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Influence on Metallomic Distribution in Brian and Vital Organs by Prolonged Consumption of Mn in Drinking Water: A Critical Review from Intercellular to Subcellular Distribution

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Abstract. The intake and concentration of metals and electrolytes in our diet are believed to be affecting our general health, in particular, the proper functions of vital organs. For example, in addition to other genetic and environmental factors, consuming water with high alkalinity over one's life time is suspected of leading to diseases such as kidney stones. There is evidence that elemental accumulation due to excessive metal intake would lead to organ failure. In this study, we summarize the data collected by using Wistar rats as experimental species in a prolonged study (750 days) of how ingestion of various amounts of Mn (heavy metal) in drinking water via regular diet intake would affect the metallomic distribution in the brain and other vital organs (e.g., brain, lung, kidney, liver, heart, spleen, and uterus) and pituitary gland as a function of time. Thirty different elements including heavy metals and electrolytes were measured at different developmental ages for the intercellular and subcellular elemental distributions. This study would provide critical insights on how our diet would affect the accumulations of unwanted elements such as heavy metals and trace elements in our vital organs. The results may help researchers and health practitioner to identify possible links between daily diet (metals and electrolytes) and diseases. Specific correlations of elements between intercellular and subcellular levels may also lead to a better understanding of diseases associated with aging such as Alzheimer's and Parkinson's diseases, neurological disorders and organ failures.

Keywords: Intercellular, subcellular, elemental distribution, manganese, vital organ, brain.

1. Introduction

In the 21th century, living conditions for humans have improved drastically; with the consequence of better living conditions and less physical activities, we are facing other aspects of health issues such as obesity and hyper immune responses (allergy). In this modern living, considerable attention has been paid to dietary intake or supplement due to health concerns. On the other hand, involuntarily consumption of unwanted chemicals and preservatives via processed food and polluted water is ever increasing due to our living style and environment. One such example would be consumption of food and/or drinking water from sources that are laden with soluble ions and heavy metals that are invisible by our naked eyes. This occurs quite frequently for those that are living in rural areas with no treatment system for their drinking water in economic disadvantage regions around the globe.

Many metals in the environment are essential for life, others are known to be highly toxic; even the essential metals can be toxic when the concentrations are too high. Metals such as chromium, cobalt, manganese, copper, and zinc are essential for life; but an excess of these can be toxic. Some metals such as arsenic, cadmium, lead, mercury, and vanadium which are found persistently throughout our environment are

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toxic to humans. Metals can come from natural sources such as volcanoes, water, bacterial activity, and also can be consequence of anthropogenic activities such as automobile exhaust, agricultural chemicals, industrial activities, and many other sources. It has been shown that in highly industrialized regions the exposure to heavy metals is extremely high [1]. In all, a major unintentional intake of heavy metals into the body is via food consumption, most likely through drinking water [2].

It is known that 26 of the 90 naturally occurring elements are known to be essential for animal life. These consist of 11 major elements or macro-nutrients, they are: C, H, O, N, S, Ca, P, K, Na, Cl, and Mg, and 15 other elements are termed as trace elements or micro-nutrients, they are: Fe, Zn, Cu, Mn, Ni, Co, Mo, Se, Cr, I, F, Sn, Si, V, and As. The molecular basis for the selection and rejection of elements is not known. Major elements, such as Na, K, Ca, and Mg, are required for functional reactions such as body fluid buffer, active transport, ionic balance, electrical transmission, and tissue development as well as the composition of body fluids and structures. Trace elements act primarily as catalyst in enzyme system in the cells where they serve a wide range of functions from weak ionic effects to highly specific associations such as metalloenzymes. In addition, the protein-metal interactions may increase the stability of the protein moiety to metabolic turnover [2].

If a person is regularly ingesting excessive heavy metals into the body, an important issue need to be addressed is how these heavy metals would affect the wellbeing of this person. Obviously, this is an issue cannot be addressed easily and economically due to the dynamics of issue involved which include time of exposure, concentration, toxicity of the metals, organs of the affected body. In essence, it is the intercellular and intracellular/subcellular elemental transport mechanism(s) need to be understood before the above question can be addressed; furthermore, to answer the question of how each heavy metal and its concentrations can affect our vital organs and the mechanism(s) requires tremendous amount of efforts intellectually and economically, if not impossible. Further question of how a particular heavy metal would affect all the metallomic distributions seems even more far-fetched.

Nevertheless, our research group has mobilized a pioneering research effort attempting to investigate how electrolytes, essential and trace elements can be affected in their accumulation in the brain and six other vital organs and pituitary gland in female Wistar rats in lifelong consumption of drinking water dosed with 3 different concentrations of Mn. Mn was used as a surrogate of heavy metals because it is a common metal found in natural environment and used in many industrial applications [3]. The 30 electrolytes and elements of interest were: Al, As, Ba, Br, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, La, Mg, Mn, Mo, Na, Rb, S, Sb, Sc, Se, Si, Sm, Sr, V, Zn; these elements represent the common electrolytes, essential and trace element found in our body, and trace elements that we often encounter in the environment. The six vital organs investigated in this study were heart, kidney, lung, spleen, liver, uterus, and pituitary gland. The pituitary gland was included in this study because it was often found to be cancerous in aged rats. The different developmental stages of the specimens before scarified were 5, 10, 23, 120 (adult), and 750 days (aged). All the elements were detected by neutron activation technique, among the 30 elements, only 20 elements were detectable, and only 14 elements were traceable in the brain [3].

In this critical review, we discussed what we had found in the open literature as well as what our contributions to the intercellular and intracellular/subcellular metallomic distribution in the brain and other vital organs and pituitary gland. It should be noted that most of the literature in open sources involved only information in limited studies in intercellular distribution, there were no organized studies in subcellular distribution. Organized study with extensive information such as ours as yet exists.

2. Background

2.1. Brian

Brain is a specialized organ of the body, it metabolizes and accumulates metals as part of its normal development and function. But in a rich metal environment, loss of metalloproteins and loss of defense against oxidative stress caused by one or more of the heavy metals could be responsible for neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD) [4]. The intake of these metals occurs via ingestion of metal-containing food and water, and/or through inhaling metal-

contaminated air. The elemental distributions in the different brain regions appear to vary for each element. Some of these metallic elements are known to increase or decrease in brains of humans with a neurodegenerative disorder [2].

Attempts in understanding the homeostasis of different elements in the brain fall significantly short due to the vast complexity of the mechanisms involved. Only an estimated 50% of the functional blood-brain barrier (BBB) transporters have been discovered [5]. There has been detailed analysis of proposed transport mechanisms of specific elements at certain stage of development of the studied species, but long term study over developmental ages is in need of further investigation. As an example a detailed analysis showed that administration of Mn chloride or sulfate resulted in more rapid brain uptake than insoluble Mn oxide or phosphate. Also consecutive studies showed that larger sample sizes are needed to observe statistical significance in the small changes over life-time exposure [3]. Taking the next step in understanding accumulation due to continuous life-time environmental exposure will help to link other detailed studies of individual mechanisms.

Other works involving elevated life-time exposure of Cu and Zn up to 17 months show very little increase in the elemental accumulation in rat brain [3]. Ingestion routes, of both water and food were investigated showing water to be more affective in uptake of both elements. Thus, this is another example that soluble form of minerals ingested via drinking water or intravenously provides faster uptake into the blood system. Zn showed a 5% increase in the rat brain compared to control. Zn showed an increase while Cu showed a decrease in the rat brain. The results of Zn replacing Cu provide evidence that similar elements compete in the same homeostatic mechanisms for accumulation and control of elemental concentrations in the brain.

2.2. Other Vital Organs

Along with the brain, other vital organs (lung, kidney, liver, heart, spleen, and uterus) can be impacted by elevated exposure to different elements; exposure consequences can be as serious as cancer or even organ failure. Other less serious exposure consequences can be physical discomfort or diminished capacity of specific organs. A more relevant aspect in relation to this paper is identifying the organs that show accumulation trends and sources or sinks for elemental accumulation in the body.

The importance of evaluating elemental accumulation is found in a number of practical applications. One application is in the understanding of neurodegenerative disorders (ND). Elements such as Cu, Zn, Fe and Mn are essential for normal brain function but evidence also shows that above normal concentrations in subjects that exhibited (ND) symptoms [5]. Additional complication that arises in this type of study is that exposure amount is not directly related to accumulation; another factor that can complicate such effect is the age of development. A second application is in understanding the possible decreasing accumulation rates with age and the potential need for supplementation. With data suggesting that improved elemental uptake by ingestion of elements dissolved in water is more effective in comparison to food creates a contradiction to currently accepted method of supplementation in pill form. In one study, a known Cu-deficient population of mice were able to be rescued from glucose-induced mortality by supplementation of Cu in drinking water [6]. This also raised the question of whether soluble organic supplement is better than inorganic supplement. A third application is in the evaluation of exposure limits and the relationship to the body's natural homeostasis of a particular species. Studies comparing exposure effects of mice compared to rats suggest that in different species, there are differences in homeostatic mechanisms resulting in tighter experimental control in mice than in rats [7].

With lifetime data on elevated exposure levels, uptake and accumulation rates can be compared to documented studies of standard and elevated levels as seen in subjects displaying ND symptoms [8]. This is a significant piece of information in evaluations of exposure limits. A second level in this analysis is the rate of change of accumulation over time with constant and regulated life-time exposure. In relating this to possible homeostatic mechanisms, various doses of Mn in water were administered to compare accumulation levels. With this experiment, elements that interact in the same mechanism would be in competition with Mn and show increasing or decreasing accumulation results with increased Mn dosage levels [9].

To better understand the possible uptake mechanisms, elements grouped by periodic table column which identifies outer shell electrons is helpful for established overall trends, such as decreasing, increasing or varying over time. Groupings help to link possible similar mechanisms and speciation characteristics. Elemental accumulations are not solely related to exposure but likely have more to do with impairment of the relevant homeostatic mechanism(s) [5]. By modeling the accumulation relationships, possible mechanisms can then be proposed and evaluated [10].

The elements showing decreasing accumulation over time would suggest possible supplementation with age. There are two aspects to this analysis. First is that even with elevated exposure levels (dietary intake) the accumulation of a particular element shows decreased levels compared to control. Decreased accumulation suggests a couple of possibilities. First possibility is that the homeostatic mechanism has been greatly impaired. Second possibility is that a similar competing element is displacing and reducing the elemental concentration of interest. The next step would be to investigate possible speciation and complex forms that would provide increased uptake of the needed elements.

3. Discussions

3.1. Influence on Organs with Mn in Drinking Water

Each organ has different characteristics as indicated by some showing overall accumulating concentrations and some showing overall diminishing concentrations and others showing mixed trends with increasing elemental concentrations and decreasing elemental concentrations in the lifetime analysis. In a few instances, there were some elements that showed a possible correlation with Mn dosage. Both increasing and decreasing elemental concentrations with increased Mn dosage were present suggesting positive and negative correlations. Three main observations can be concluded from our analysis of previous results:

1) Elements showing increased concentrations by organs;

2) Elements showing decreased concentrations by organs;

3) Organs that showed an overall accumulating concentration, diminishing concentration, or mixed with a similar number of elements increasing and decreasing.

3.1.1. Elements showing increases

In most cases, treatment with Mn showed increased elemental accumulation in organs. Elements showing accumulation in at least four organs are: Ca, Mg, Co, Cr, Cu, Mo and Se. In this group of elements, there are some elements with known impacts on human health and degenerative diseases (i.e. Cu related to Alzheimer's and Parkinson's diseases). The findings emphasize the importance of more extensive studies where Mn may have been used in an application that may have increased human ingestion, such as in treatment of drinking water.

Elements showing accumulation in two or fewer organs are: F, K, Na, Br, Hg, I, Rb, V and Zn. Even though accumulation was shown for this group of elements, only certain organs displayed accumulation. This information is important to help identify elements that may have more complicated homeostatic mechanisms and are more selective to individual organs. Within this group, there also are elements with known health impacts.

3.1.2. Elements showing decreases

The liver and uterus show more overall decreased elemental concentrations. The spleen and pituitary also had several elements that decreased in elemental concentration with Mn treatment. Elements that show decreasing concentrations in three or more organs are: Br, Hg, Se, V and Zn. Elements that show decreased concentration in 1 or fewer organs are: Cl, Fe, K, Mg, Na, Al, Cr, Fe, I and Rb. Overall, more elements showed increased concentrations or accumulation due to Mn treatment than elements that showed decreased concentrations.

3.1.3. Overall accumulating, diminishing, mixed

The organs that showed significant decreasing elemental concentrations with added Mn were the liver and uterus. The uterus, showing a decrease in overall concentration, may provide a link to the impact of Mn

exposure and possible birth defects. The liver, showing decreased concentrations, may have two possible impacts. First is the possibility that the elements are being excreted from the body. This may be a positive impact in regard to applications that would need to reduce the concentration of a particular toxin in the body. The second possible result in the liver showing reduced elemental concentrations is that the function of the liver is being reduced (overloaded) and therefore is not pulling contaminants from the body.

From the literature search listed above, it is evident there are many focused and detailed studies showing the health impacts due to increased elemental concentrations of several particular elements. One study identified Cu, Zn, Fe and Mn are essential for normal brain function but also show that above normal concentrations may lead to ND symptoms [6]. In our studies, we found the concentration of Cu is increasing in many organs due to Mn treatments. In an evaluation of all brain data in our experiments, Cu decreases by 100% from adult control (AC, 120 days) to old control (OC, 750 days) rats (see Figure 1). This corresponds to the loss of Cu with old age and the possible onset of ND with old age. But then looking at all brain data for Mn treated OC to old treated (OT, 750 days), there is a slight increase in Cu concentration by slightly over 10%. Mn increased Cu in the brain and in many other organs.

The treatment of Mn could be beneficial in some cases and detrimental in other cases. An increase in Cu concentration (≈ 10%) in the brain may be beneficial but an increase in Hg in the heart (≈ 1500%) may be detrimental to humans. This data set will provide a key piece in understanding human health effects due to elevated elemental ingestion over the full life span [11]. The following figure displays a fractional change between adult control (AC, 120 days) sample, old control (OC, 750 days) samples, and old Mn treated (OT, 750 days) samples for the all (whole) brain of rats by comparing AC vs. samples (e.g., OC) (AC/OC).

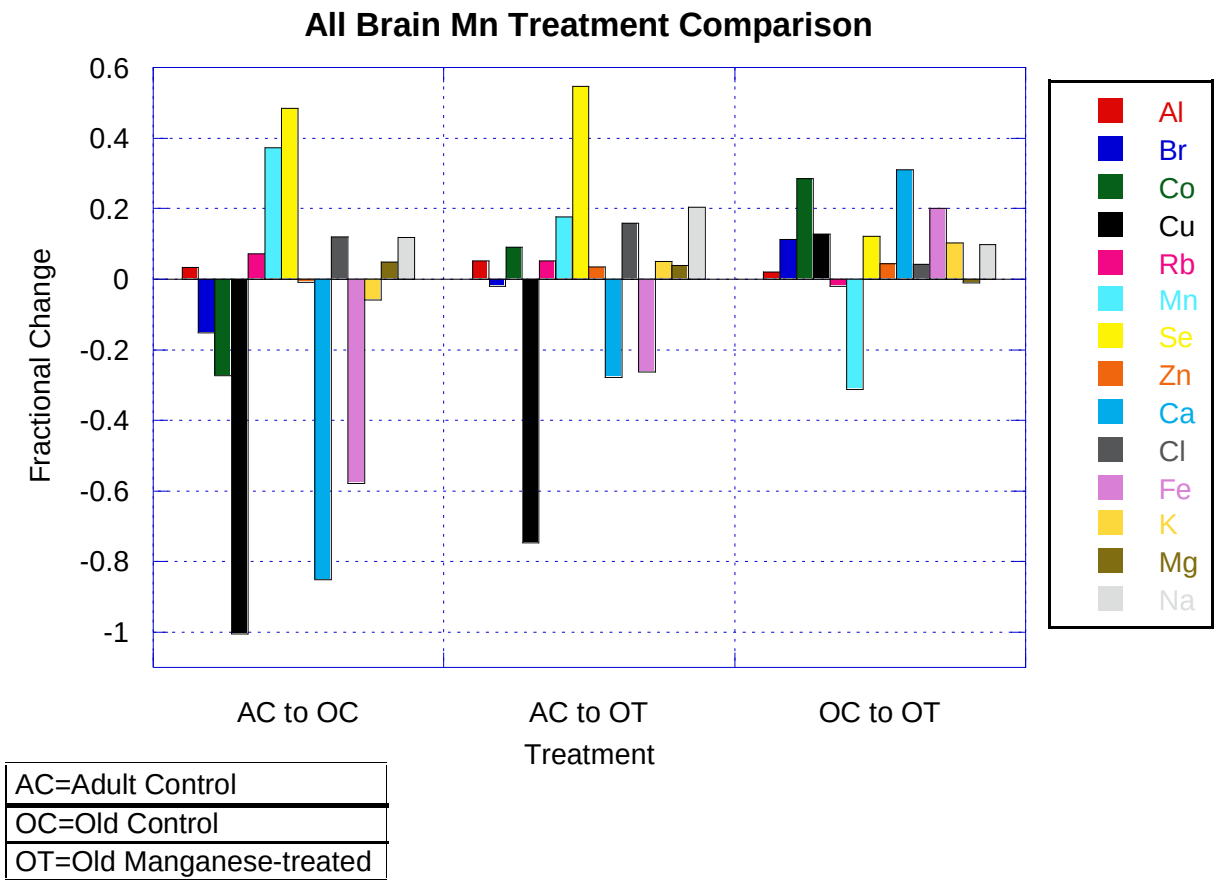

Fig. 1: Comparison of elemental distributions of the brain in fractional changes for adult and aged rats: AC/OC, AC/OC, and AC/OT.

The whole brain result shows significant change on Cu concentrations with age. For the adult control to old control (AC to OC) there is significant loss in the concentration of Cu. This relates other studies that found reductions in Cu for subjects that exhibited neurodegenerative diseases (i.e. Alzheimer's and

Parkinson's). Comparing AC to aged rats with Mn treatment (OT), the reduction in Cu is less by about 20%. This supports current studies involving Cu but may suggest that Mn (heavy metals) also has an impact on the Cu accumulation in brain [12].

3.2. Transition from Intercellular to Subcellular Distribution

Extensive literature search was also conducted to obtain elemental subcellular/intracellular distribution in the brain and other vital organs [13]. Unfortunately, as mentioned in previous intercellular elemental distribution search, there were only sparse reports on selected elements on a specific organ of animals, reports on systematic study on subcellular/intracellular elemental distribution with multi-elements, multi-organs, and prolonged duration are not existing. With such, in this conference, we are also giving a report that is parallel to the topic of this study that reveals detailed subcellular information of different parts of the adult rat's brain for 14 detectable essential and trace elements, and the influence of Mn, while ingested via daily drinking water, on the brain's subcellular elemental distributions [14]. It is anticipated that these article series would enhance the understanding of the intercellular and subcellular elemental distribution in the brain and other vital organs, as well as the influence of heavy metal (Mn) on the elemental distributions if it is ingested via the daily diet of drinking water. Furthermore, the information of dietary intake of Mn provides insights of how dietary supplements would affect the elemental distributions of all the vital organs. Likewise, similar effects may be extrapolated if polluted water laden with heavy metals as part of the regular diet is consumed.

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