CLINICAL STUDY

OLIGOELEMENTS OF THE CEREBRAL ARTERIAL WALL AND MODIFICATION OF THE CONCENTRATIONS IN CONNECTION WITH AGE

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ABSTRACT

The oligoelements known also as trace elements represent 1% of body weight corresponding to a weight of approximately 10 g and had an essentials role in many structural cell membranes and functional processes. They play important roles in brain function such as: catalysts, second messengers, and gene expression regulators. Take into account the favourable effects of the oligoelements complex therapy in the ageing process we have investigated the variations in concentration and distribution of some macro and microelements in the cerebral vessels walls in connection with aging. The oligoelements measurements have been done though two methods: spectrometry of atomic absorption under fire and that of emission. We can conclude that cerebral artery aging was associated with an increased mineralization mainly due by the increase of Na and Ca. This mineralization probably decrease wall elasticity by decreasing the hidratation of elastin fibres and the increasing the amount of metallic ions. The minerals that increase with age are Na, Ca, Mg, Fe, and Se; by contrast Zn, Mn present a decrease concentration with ageing. Ni, Co and Cu present a more constant evolution with no statistical significant modification with age. We couldn't identify differences between male and female regarding minerals evolution with age. There are many mechanisms that involve trace elements in ageing process of arterial wall but a clear role of each of them need to be establish after future studies.
Key words: oligoelements, cerebral vessels wall, spectrometry of atomic absorbtion

The importance of the bio-organic chemistry when explaining the phenomena specific to life has been neglected or minimalised for a long time.

At the beginning of the century, Bertrand, on the basis of the investigation over the activity of the lactase (oxidase) has discovered that its catalytic reaction is not possible unless the manganese is present. This discovery led to the hypothesis according to which the link between enzyme and substrate is possible only with the help of an inorganic element.

After 1960, the elucidation and the practical validation of electrolysis laws permitted the clarification of the energetic role of the cation inside the complex formed by enzyme-metal-substrate. In these reactions, the main role is held by the structure of the metallic cation atom with special attention to a certain group of elements with a significant physiologic importance (Popa, 1976) if we compare it with that of the vitamins (Goudot and Picard 1982). There are only 52 in the composition of the live material from all the chemical elements known so far. Among these the most important are: iron, magnesium, manganese, copper, zinc, calcium, cobalt, molybdenum, vanadium and cadmium which have been ranked according to quantity and partially to quality. With the exception of the molybdenum (Z=42) and of the iodine (Z=53) in the human physiology appear only light elements from Mendeleyev chart up to N=30 (Z=30). They represent parts from the following group: alkaline metals, alkaline-earth metals and transition metals. The alkaline metals are represented by sodium, potassium and lithium. In the group of alkaline-earth metals there are calcium, magnesium, strontium, barium and radium while in the group on the transition metals there are vanadium, chromium, manganese, iron, cobalt, copper and nickel which are all very important in the normal functioning of some enzymes (Popescu CD 1997).

According to their role (Goudot & Picard 1982) the macro and microelements can be classified in: ***essential elements*** (Fe, K, Ni, Cu, Zn, Cr, Mo, V, Bn, Si, F, I, Se), ***possible essential*** (Al, Br, Ba, Br, Rb, Ag, B, Ri, Ge, Ti) and ***not essential.*** Defining as essential element is quite difficult and it has to meet at least a few criteria: (a) to be present in all normal tissues, (b) its lack to lead to the same structural or biological modification, (c) the substitution in case of deficit, to remove the secondary

dysfunctions related to its lack. At the same time we must take into consideration the fact that there are variable essential characteristics for the same human body. Sodium, potassium, magnesium and calcium are characterized by high concentration (about 99%) from the inorganic ions.

The oligoelements known also as trace elements, represent 1% of body weight corresponding to a weight of approximately 10 g. The aport of the oligoelements is a digestive one. Their absorption depends on the oligoelements concentration in water and food. In their turn the concentration of the oligoelements in water and food varies according to the composition of the soil, so that there are diseases with a local character, caused by the abnormal soil concentration.

Researches that have been conducted in the two decades ago (Georget A & Durif F 1990, Giampalmo A et al 1989) have established that the most important oligoelements involved in the cardiovascular pathology are: ***magnesium (Mg), manganese (Mn), copper (Cu), cobalt (Co) , vanadium (V), cadmium (Cd), chromium (Cr), selenium (Se) and nickel (Ni).*** At the same time for most of them there have been highlighted some connections, tolerance rate as well as accepted limit dosage. Further research on these results lead to developing oligoelements therapies but for which has been stipulated the very close limits between overdosage and underdosage.

Thus, favourable results in Keshan disease have been reported (local cardiopathy through lack of selenium in food) by using substitution therapy with this metalloid (Georges P 1986). There have been reported also data that support the prophylaxis for arterial hypertension by administrating the manganese-calcium-magnesium complex, as well as the favourable effects after cobalt-manganese therapy in cardiovascular diseases associated with muscles kinetics abnormalities. An important direction is represented by the observations that have revealed the fact that the aging process is the beneficiary of the bioorganic treatment based on the therapies with copper-silver-manganese-cobalt, fluorine-sulphur, phosphorous-lithium which contributes to the harmonization of the disturbed functions (Goudot & Picard, 1982).

Many essential metals (e.g. calcium, copper, magnesium, manganese, iron, zinc, cobalt, and molybdenum) are required also for optimal CNS function. They play important roles in brain function such as: catalysts, second messengers, and gene expression regulators. Being essential cofactors for functional expressions of many proteins, trace elements are needed to activate and stabilize enzymes, such as superoxide dismutase, metalloproteases, protein kinases, and transcriptional factors containing zinc finger proteins. Clearly, metals must be supplied to the CNS at an optimal level, because both deficiency and excess can result in aberrant CNS function.

Iron

The essential element iron, plays a wide variety of physiological and biochemical roles, the most known being ***oxygen transport*** in which iron is associated with heme forming hemoglobin found in circulating red blood cells. However, there are many other important heme-containing proteins such as myoglobin, cytochrome P450, and the cytochromes a, b, and c in the electron transport chain that are essential for ***mitochondrial respiration*** and ATP synthesis (Gordon, 2003). There are also a variety of ***enzymes*** that require iron for their ***catalytic activity*** including polylhydroxylase (Vranka et al., 2004), cytochrome oxidase (Blomberg and Siegbahn, 2003), and tyrosine hydroxylase.

The functions of iron are also illustrated by the fact that iron deficiency has been implicated in cognitive deficits. This may be due to the role of iron in ***myelin synthesis,*** the development of dendritic spines in the hippocampus, hippocampal metabolism (Jorgenson et al., 2003), synthesis of monoamine transmitters and GABA. Changes in iron metabolism with age are well known (Martin et al., 1998), particularly in women.

The role of iron as an essential nutrient during development and aging cannot be disputed. However, a controversy has arisen surrounding the possibility that excess iron may contribute to the aging process and to the development of disorders associated with aging. The role of dietary iron in these processes has not been fully explored, but it appears that many of the deleterious effects of high iron may not be due to increased intakes of iron but rather to abnormal cellular iron metabolism, transport, or storage.

Magnesium

Magnesium is another essential elements which represents about 0.04% from the body mass, approximately 29 g at an adult of 70 kg. Under diffusible form it is found in a proportion of 80% and the rest of 20% as a not diffusible fraction. It interferes in a series of metabolic and visceral processes being predominant in intracellular form. Magnesium act in lipid, proteinic, glucidic

metabolism, in DNA synthesis and in maintaining the integrity of the ribosomes. Daily needs are off about 200-300 mg/day. When food is deficient in magnesium (under 15 micrograms/l of water) there is favoured the appearance of coronary disease. At least is well documented that the arterial tonus is connected by the magnesium ion which regulates the membrane and intracellular transport at the level of soft muscular cell. The balance Mg^{2-}/E^{2+} constitutes a way of regulating intracellular activity. The muscular cells contractibility is under the connection of action and interaction of two bivalent cations Ca^{2+} and Mg^{2-}.

A series of studies claim the fact that Mg^{2+} is an antagonist for Ca^{2+} which is genuine and natural (for the soft muscular cell). Nowadays magnesium is attributed the following functions: a) the blocking of calcium entrance and diminishing the peripheral and cerebral vascular resilience; b) improving the peripheral and cerebral blood flow; c) protection against the cerebral coronary and peripheral spasm; d) lowering the arterial pressure on the condition to be administrated in sufficient quantities.

In the plasma it is found that at the concentrations starting from 1.2 to 3.2mEq/l and in erythrocytes of 4-6mEq/l the magnesium ion interferes in the activity of the ATP and at the same time is indispensable to some enzymes from the hydrogenesis class. As a rule, the magnesium has an antithetic action comparing to calcium with the exception of the muscular contraction where both ions have a synergic action. The lack of magnesium leads to the same phenomena which were observed in the case of hypocalcaemia. The role of this ion would also be to correct some metabolic deviations by synchronization of the two fundamental processes: energy releasing and storage.

Low magnesium (Mg) affects endothelial function, thus playing a role in cardiovascular diseases, including atherosclerosis; Mg deficiency acts through induction of the pro-inflammatory cytokine (interleukin-l alpha IL1α) in cultured human endothelial cells. Indeed, the inhibition of IL-l alpha prevents low Mg-induced adhesion of monocytoid cells to the endothelium as well as the upregulation of the cdk inhibitor p21. Mg deficiency induces also several features typically associated with endothelial senescence, affects gene expression at the transcriptional level, and modulates the activity of the proteasome.

Zinc

Zinc (Zn) is a trace element essential for cell proliferation and differentiation. It is a structural constituent of enzymes and proteins, including metabolic enzymes, transcription factors, and cellular signaling proteins. There is increasing evidence for a direct signaling function of Zn at all levels of cellular signal transduction (Beyersmann 2002). Zinc is an important element in preventing free radical formation, in protecting biological structures from damage and in correcting the immune functions. Zinc deficiency produces growth retardation, anorexia, delayed sexual maturation, iron-deficiency anemia, and alterations of taste (Barceloux 1999). Zinc-binding proteins, such as metallothioneins (MTs), belong to the family of intra-cellular metalbinding proteins that are present in virtually all living organisms and they play a key role in the Zn effect upon the immune system. MTs are protective against stress and increase in ageing (Mocchegiani et al. 2001). Zinc plays three major biological roles in the organism: as catalyst, and as structural and regulatory ion (Mocchegiani et al. 2000). Apoptosis is induced by several extracellular or intracellular stimuli with an important role for trace metals like Zn or calcium (Seve et al. 2002). The dysregulation of apoptosis is central to pathogenic mechanisms in many diseases such as neurodegenerative disorders, acquired immune deficiency syndrome, autoimmune disease and cancers (Thornberry and Lazebnik 1998; Tapiero and Tew 2003). Increased apoptosis in vivo may occur as direct or indirect consequence of a decrease in intracellular Zn concentrations. Therefore, cellular Zn is described as an inhibitor of apoptosis, while its depletion induces death in many cell lines (Seve et al. 2002). In ageing MTs preferentially bind Zn rather than copper and they are unable to release Zn. Indeed, during ageing the stress like-condition is persistent provoking a sequester of intracellular Zn with subsequent low Zn ion bioavailability for immune efficiency and for the activity of Zn-dependent enzymes and proteins (Mocchegiani et al. 2000, 2002). Low Zn ion bioavailability and high MTs levels are present in aging and stress (Mocchegiani et al. 2000). A low Zn ion bioavailability may also trigger impaired cognitive functions, via altered thyroid hormones turnover.

Selenium

Selenium (Se) was recognized as an essential trace element in 1957 (Schwarz & Folz 1957), but Se deficiency could partially be compensated by vitamin E. However, data from nutritional sciences were hard to interpret without the knowledge of molecular players until the discovery of Se as an essential part of mammalian enzymes like glutathione peroxidases (GPx), thyroid hormone

deiodinases, and thioredoxin reductases. Selenium depletion in animals is associated with decreased activities of Se-dependent enzymes and leads to enhanced cell loss in models of neurodegenerative disease. Genetic inactivation of cellular glutathione peroxidase increases the sensitivity towards neurotoxins and brain ischemia. Conversely, increased GPx activity as a result of increased Se supply or over-expression ameliorates the outcome in the same models of disease. Genetic inactivation of selenoprotein P leads to a marked reduction of brain Se content, which has not been achieved by dietary Se depletion, to a movement disorder and spontaneous seizures.

Manganese

Manganese is an essential mineral that is found at low levels in virtually all diets. Mn ingestion represents the principal route of human exposure, although inhalation also occurs, predominantly in occupational cohorts. Regardless of intake, animals generally maintain stable tissue Mn levels as a result of homeostatic mechanisms that tightly regulate the absorption and excretion of this metal (Chen et all, 2002). However, high dose exposures are associated with increased tissue Mn levels, resulting in adverse neurological, reproductive, and respiratory effects. In humans, Mn-induced neurotoxicity, commonly referred to as manganism or Parkinson's disease-like syndrome, is of paramount concern and is considered to be one of the most sensitive endpoints. Mn neurotoxicity is associated with motor dysfunction syndrome that is recognized as a form of parkinsonism (Tomas-Camardiel et all 2002).

Nickel

Nickel represent a trace elements with a plasma level of 0.002-0.003 microg. It is usually associated in blood flow with albumin or metalloproteins. Daily intake of Ni is around 0.3-0.5 microg, but a big amount is lost via digestive, renal and skin route. Ni has an important role in thermo stability of DNA and RNA; also it plays a key role in mention of structure and functioned of cells membranes (Ulmer 1984). The combination between Ni and carbon monoxide is extremely dangerous inducing pulmonary inflammation and hepatic necrosis.

PERSONAL RESEARCH

Starting from the favourable effects of the oligoelements complex therapy in the ageing process we have investigated the variations in concentration and distribution of some macro and microelements in the walls of cerebral vessels in connection with the ageing. When conducting these researches the following steps where required: collection, weighing, conservation, disintegration, determining the concentrations and establishing some similarities (Popescu CD 1991).

MATERIALS AND METHODS

Our research has been conducted on a necroptic material which included 10 cases with ages varying from 2 to 80 years old. In all cases there have been collected fragments with lengths between 2-3 cm from the big arteries of the brain or the arteries with cerebral destination. After the collection there has been performed the longitudinal section of the vessels and cleaning the blood stains or serosity, by light cleaning the surfaces. The samples have been bottled in glass recipients with a stopper (previously weighed). After, the fragment has been introduced the tube have been once more weighted. The weight differences, established with the help of analytical scales, have permitted to weight the samples. They have been kept for 2-10 days at a temperature of 4oC and then we preceded the next step with sulphuric acid and peroxide that permit to obtain a clear solution for analytical tests in order to evaluate the concentrations of oligoelements. The oligoelements measurement have been done though two methods: spectrometry of atomic absorption under fire and that of emission.

Iron

In cerebral artery the amount of Fe vary between 1.1-8.6 mg% being the fourth after Na, Ca and Mg. Commune and internal carotid artery present comparable concentration; the biggest amount was found in Willis polygon artery, and the lowest in vertebro-basilar arteries and anterior cerebral artery. There is a clear tendency of increase iron concentration in cerebelar artery with age especially after 35 years. The increase amount of Fe with age both in intra and extracerebral artery could be explained by metabolic disturbances and enzyme dysfunction associated with accumulation of catabolism productions. There is also possible the sequestration of erythrocytes with reduce elasticity at vasa vasorum level.

Magnesium

In the walls of the cerebral arteries it is found in significant amounts comparing to the oligoemelemets having values between $47.45 \cdot 10^{-4}$ and $383 \cdot 10^{-4}$ g%.

In the walls of the cerebral arteries the magnesium is found in big quantities which could determine us to conclude the fact that there is a slight accumulation in all the arteries in connection to age. This accumulation is relatively proportional both for extracerebral as well as intracelebral arteries. The highest values of the magnesium concentration are met in decreasing order at the common carotid artery, carotid bulb, and internal carotid artery. So we can consider that magnesium marks a slight accumulation in the wall of cerebral arteries in connection with age without reaching the values of sodium and calcium.

Zinc

In comparison with the calcium, the magnesium or the sodium, the zinc is found in cerebral arteries in very small quantities. At cases A.L. 55 years old and B.R. 42 years old, zinc concentrations could not be highlighted by the method used for the vertebra-basilar trunk because the values were under the apparatus detection limits. Zinc regulates the activity of oxide reductive enzymes (alcohol hydrogenise and carbonic anhydrase) and it favours the triglycerides synthesis in the liver followed by hyperlipemia, a situation which favours the formation and extension of the atheroma layer. The concentrations of this ion decrease in connection with age in the walls of cerebral arteries; this is a characteristic element to the blood serum which undergoes the same phenomenon.

Selenium

The blood selenium concentration is in connection with the food, varying between 0.10-0.34 micrograms/ml. Using the identification methodology for oligoelements we have observed the presence of this metalloid in quantities which are at the sensibility limit of the method. The values found in the walls of the cerebral arteries are between $3.9 \cdot 10^{-4}$ and $10 \cdot 10^{-4}$ g%. It is to be noted the uneven distribution of the concentration of the selenium in cerebral arteries, so: Willis polygon vertebro-basilar trunk and anterior cerebral artery have values at least second times smaller than the other structures that have been investigated. The uneven distribution can be explained by the different histological structure. The arteries of which medium layer is better represented concentrate selenium as well in order to sustain their specific metabolism. The highest concentrations are in the carotid artery. The values of the selenium rise in connection with the age at both types of arteries. At the age of 3 years old the values of the selenium are almost the same for all arteries suggesting as relatively equal distribution of this element after the birth and then accumulations to appear in connection to age that seem to be necessary in order to annihilate the free radicals which are present in increasingly higher numbers. The selenium is also accumulated in the hypophysis influencing its secretion. In the polymers chemistry selenium is used to inhibit the lateral chains, phenomena which are met in the human pathology in the abnormal synthesis of the proteins. Selenium could be a limiter for the abnormal synthesis of the proteins.

Manganese

Manganese has a very low concentration in cerebral arterial wall being under the limit of detection in 4 cases for verterbro-basilar trunk and 5 cases for anterior cerebral artery. The extracerebral artery presents larger amount especially in commune carotid arteries. Mn concentration has a progressive decrease with age; maximum concentration was found in cases of 42 and respectively 29 years. The Mn concentration decrease with age both in intra- and extracerebral artery.

Nickel

We found very small amounts of Ni in cerebral arterial wall vary between 0.4-2.1 mg. The Ni concentration is two to three fold lower in intracerebral artery in comparison with extracerebral artery. There are no significant modifications of Ni concentration in relation with age. In 4 extracerebral and 14 intracerebral arteries the concentration was under the limit of detection.

CONCLUSION

We can conclude that cerebral artery ageing was characterised by increase mineralization mainly as a consequence of increased Na and Ca that will probably decrease wall elasticity. The elasticity is dependent of degree of hidratation of elastin fibres and the amount of metallic ions. The minerals that increase with age are Na, Ca, Mg, Fe, and Se; by contrast Zn, Mn presents a decreased concentration with ageing. Ni, Co and Cu present a more constant evolution with no statistical significant modification with age. We could not identify differences between male and female regarding minerals evolution with age.

There are many mechanisms that involve trace elements in ageing process of arterial wall but a clear role of each of them needs to be established following future studies.

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