the intracellular accumulation of trace elements. These make hair an excellent

choice as a screening tool in certain situations (Senofonte *et al.*, 2000; Shamberger, 2002; Forte *et al.*, 2005).

This work therefore aims to use scalp hair for analysis of trace element contents in $\geq$60-year-old dementia patients by comparison with a control group of normal elderly using the combined techniques of synchrotron X-ray absorption near-edge spectroscopy (XANES) and wavelength-dispersive X-ray fluorescence spectrometry (WDXRFS).

2. Experimental

2.1. Sample preparation

Natural hairs, which had not been dyed, bleached or straightened, were taken from the scalps of 15 dementia patients who were over 60 years old, composing one mild Parkinson's disease dementia (PDD) patient, two moderate and one late Alzheimer's disease (AD) patients and 11 vascular dementia (VaD) patients. Hair samples from 15 normal persons who were over 60 years old were also taken, to be used as a control group. The dementia patients had been diagnosed by a psychiatric doctor at Nakhon Ratchasima Rajanagarindra Psychiatric Hospital.

Scalp hairs were cleaned following the method of Baranowska *et al.* (2004). Hair samples, weighing approximately 2–3 g, were treated in four stages, each of length 15 min: in 20 ml of acetone, twice in 40 ml of distilled water, and then again in 20 ml of acetone. After washing, they were dried at 353 K. The clean dry hairs were then kept in a desiccator until the measurements were taken.

2.2. WDXRFS measurements and statistic calculations

X-ray fluorescence (XRF) measurements of hair fibres contained in a sample cup (diameter 27 mm) were performed using a Philips MagiX (WDXRF) equipped with a rhodium X-ray tube. The data were analyzed using *SuperQ V3.0* software.

Statistical calculations were conducted using the *SPSS for Windows* package (version 11.5.0). The normality of the data distributions was analyzed using Shapiro–Wilk statistics (Shapiro & Wilk, 1965). An independent sample *t*-test was applied for group comparisons.

2.3. XANES measurements

For each measurement the types of sulfur, calcium and phosphorus along the length of the hair strands of all groups were determined using XANES. The hair strands of length 8 cm and width 2 mm were attached to kapton tape and the other side was covered with Mylar X-ray film (Chemplex, USA) and exposed to X-rays. XANES measurements were analyzed every 2 cm along the length of the hair strands. The XANES experiment was performed at the XAS station of beamline 8 of the Siam Photon Laboratory, Synchrotron Light Research Institute, Thailand. The flux at the sample is $\sim 10^8$–10^{10} photon s^{-1} at 100 mA beam current (Klysubun *et al.*, 2007). *K*-edge XANES of sulfur, calcium and phosphorus contained in the hair samples were measured in fluorescent mode. A double-crystal monochromator with Si(111) crystals was used to monochromatize the X-ray beam for the *K*-edge measurements of sulfur and calcium, while InSb(111) crystals were used for the measurements of phosphorus. The XANES spectra were recorded using an ionization chamber and a Lytle detector. The ionization chamber was filled with nitrogen gas (16 mbar for the phosphorus, 37 mbar for the sulfur and 160 mbar for the calcium). A polypropylene window was used to separate the sample chamber from the ionization chamber. The Lytle detector or five-grid ionization detector was placed on the left-hand side of the sample. The sample chamber was flowed with He gas. The measurements took $\sim$8–10 min per sample. All of the XANES spectra were averaged and normalized using the *IFEFFIT* package, version 1.2.11 (Ravel & Newville, 2005). Radiation damage to the samples was observed to be negligible by visual inspection of the measured samples and the reproducibility of the XANES spectra.

Table 1
Trace element content in hair samples of dementia patients and control normal persons.

	Concentration (wt%)	
Element	Normal	Dementia
O	58.60 ± 0.64	58.05 ± 0.88
S	38.06 ± 0.69	37.80 ± 0.59
Cl	1.67 ± 0.87[a]	2.94 ± 0.92[b]
Si	0.31 ± 0.12	0.23 ± 0.03
Ca	0.79 ± 0.24[c]	1.04 ± 0.31[d]
P	0.76 ±. 07[e]	0.95 ± 0.28[f]
Mg	0.37 ± 0.07	0.43 ± 0.11
Al	<0.01[g]	<0.01[g]
Na	<0.01[g]	<0.01[g]
Fe	<0.01[g]	<0.01[g]
K	<0.01[g]	<0.01[g]

The letters (*a*, *b*, *c*, *d*, *e*, *f*) denote values which are significantly different from one another using the independent samples *t*-test. Letter (*a*) significantly different from letter (*b*) at $P < 0.05$. Letter (*c*) significantly different from letter (*d*) at $P < 0.05$. Letter (*e*) significantly different from letter (*f*) at $P < 0.05$. No statistical tests were performed with values lower than 0.01% (letter *g*).

3. Experimental data and discussion

The chemical compositions of scalp hair samples were initially identified using WDXRFS. Significant amounts of seven elements (O, S, Cl, Si, Ca, P and Mg) were found in both dementia and control samples. Small amounts of four elements (Al, Na, Fe and K) were also present in both groups. The results are shown in Table 1. There are significantly elevated amounts [with $P < 0.05$ (where P is the P-value, the probability of obtaining a test statistic)] of Cl, Ca and P in the dementia group compared with the control group. This indicates the imbalance of macronutrients in dementia patients.

The WDXRFS results coincide with the results reported previously. Instrumental neutron activation analysis (INAA) was used to determine the concentrations of 16 elements in selected brain regions and grey- and white-matter specimens from histologically verified AD patients and the age-matched control group, where significantly different ($P < 0.05$) mean concentrations of Br, Cl, Cs, Hg, N, Na, P and Rb were

observed in AD bulk brain samples compared with those of the control group (Ehmann *et al.*, 1986). Vance *et al.* (1988) reported studies of concentrations of elements in hairs and nails of AD and control subjects using INAA, where elevated levels of six elements (Br, Ca, Co, Hg, K and Zn) revealed significant imbalances between AD and control groups. In contrast, Ca content in hair samples from a Parkinson's disease patient without dementia analyzed by inductively coupled plasma atomic emission spectrometry was reported to be slightly, though not significantly, lower than that of the control group (Forte *et al.*, 2005). These results indicate that the elevated level of Ca may be an indicative factor for dementia.

Calcium may be involved in the mechanism of dementia since it is essential for neuronal development, synaptic transmission and plasticity and metabolic pathway regulation (Ramonet *et al.*, 2002). Dysregulation of intracellular calcium homeostasis, for instance calcium precipitation, has been found to play a role in neurodegenerative processes and neuron death (Mattson & Chan, 2001; Ramonet *et al.*, 2006). In humans, basal ganglia and choroid plexus calcifications were found by computer tomography and other techniques to be associated with neuronal death in the neurodegenerative disorders, such as AD, Parkinson's disease (PD) (Friedland *et al.*, 1990; Sebeo *et al.*, 2004) and VaD (Ramonet *et al.*, 2002). The calcium precipitation process in brain and tissues including hair has been reported to affect the blood calcium level by lowering serum calcium in early (Landfield *et al.*, 1991) and moderate (Rípová *et al.*, 2004) AD and VaD (Rípová et al., 2004).

Moreover, the abundance of phosphorus has been observed together with calcium in brain calcification of AD and VaD (Ramonet *et al.*, 2006). Elemental X-ray microanalysis revealed that calcium associates mainly with P, O and/or other minor components such as Fe, Zn or S in the brain calcification (Ramonet *et al.*, 2006). Co-aggregation between heterogeneous elements can be formed into biological hydroxyapatites (Honda *et al.*, 1994; Kim, 1995). In human tissues phosphorus exists in both organic and inorganic forms (Yu & Lee, 1987). Organic phosphorus comprises the phospholipids, nucleic acids and phosphoproteins that are needed for cellular integrity and metabolism. Intracellular inorganic phosphorus provides substrate for the synthesis of energy-generating compounds including adenosine triphosphate. Serum phosphorus is mostly in inorganic forms consisting of orthophosphate ions. Dietary intake and excretion in urine and faeces maintain homeostasis. Parathyroid hormone regulates renal phosphorus reabsorption with the help of calcitonin, thyroid hormone and growth hormone (Yu & Lee, 1987). There is also an internal homeostasis kept between intracellular and extracellular levels. Phosphorus also has a role in the hyperphosphorylation process of tau and amyloid precursor protein in AD (Pierrot *et al.*, 2006). The level of protein phosphorylation is controlled by the opposing actions of protein kinases and phosphatase. Abnormal protein phosphorylation may contribute to the progression of AD by modifying substrates in various processes such as enzymatic activity, subcellular localization, ligand binding or by interaction with other proteins (Chung, 2009). Consequently, elevated levels of phosphorus may be associated with aberrant phosphorylation processes and/or co-precipitation with calcium or other elements. These results coincide with the WDXRFS results of the present work which show significant elevated levels of Ca and P in dementia patients.

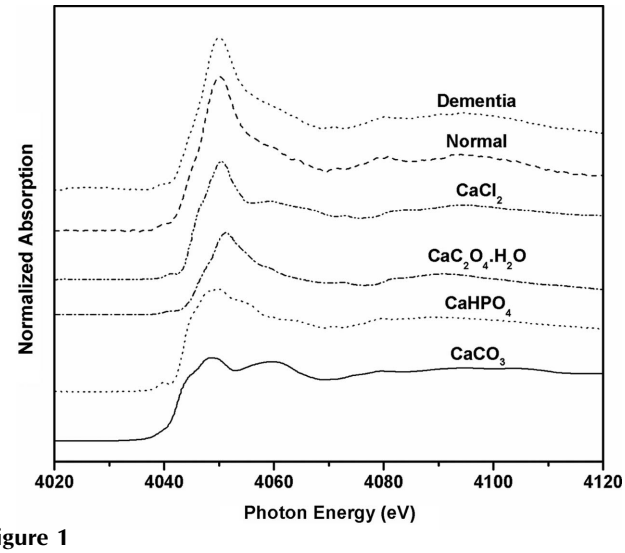

Figure 1
Calcium *K*-edge XANES spectra of human hairs from dementia and normal samples, together with the reference compounds used. The reference compounds are calcium carbonate ($CaCO_3$), calcium hydrogen phosphate ($CaHPO_4$), calcium oxalate monohydrate ($CaC_2O_4{\cdot}H_2O$) and calcium chloride ($CaCl_2$).

Sulfur is an element found in all cells of the body. It is needed for the production of keratin, a protein found in all cells, which is essential for the formation of bones, cartilage and tendons. It is also needed for digestion, elimination and bile secretion. Moreover, it is necessary for the production of hormone insulin, which balances blood sugar levels. Studies have shown that adequate levels of sulfur have a detoxifying effect on the body and help it to get rid of harmful toxins (Headfield *et al.*, 1990). Sulfur deficiency could result in skin problems and poor growth, especially of the hairs and nails. It could also affect functioning of the body. A comparison of sulfate concentrations in plasma between AD patients and normal persons has been reported (Edwards *et al.*, 1993). The study showed that an averaged concentration of sulfate in ten patients with AD was not significantly different from that in the age-matched control or young healthy control group. These results indicated that plasma sulfate concentration was not altered in AD cases. WDXRFS results of the present work similarly show that concentrations of sulfur in hair samples of the dementia and control groups were not significantly different.

The measured XANES spectra of Ca *K*-edge from hair samples are shown in Fig. 1. The Ca *K*-edge spectra of dementia and control samples match that of standard calcium chloride ($CaCl_2$), with the first peak located at 4050.30 eV. Fig. 2 shows the measured P *K*-edge XANES spectra. The results show that the phosphorus contained in hair samples of

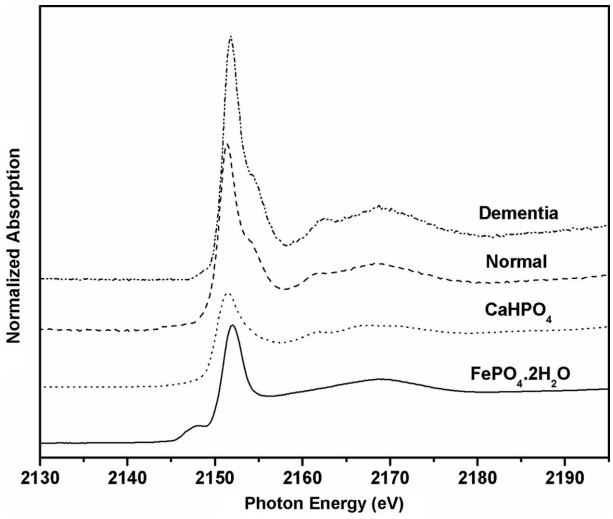

Figure 2
Phosphorus *K*-edge XANES spectra of hair samples from dementia and normal persons, together with a spectrum of the reference compounds: ferric phosphate dihydrate ($FePO_4{\cdot}2H_2O$) and calcium hydrogen phosphate ($CaHPO_4$).

both the dementia and control groups are in the form of phosphate (PO_4^{3-}), where the highest peak is located at 251.4 eV. These results indicate that elevated levels of Ca and P, shown above to be a possible indication of dementia, are present in the same chemical forms as in normal persons.

The sulfur *K*-edge XANES spectra are shown in Fig. 3. Four spectra of S *K*-edge standards are also shown indicating sulfur species with peaks at 2472.30, 2475.04, 2479.93 and 2481.13 eV. These peaks can be assigned to the thiol (−SH), σ^* (S−O), sulfite ($R-SO_3$) and sulfate ($R-SO_4$). The assignments of the peaks are based on the studies of Hitchcock *et al.* (1986), Sze *et al.* (1988), George & Gorbaty (1989), Prange *et al.* (2002) and Jalilehvand (2006). All the XANES results of hair samples from both the dementia and control groups show the first peak, located at 2471.6 eV, which describes the excitation of electrons to the valence state (white line). However, the spectra of the samples from AD and PDD patients additionally contain the sulfate peaks located at 2481.01 eV. This indicates that there are abundances of sulfate deposited in hairs of AD and PDD patients. Elevated level of deposited sulfate may therefore indicate the condition of AD and PDD. These results correspond to previous reports that the increases of plasma sulfate compound, such as heparan sulfate (Lindahl *et al.*, 1995) and dehydroepiandrosterone sulfate (Genedani *et al.*, 2004), were found in AD and PD patients. This may indicate that the sulfation process may be involved in the cause of dementias in the AD and PDD cases. The result, however, seems to be in contrast with another report which suggested that the deficiency of plasma sulfate may lead to reduced xenobiotic detoxification and can cause AD and Parkinson's disease (Headfield *et al.*, 1990).

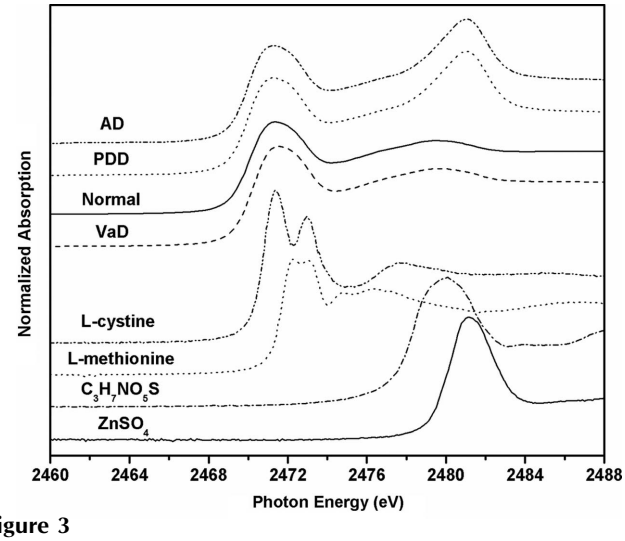

Figure 3
Sulfur *K*-edge XANES spectra of hair samples from AD, PDD and VaD dementia patients and normal persons, together with the reference compounds used. The reference compounds are zinc sulfate ($ZnSO_4$), cysteic acid ($C_3H_7NO_5S$), L-methionine and L-cystine.

Since hair grows approximately 1 cm per month (Yoshinaga *et al.*, 1993), the types of S, Ca and P have been monitored every 2 cm along the 8 cm length of hair strands in this study. All XANES results of each length along the hair samples from both dementia and control groups show the same spectra and the same compounds described above. This indicates that the chemical elements in hair are not changed over time. In addition, hair is a potential biomarker for diagnosis, following up and planning to prevent disease, and is easily collected and does not require any special storage or preservation. Keratin is the major protein of hair which makes it stable (Gellein *et al.*, 2008).

4. Conclusions

The combined techniques of WDXRFS and XANES were used to determine the concentrations of elements and their chemical forms contained in hairs of $\geq$60-year-old dementia patients in comparison with those of age-matched normal persons. The WDXRFS data indicate that concentrations of calcium, chlorine and phosphorus were significantly elevated in hairs of dementia patients. The chemical forms of deposited elements are identified by the XANES results. The *K*-edge XANES spectra show that calcium is deposited in the form of $CaCl_2$, while phosphorus is in the phosphate form. Elevation of these compounds may be associated with dementias *via* the mechanisms of element co-precipitations and aberrant phosphorylation processes. The amount of sulfur in hairs of dementia patients was not significantly different from that of normal persons. However, the sulfur *K*-edge XANES spectra show that sulfur in the form of sulfate is significantly abundant in AD and PDD patients, by comparison with that in normal persons. This indicates that the sulfation process may be involved in the cause of dementias in the AD and PDD cases.

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