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ALTERATIONS IN NICKEL AND CADMIUM CONCENTRATIONS IN ERYTHROCYTES AND PLASMA OF PATIENTS WITH PARKINSON'S DISEASE

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ABSTRACT: Heavy metals are possibly implicated in the pathogenesis of Parkinson's disease. Inductively coupled mass spectrometry (ICP-MS) is a useful method for measuring elemental profile in tissues and body fluids. The present experiments were designed to analyze concentrations of cadmium (^{114}Cd), nickel (^{60}Ni), cobalt (^{59}Co), rubidium (^{85}Rb), strontium (^{88}Sr) and molybdenum (^{98}Mo) in erythrocytes and plasma of patients with Parkinson's disease using ICP-MS technique. Samples were obtained from twelve Parkinson's patients receiving L-dopa and twelve healthy controls. Elevated levels were observed for, ^{114}Cd ($p < 0.05$) and ^{60}Ni ($p < 0.05$) in the erythrocytes. Concentration of ^{60}Ni was significantly increased ($p < 0.01$) in the plasma. Nickel and cadmium ions could interfere with copper and zinc in the synthesis of eumelanin, pheomelanin and opiomelanin. Ferrochelatase has rather low specificity for iron and nickel may enter its place in the haem group with lowered oxygen capacity. If other metal ions with similar ion radii e.g. Mg, and Zn enter haem group redox capacity is lost. Nickel ions may as well enter substantia nigra if iron concentration is lowered. Cadmium may act as competitive inhibitor of haem iron. Nickel also stimulates dopachrome oxidoreductase thus decreasing L-dopamine availability. Furthermore catalytic effects nickel and cadmium possibly lead to the formation of oxygen free radicals that oxidize lipids to lipofuscin and destroy tissues. It could be suggested that observed changes in trace element profile may indicate disruption of cellular function.

Introduction

Heavy metals like iron and manganese are possibly implicated in the pathogenesis of neurodegenerative disorders such as Parkinson's disease. It has been suggested that agents inducing neurodegenerative processes such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) may impact transition elements and thereby leads to dementia in Parkinson's disease Sokolowski [1, 2]. Transition metals are among the most important factors that participate in the production of oxygen reactive radicals and disrupt cellular function possibly via induction of mitochondrial damage. Several reports have implicated the involvement of redox-active metals with the onset of different neurodegenerative diseases such as Alzheimer's Disease, Progressive Supranuclear Palsy, Amyotrophic Lateral Sclerosis and Parkinson's Disease, Egana et al. [3].

For instance elevated concentration of free iron and decreased levels of ferritin is believed to contribute significantly to the development of Parkinson's like symptoms. Iron is suspected to contribute to Parkinson's disease because it is known to promote oxidative damage. Studies on postmortem brains from Parkinson's patients revealed increased iron levels in the substantia nigra, Kaur et al. [4]. It may be postulated that selective cell death in this brain region is associated with oxidative stress and may be exacerbated by the presence of excess iron. Iron accumulation, together with a lack of up-regulation of the iron-storing protein, ferritin, contribute to increased oxidative stress in substantia nigra leading to the manifestation of extra pyramidal symptoms. Recent studies suggest additional mechanisms by which iron might contribute to Parkinson's disease. One of these mechanisms is inducing aggregation of the alpha-synuclein which is a protein that accumulates in Lewy bodies in Parkinson's disease, Wolozin and Golts [5].

Other metals have also significant role in the development of neurodegenerative disorders. Chronic exposure of miners to manganese dust may also induce Parkinson's disease like symptoms. Increasing evidence suggests that astrocytes are a site of early dysfunction and damage. Chronic exposure to manganese leads to selective dopaminergic dysfunction, neuronal loss, and gliosis in basal ganglia structures together with characteristic astrocytic changes known as Alzheimer astrocytosis, Normandin and Hazell [6]. Cirrhosis-related parkinsonism represent a unique, consistent, and common subset of acquired hepatocerebral degeneration, whose features are permanent and entirely different from acute hepatic encephalopathy episodes. Recent studies also support the concept of the toxic effects of manganese being the major determinant of basal ganglia dysfunction leading to the predominantly extrapyramidal central nervous system contribute to the manifestations of cirrhosis observed in these patients, Burkhard et al. [7].

Medical treatment with elements like Sibelium may also induce Parkinson's disease like symptoms. Furthermore in conditions such as Wilson's disease the increased copper concentration may give Parkinson's disease like symptom. Several forms of neurodegenerative diseases, either arising as inherited disorders of copper

metabolism, such as Menkes' and Wilson's disease Portala et al. [8], or as conformational diseases such as Alzheimer's disease and prion diseases. Although copper is an essential trace element but its redox reactivity leads to risks of damage to cell and tissues, Rotilio et al [9].

Although there had been many studies on iron other metals like nickel and cadmium were not extensively studied. Most previous studies investigated Parkinson's symptoms but not much emphasis was made on the utilization of these elements as biomarkers for disease development. The current project aimed to study trace elements changes in Parkinson's disease. We decided to investigate changes in trace elements profile of erythrocytes and blood plasma in patients with Parkinson's disease to identify if any element is possibly associated with neurodegeneration. Secondly we aimed to search for elements that are possibly useful as biomarkers of the disease. In the present study we included cadmium (^{114}Cd), nickel (^{60}Ni), cobalt (^{59}Co), rubidium (^{85}Rb), strontium (^{88}Sr) and molybdenum (^{98}Mo) in the erythrocytes and plasma of patients with Parkinson's disease using inductively coupled mass spectrometry (ICP-MS) technique.

Material and methods

Patients. Twelve patients (mean age 65 y) and age matched controls (mean age 58 y) were selected for the study. All of the patients were receiving L-dopa. All persons participated voluntarily according to the Helsinki declaration.

Samples. Whole blood (2×7 ml) was drawn into vacutainer tubes for trace element analysis (BDH, with EDTA as anticoagulant). Centrifugation was started half an hour after venopuncture. The erythrocytes were separated by centrifugation at 1000×g for 9 min, 4°C. After removing the buffy coat the erythrocytes were washed twice with 0.9% NaCl at 1000×g for 5 min. The erythrocytes were transferred to cryo vials (Nunc), weighed and freezed at -18°C. The blood plasma was centrifuged at 3000×g for 10 min and the pellet was removed. After another centrifugation at 4000×g for 5 min blood plasma was transferred to CryoTube vials, weighed and frozen at -18°C. The samples (0.6-0.8 g, wet weight) were spiked with indium (25 ppb) as internal standard and then digested in 3 ml of nitric acid and 1 ml of hydrogen peroxide both ultra pure. After digestion in a microwave oven (Miele) for 7 min in Teflon vessels, the samples were diluted with quartz double distilled water in 25 ml polypropylene flasks.

ICP-MS instrumentation. For the elemental analysis inductively coupled mass spectrometry (ICP-MS) PQ3 from Thermo Elemental, LTK was used. ICP-MS operating parameters included the followings: standard torch, nickel cones (sample cone 0.7 mm, skimmer cone 1 mm), RF forward power 1360 W, reflected power 5.2 W, Argon flow: nebulizer 0.83 ml/min, cool gas 11.5 ml/min, aux 0.99 ml/min, Spray chamber 5.2°C, Scan mode, dwell time 320 μs, 19 channels/AMU, 3 replicates Sample rate 0.9 ml/min, anal. pressure 1.4×10^{-6} mBar, expansion 1.87×10° mBar, Aquisition 45 sec uptake, 60 sec wash.

Reagents and standards. Nitric acid and hydrogen peroxide, Suprapure was obtained from Merck. ICP-MS standards were obtained from Johnson & Matthey, Specpure isotopes used for the analysis: ^{59}Co, ^{60}Ni, ^{85}Rb, ^{88}Sr, ^{98}Mo, ^{114}Cd. Trace elements in water 1643d and Oyster tissue 1566a, was obtained from Natural Institute of Science and Technology, USA. 20 ppb of Co, Ni, Rb, Sr, Mo and Cd was obtained from stock solutions, Spex 2A, Promochem, Sweden. Double quartz distilled water was used for dilution.

Results

Validation of the elemental analysis. For the quantitative analysis of the elements scan mode was selected. Standard Reference Materials from National Institute of Science and Technology were used to validate ICP-MS analysis. The estimated relative error of SRM was less than ± 10% in the different runs. The mean error in the analysis of samples was ± 15% or less generated in the sampling procedure, preparation, digestion, volumetric and weighing error and error in the ICP-MS analysis, Table 1.

Elemental profile of blood plasma. The concentrations of Co, Rb, Sr, Mo and Cd of blood plasma were not significantly different in blood plasma from that of controls but the concentrations of Ni was significantly increased ($p < 0.01$, Figure 1). The mean concentration of nickel was 161 μg/L compared with that of controls 64 μg/L. The nickel concentration in serum should decrease with age Hindsen [10]. The increased nickel concentrations indicate changes in the metabolism of nickel in Parkinson's patients. Further studies are needed to understand the pathophysiology of these changes.

Elemental profile of erythrocytes. The concentrations of Co, Rb, Sr and Mo were not significantly different from that of controls but there was a significant increase of the concentration of Ni ($p < 0.01$) and Cd ($p < 0.05$), Figures 1-2. The mean of erythrocytes was 212 μg/L in comparison with controls 38 μg/L. The changes of elemental profile may explain difficulties experienced with the diagnosis of Parkinson's disease, Holmberg [11] and Hughes [12]. Ni and Cd are known to interact with sulphur and selenium e.g. thiol and selenol groups in GSH, GSH-Px.

Discussion

Heavy metals like iron and manganese are among

Table 1. Validation of analysis by ICP-MS, 6 runs. Certified and analysed value was in reasonable agreement

SRM	Ni(60)	Cd(114)
NIST 1643d, certified	58.1 μg/L	6.47 μg/L
Obtained value, n=6 ($\bar{x}$)	56.9	6.8
NIST 1566a, certified	2.25 μg/g	4.15 μg/g
Obtained value, n=6 ($\bar{x}$)	2.2	3.8
Spex 2A, recommended	20 μg/L	20 μg/L
Obtained value, n=6 ($\bar{x}$)	19.4	20.4

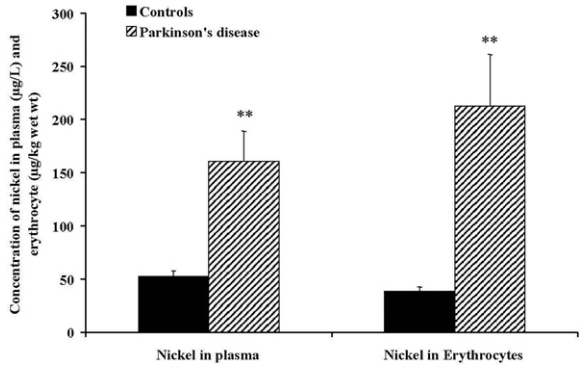

Figure 1. Concentration (mean) of Ni in blood plasma and erythrocytes of controls (n = 12) and patients with Parkinson´s disease (n = 12). Error bars 15%. The concentration (mean) of Ni was significantly higher (p < 0.01, Students t-test) in both blood plasma and erythrocytes. Standard error of plasma was in controls 5.3, in Parkinson patients 28.3, standard error of erythrocytes in controls 3.9, in Parkinson patients 48.7

the important factors involved in the pathogenesis of neurodegenerative disorders such as Parkinson's disease. Transition metals may participate in the production of oxygen reactive radicals and disrupt cellular function possibly via induction of mitochondrial damage. Although there had been many studies on iron other metals like cadmium, nickel and were not extensively studied previously. The present experiments analyzed concentrations of, cadmium, nickel, cobalt, rubidium, strontium and molybdenum in the erythrocytes and plasma of patients with Parkinson's disease. The current study revealed significant increased concentration of nickel and cadmium in the erythrocytes of patients with Parkinson's disease. Furthermore the concentration of nickel was significantly increased in blood plasma of patients with Parkinson's disease.

Storage of metal ions in melanin. The mechanism for nickel's involvement in Parkinson's disease is not well understood. Nickel binds to melanins and may

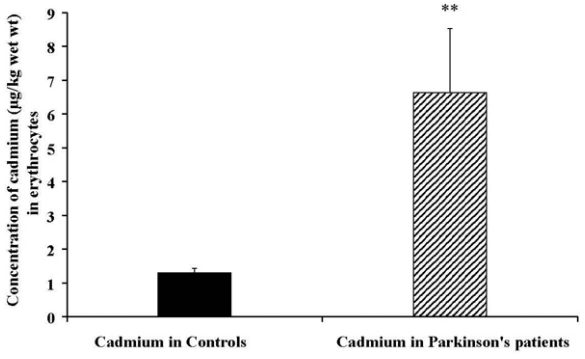

Figure 2. Concentration (mean) of Cd in the erythrocytes of controls (n = 12) and patients with Parkinson´s disease (n = 12). Error bars 15%. The concentration of Cd in the erythrocytes was significantly higher (p < 0.01, Student´s t-test) than that of controls. Standard error of erythrocytes was in controls 0.1, in Parkinson patients 1.9

interfere with cellular protection mechanisms. Metal ions like nickel may bind to OH-groups and impact synthesis process of melanin, tyrosine and dopamine, Tallkvist [13]. There are metal ions necessary for the synthesis of the melanin and intermediates such as eumelanins and pheomelanin from tyrosine via dopamine, Kronstrand [14]. However melanin have storage capacity of different metal ions and may not only store necessary ions but also excess of other ions, Mars and Larsson [15]. When melanins are oxidised associated metal ions may be released and interact with enzymes and amino acids. Oxidation may further modify the binding capacity of melanin due to fewer binding groups or changed form of the ligands, Leonard et al. [16], Baez [17]. The increased concentration of nickel may interfere with tyrosine hydroxylase enzyme that is believed to be copper dependent. It is possible that the enzyme urease which is nickel dependent may be triggered to produce more ammonia. Ammonia is toxic and must be used in further reactions or neutralized.

Interaction with thyroid enzymes. Cadmium and nickel ions may interact with tyronindeiodinase enzymes in the thyroid gland making iodine and selenium not biological available. The result of heavy metal interaction in thyroid is not well understood but decreased production of ATP may be one consequence. Nickel uptake was increased in ATP-depleted cells, Tallkvist [13]. This phenomenon was also well demonstrated with low iron concentration, Tallkvist [13]. It is possible that increased nickel and cadmium concentration in erythrocytes of patients with Parkinson's disease represent a phenomenon of depleted cellular energy.

Interactions of metal ions in ion channels. Metal ions with similar ionic radius may interfere with ion flux into nerve cells and ion channels and therefore interfere with neuronal function. Cadmium ions, radius 0.97 Å, may interfere with calcium ions (0.99 Å) in calcium channels. Effects on energy systems and on transmitter function may be expected.

As Ni^{2+} also affects Ca^{2+} and K^{+} channels will Cd and Ni together reinforce interactions on transmitter functions.

Nickel ions with radius 0.69 Å may interact with sodium channels in epithelial cells Sheng et.al. [18] which may have effect on blood pressure.

Possible exchange of Ni in ATP and B_{12}, zinc finger. Some reactions may result in forming association with proteins thus changing the elemental status by presenting loosely bound metal ions, only a few examples are suggested here.

If magnesium (0.66 Å) is changed to Ni (0.69 Å) in the ATP metabolism the production of energy may be decreased.

Due to similar radius of nickel and cobalt, cobalt ion may be replaced in B_{12} giving reduced capacity. If B_{12} has reduced capacity, the folate system can not balance the methionine and cysteine requirement and sulphur metabolism will be disturbed.

If Zn^{2+} in zinc finger is replaced by Ni^{2+}, the transcription process in gene activation will be disturbed.

Nickel intake and mobilization. Changes in

diet, increased environmental exposure or changed pathophysiology may be involved in the increased nickel concentration in blood plasma and erythrocytes of Parkinson's patients. Nickel intake from food and drinking water has been estimated to be 2-10 μg/kg body weight, Myron et. al. [19], Smart and Sherlock [20]. The concentration of nickel in drinking water is normally <10μg/L but sometime it may reach 200-2500 μg/L. High nickel concentration is found in cacao, dark chocolate, soya beans, hazel nuts, oatmeal, buck-wheat, Ellen et. al. [20], Nielsen and Flyvholm [22], Anke et. al. [23]. The contribution of nickel from environment, dental contacts, jewellery, coins, tobacco smoke, dialytic fluids is not well known. Although usually the concentration is considered to be low prolonged exposure may constitute a risk.

Nickel transport in plasma. Modification in carrier system may contribute to changed nickel concentration in the erythrocytes and plasma of Parkinson's patients. Nickel is transported by albumin in plasma. Under conditions where iron status in the body is low more nickel is transported by both transferrin and non-transferrin carriers, Tallkvist [13]. It has been observed that non-transferrin bound iron is inhibited by nickel, cobalt, manganese and zinc, Tallkvist [13]. Other molecules like α_2-macroglobulin, histidine also may bind to nickel. If histidine binds nickel then all proteins with an exposed histidine should bind nickel. The influence of modification on binding to these carriers in the development of neurodegeneration needs further investigation.

Despite various studies of the mechanism by which heavy metals are involved in the pathogenesis of Parkinson's disease in not yet clear. Possibly selective cell death in various brain regions is associated with oxidative stress and may be exacerbated by the presence of excess heavy metals. As previously reported with iron metal accumulation, together with a lack of up-regulation of the iron-storing protein, ferritin, contribute to increased oxidative stress in substantia nigra leading to the manifestation of extrapyramidal symptoms. Recent studies suggest additional mechanisms by which iron might contribute to Parkinson's disease. One of these mechanisms is inducing aggregation of the alpha-synuclein which is a protein that accumulates in Lewy bodies in Parkinson's disease, Wolozin and Golts [5], another mechanism may involve frataxin and ferrochelatase.

Ferrochelatase and frataxin. Frataxin is a protein required for the cellular regulation of iron homoestasis together with ferritin, Karlberg [24]. It has been suggested e.g. that frataxin act as a chaperone in haem production in mitochondria. Ferrous iron is inserted into porphyrin by ferrochelatase to produce haem prostehetic group Taketani [25]. Ferrochelatase can chelate divalent metal ions besides Fe^{2+}. Ions with similar ion radius e.g. Ni^{2+}, Mn^{2+}, Mg^{2+}, Zn^{2+} may enter the haem group, Daily [26] and disturbe the redox capacity. If a metal ion with no redox capacity (Mg^{2+}, Zn^{2+}) is introduced in haem severe disturbances may be expected. It appears that heavy metal ions have significant role to play and they are among the most important factors that are possibly involved by association of metal ions or catalyzing neurodegenerative conditions. Metal ions may participate in the production of oxygen reactive radicals and disrupt cellular function possibly via induction of mitochondrial damage. Several reports have implicated the involvement of redox-active metals with the onset of different neurodegenerative diseases such as Alzheimer's Disease, Progressive Supranuclear Palsy, Amyotrophic Lateral Sclerosis and Parkinson's Disease, Egana et al. [3].

In summary heavy metals are possibly implicated in the pathogenesis of neurodegenerative disorders such as Parkinson's disease. Selective brain cell death may be associated with oxidative stress, which may be exacerbated by the presence of excess metal ions. Alterations in elemental profile may indicate disruption of cellular function at several levels for instance erythrocytes, storage sites in mitochondria, carrier systems and enzyme activity.

Although the production of free radicals might be a consequence of changed elemental profile or associated factor further studies are essential to investigate mechanisms controlling target systems at the cellular level and any protective role for antioxidants.

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