Chapter 21

Metabolism Changes as Indicated by the Erythrocytes of Patients with Alzheimer's Disease

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/57511

1. Introduction

The aging processes proceed in small steps making it difficult to separate aging symptoms from early pathological processes. Cell metabolism involves metal ions and trace elements together with compounds necessary for optimal function. In case of imbalance in cells both compounds and distribution of metal ions may be changed but the change of metal ions and trace elements may probably be easier to identify than the large quantity of metabolized compounds. In cells there are distributions of elements, an elemental profile, indicating normal metabolism. Brain has a high demand of oxygen supply and need optimal supply of protection of reactive products. The erythrocytes pass brain many times and other organs accumulating information of elements during their way. We have chosen erythrocytes because oxygen dependent cells need regular supply of oxygen for a stable metabolism. Erythrocytes collect information for about 120 days and then will be replaced by new cells. Elemental profiles of erythrocytes reflect information in their own metabolism (limited metabolism) but may also give important information from other cells by deviations in the homeostasis, the element profile. For monitoring elemental profiles of the erythrocytes we have used inductively coupled plasma mass spectrometry (ICP-MS) which is selective, sensitive and allow analysis of elements in low concentration in a short time.

2. Material and methods

2.1. Patients

Thirteen diagnosed Alzheimer patients, 9 men and 4 women (mean age 61 y) and age matched controls (mean age 58 y) were selected for the study living in the same area. All patients participated voluntarily according to Helsinki declaration.

2.2. Samples

Whole blood (2x7 ml) was drawn into Vacutainer tubes for trace element analysis (BDH, with sodium heparin as anticoagulant). Centrifugation was started half an hour after venopuncture. The erythrocytes were separated by centrifugation at 120xg at 4 C for 15 minutes. After removing the buffy coat the erythrocytes were washed twice with 0.9 % NaCl at 1000xg for 5 minutes. The erythrocytes were transferred to cryo vials (Nunc) weighed and freezed at-18 C. The samples (0.6-0.8g, wet weight) were digested with nitric acid and hydrogen peroxide (both ultra pure) in microwave oven. The samples were diluted with Milli-Q water in 25 ml poly-propylene bottles.

2.3. ICP-MS instrumentation

For the elemental analysis of the erythrocytes ICP-MS, Elan 6100 DRC was used in peak hopping mode. The isotopes monitored: ^{107}Ag, ^{114}Cd, ^{208}Pb and ^{238}U. Integration time: 5 sweep, 100 msek. Internal standard 10 ppb Rh was supplied by externally feeding. ^{114}Cd was corrected for ^{114}Sn. The analytical technique used is described in more details [1, 2, 3].

2.4. Reagents and standards

Ultra pure nitric acid and hydrogen peroxide was obtained from Merck. ICP-MS standards were obtained from Johnson & Matthey, SRM 1566a and 1577a from NIST. The different isotopes were validated against Oyster tissue 1566a, Bovine liver 1577a.

The estimated accumulated mean error in the analysis of samples was ± 10 % or less generated in the sampling procedure, preparation, digestion, volumetric and weighing errors and error in the ICP-MS analysis. For statistical calculations of ICP-MS results Wilcoxon's nonparametric test was used.

3. Results

3.1. Elemental profiles of erythrocytes

The main transport of erythrocytes is oxygen from the lungs to cells and carbon dioxide back to lungs serving different organs including brain. The elemental profile of the erythrocytes reflect metabolism of oxygen supplied cells but also the metabolism of the erythrocyte itself.

http://dx.doi.org/10.5772/57511

As erythrocytes carry ferro ions in hemoglobin and other divalent elements e.g. zinc, magnesium, calcium they will also reflect external influence of metabolism. The elemental profile of erythrocytes will be a status report of cell metabolism translated in terms of metal ions and trace elements. In this study we report accumulated ions: silver, cadmium, lead and uranium in the erythrocytes of patients with Alzheimer's disease. The concentration of lead (wet weight) in the erythrocytes of patients with Alzheimer's disease is demonstrated in Figure 1. The result of cadmium analysis (wet weight) of the erythrocytes is shown in Figure 2. The concentration of silver in the erythrocytes is indicated in Figure 3. The concentration of uranium in the erythrocytes was significant higher that of controls Figure 4. In summary the concentrations of Pb, Cd, Ag and U were significant higher than those of controls.

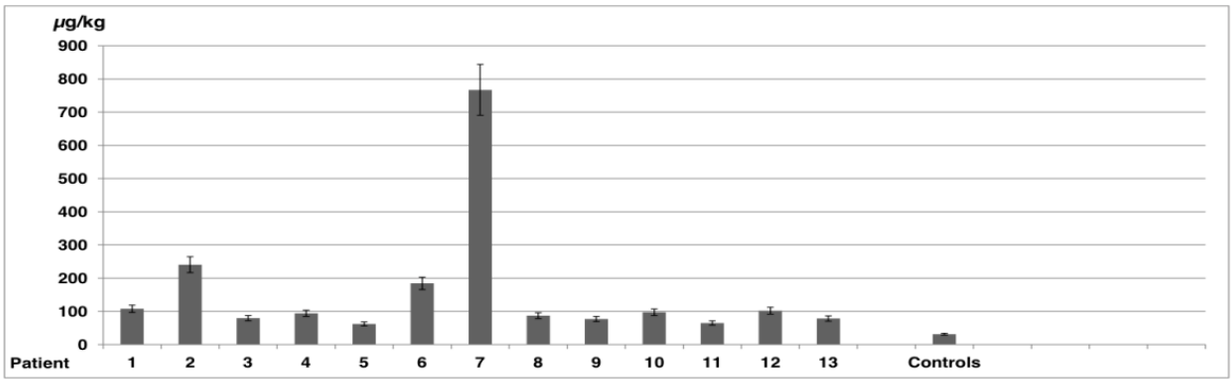

Figure 1. Lead concentration in erythrocytes of 13 patients with Alzheimer's disease. Patient 1-9 men, 10-13 women. Error bar 10%. The mean value of Pb in Alzheimer's patients was 157 µg/kg, mean of controls 31 µg/kg, standard error 52.8 µg/kg. Pb increases normally with age but the Alzheimer group indicate significant (Wilcoxon, $p < 0.005$) higher accumulation.

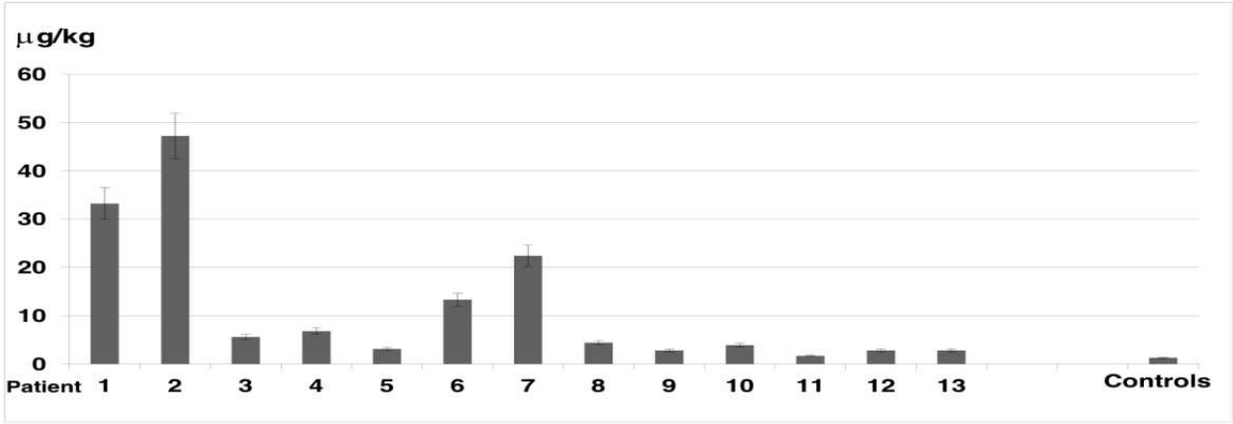

Figure 2. Distribution of cadmium in erythrocytes of 13 patients with Alzheimer's disease. Patient 1-9 men, 10-13 women. Error bar 10%. The mean value of Cd in Alzheimer's patients was 11.5 µg/kg, standard error 3.9 µg/kg, mean of controls 1.3 µg/kg, The concentration of Cd in the erythrocytes of patients with Alzheimer's disease was significant higher (Wilcoxon, $p < 0.05$) than that of controls.

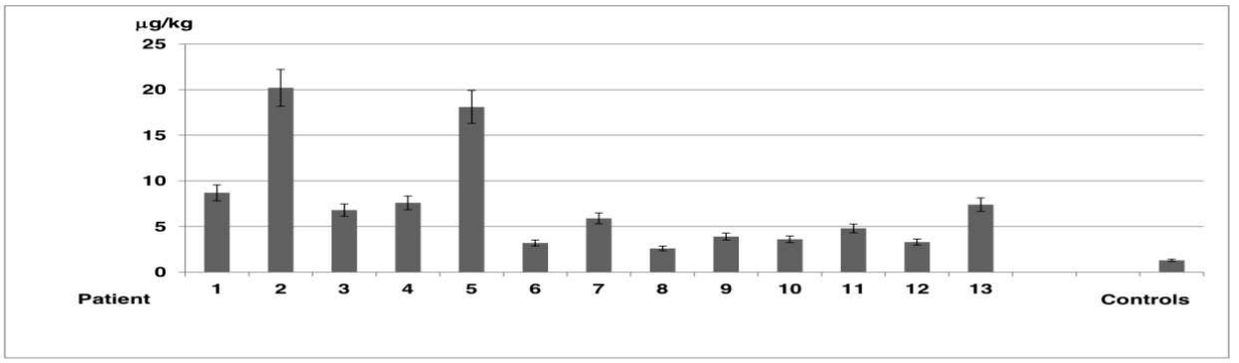

Figure 3. Distribution of silver in erythrocytes of 13 patients with Alzheimer's disease. Patient 1-9 men, 10-13 women. Error bar 10%. The mean value of Ag in Alzheimer's patients was 7.4 µg/kg, standard error 1.5 µg/kg, mean of controls 1.3 µg/kg, The concentration of silver in the erythrocytes of patients was significant higher (Wilcoxon, $p < 0.005$) than that of controls.

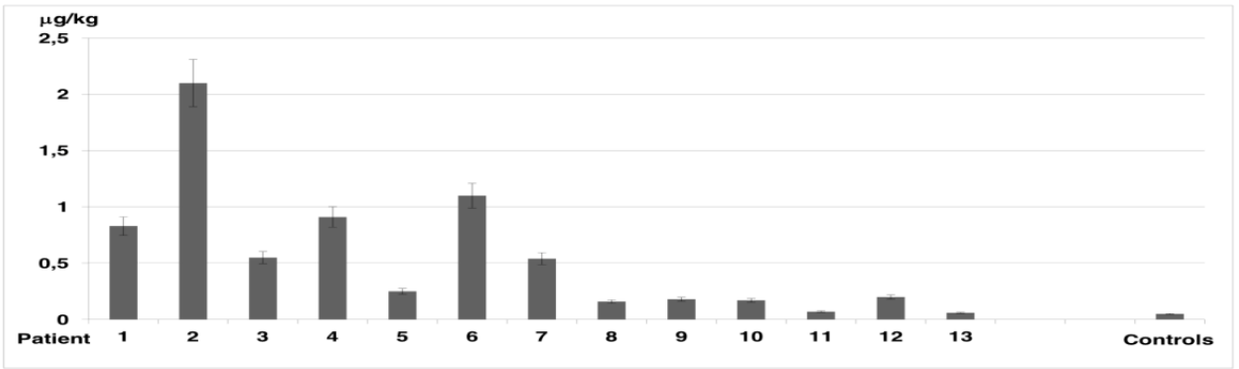

Figure 4. Concentration of uranium in erythrocytes of 13 patients with Alzheimers disease. Patient 1-9 men, 10-13 women. Error bar 10 %. The mean value of U in Alzheimer's patients was 0.55 µg/kg, standard error 0.16 µg/kg, mean of controls 0.11 µg/kg, The concentration of uranium in the erythrocytes of patients with Alzheimer's disease was significant higher (Wilcoxon, $p<0.01$) than that of controls

4. Discussion

Brain has low storing capacity of oxygen though it is a demanding consumer: brain uses about 20 % of oxygen total supply. In degenerative diseases like Alzheimer's, Parkinson's disease the etiology often are not fully known. In Alzheimer's disease pathophysiological theories have been proposed e.g. heat shock protein interactions [4], Tau-protein abnormality [5] neuroinflamation and neuronal loss [6, 7], β-amyloid interactions [8], mitochondrial misfunction [9]. An overlooked possibility may be using ICP-MS for analysis of elemental profiles of erythrocytes as biomarker for Alzheimer 's disease. Small changes in metabolism of oxygen dependent cells may give symptoms difficult to identify due to lack of suitable biomarkers [10, 11, 12. 13, 14]. Erythrocytes carrying excess lead, cadmium, silver and uranium ions may induce damages

http://dx.doi.org/10.5772/57511

in cells including brain cells. Accumulation of elements in the human erythrocytes are a result of low exposure and not of a sudden insult which calls for sensitive biomarkers.

4.1. Effects of accumulated lead, cadmium, silver and uranium in the erythrocytes

At present there is lack of methods to describe the accumulated effects of metal ions in erythrocytes in terms of stability constants or conditional constants. There is lacking information of e.g. solvent properties, pH, temperature, redox properties, lack of knowledge of interacting compounds. Elemental profiles of erythrocytes may be an alternative to estimate changes of trace elements and metal ions at the cellular level. The discussion below will be focused on some effects on membrane integrity by lead, cadmium, silver and uranium because it is not fully known how these ions are associated to other compounds.

4.2. Initiation of eryptosis by accumulated lead, cadmium silver and uranium in erythrocytes

It has been suggested that mature erythrocytes may enter programmed death without the aid of caspase systems [15,16]. Heavy metal ions and organic compounds may be involved in early senescence [17, 18, 19]. When erythrocytes are growing old they will change gradually, fragments will be cleaned up [20, 21], mainly in spleen, liver, bone marrow by the macrophages. Most material of captured senescent erythrocytes e.g. iron and metabolized organic material will be reused [22, 23].

Silver, cadmium ions induce suicidal erythrocyte death [24, 25] and it is likely that erythrocytes and cells dependent on erythrocytes of patients with Alzheimer's disease with accumulated silver and also lead, cadmium and uranium may show early eryptosis. Eryptosis may also be initiated via formation of phosphatidylserine by lead, cadmium, hemin as stimulator [26, 27, 28].

4.3. Erythrocyte channels exposed to accumulated ions of lead, cadmium, silver and uranium

The accumulated lead, cadmium, silver and uranium may interfere the activity in the erythrocyte channels for cations, anions and water. Cadmium, lead, silver may interfere with potassium, calcium [29, 30] and other ions in erythrocyte membranes [31, 32]. Binding in vitro of cadmium to β-amyloid channels was reported by [33]. Exposure to accumulated lead, cadmium, silver, uranium ions to β-amyloid or other compounds is likely to support the pathophysiological process by association to available binding sites.

Anions e.g. chlorides, carbonates ions are transported in anion channels [34, 35, 36] may react with silver, lead, cadmium and uranium disturbing channel transport and oxygen handling.

Aquaporin channels in the erythrocyte membrane are important for flow of water and shape [37, 38, 39]. Accumulated cadmium, lead, silver, uranium ions associated to erythrocyte aquaporin channels may decrease the capacity to shrink and expand. Water exchange may also be important for osmotic regulation when the erythrocytes enter capillaries. It cannot be excluded that lead, cadmium, silver, uranium may release important elements. e.g. calcium from their association sites. Released calcium may form non-selective channels in membranes [40].

4.4. Accumulated cadmium, lead, silver and uranium ions may compete with essential elements in carrier systems

Cadmium is known to compete with zinc in e.g. metallothionein (MT), carrier of zinc. Metal ions having higher affinity than zinc may interact with the binding sites in MT or other carrier. Lead and uranium ions is known to compete with calcium metabolism. Magnesium and calcium ions in calmodulin (calcium carrier) may be displaced by accumulated lead, cadmium, silver, uranium ions and release essential elements e.g. Mg, Ca, Zn. Release of secondary messenger e.g. Mg may disturb ATP metabolism, Ca may activate translocases flippases, floppases, scramblases and start apoptosis signals. Ca may also activate platelets and make them sticky, ready for clot. Sticky platelets may associate to proteins and form placks.

4.5. Sources of cadmium, lead, silver and uranium — Food and implants

The food intake is not known for individual patients. In Finland cadmium uptake from food is about 5-10 μg/day, lead 20-66 μg /day, uranium and, silver was not mentioned [41]. In Sweden is reported cadmium uptake from wheat, rice, potatoes, root-crops 10-20 μg /day, lead 15-30 μg /day, uranium 1-4 μg /day, silver not indicated [42]. Cd uptake from nutrition may disturb the heme synthesis. Cadmium rich diet in Nigeria decreased Hb and erythrocyte counts in mice [43]. It cannot be excluded that cadmium, lead, silver, uranium contaminated food may accumulate in human erythrocytes and in similar manner decrease erythrocyte counts and hemoglobin synthesis. Smoker may have higher cadmium values in blood but there was no information about smoking habits. Elevated uranium in drinking water may damage the kidneys and increase protein loss in urine [44, 45].

In Finland some districts have drinking water with high uranium concentrations (> 100 μg/L) which together with cadmium, lead and silver may increase kidney damage and disturb erythrocyte metabolism. Uranium in the drinking water may explain the significant elevated uranium concentrations of erythrocytes of Alzheimer patients.

Implants may be a source of metal ion supply. Amalgam is an alloy which is not stable [2, 3, 46, 47, 48] (release mercury, silver). Guttapercha may sometime contain cadmium [2] but the released amount is not known for the examined patients. Mercury was not analyzed as mercury will associate strong to liver, kidney also to pituary, low mercury will be found in blood. Mercury has also capacity to displace secondary messenger e.g. Mg, Ca, Zn, Fe when not properly associated.

4.6. Elemental profiles of erythrocytes as biomarkers of Alzheimer's disease by ICP-MS

The early diagnosis of a neurological disease like Alzheimer's and Parkinson's disease is difficult. In Parkinson's disease about 25 % of the patients may get wrong diagnosis [49]. The present study indicate that the elemental profile of erythrocytes may be used as a support in the diagnosis and pathophysiology of Alzheimer's disease. Changes in the elemental profile should be possible to identify earlier due to high sensitivity of ICP-MS. Monitoring elements in the erythrocytes may also be used to observe effects of applied pharmacy and effects on

http://dx.doi.org/10.5772/57511

secondary messengers. More research is needed to interpret early symptoms of Alzheimer disease and pathophysiological processes.

5. Conclusions

Monitoring changes of elements in the erythrocytes by ICP-MS may be used as an early biomarker of Alzheimer's disease and may support the pharmaceutical treatment. The accumulated lead, cadmium, silver and uranium may interfere with channel activities, have effects on apoptosis, react with secondary messenger and support pathophysiological processes.

Acknowledgements

We thank Crafoord foundation for economic support and Åbo Academy for putting instrumental facilities to our disposal. We also thank dr Sture Brishammar, Evert Karlsson for discussions.

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