Distribution and role of metals in sclerotic hippocampi of patients with mesial temporal lobe epilepsy

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The accumulating evidence on the relation between the disturbed metal homeostasis and epilepsy urges the need for data regarding the total metal concentrations, as well as metal distribution in the brain itself, in order to indicate where to direct the potential therapy, to metal supplementation or chelation. This paper summarizes our results on the measurements of some important essential metals in hippocampi of patients with mesial temporal lobe epilepsy (mTLE) who underwent amigdalohippocampectomy. The key findings point out that levels of copper and manganese are deficient in hippocampi of mTLE patients, and that their concentrations correlated positively with neuronal loss in affected regions of sclerotic hippocampus. In addition, the Cu concentration was decreased in the areas of total neuronal loss. Iron and zinc total hippocampal levels were neither accumulated nor deficient compared to control. Our results contribute to deeper insight of metals biology in the epilepsy and may represent the initial point of new and non-invasive therapy of drug resistant epilepsy.

Introduction

Epilepsy is neurological condition which is clinically manifested as spontaneous, recurrent epileptic seizures, due to short-term, uncontrolled and highly synchronized outburst of electrical activity of larger group of neurons in the brain. As a result, balance between brain excitability and inhibition is disturbed and shifted in favor of excitatory state. Epilepsy is accounted for one of the most common among serious neurological disorders. It affects about 50 million people worldwide, with 50 new cases per 100,000 per annum [1]. It is estimated that 1% of each population suffers from epilepsy at any time point. Despite that, many uncertainties considering the pathophysiology follow this condition, making therapeutic strategy inadequate or insufficiently good for one third of patients that enter chronic state of this disorder. In line with that, there are multiple causes of epilepsy which may have a genetic, structural or metabolic background and often the underlying cause is indefinite and remains to be identified. Epileptic seizures could be generalized, when rapidly affecting neuronal networks of both hemispheres, or they could be focal when affecting neuronal networks unilaterally [2].

Focal epileptic seizures frequently arise in temporal lobe, mostly in its mesial part comprised from amygdala, hippocampus, uncus, gyrus dentatus and parahippocampal gyrus. Although mesial temporal lobe epilepsy (mTLE) is one of the most common types of focal epilepsies, portion of the mTLE patients is difficult to treat with available antiepileptic drugs. Unfortunately, in time they develop drug resistant epilepsy. The most common cause of drug resistant mTLE is structural changes within hippocampus that involve different level of neuronal cell degeneration and loss (mTLE with hippocampal sclerosis – mTLE-HS), as well as abnormal reorganization of neuronal circuits [3]. Therefore, surgical treatment, *i.e.* removal of sclerotic hippocampus, was shown to be helpful in attaining the control over epileptic seizures in 60 – 80% of patients [4]. Surgical failures emphasize the complexity of the condition indicating the role of other mesial structures in seizure development, as well as the constant need for new therapies. On the other side, specimens gained through these surgical interventions offer direct look into the "crime scene", which could contribute to better understanding of the pathophysiological processes associated with mTLE-HS. There are four types of HS based on the affected regions and the level of neuronal loss according to International League Against Epilepsy (ILAE) [4].

Metals biology in epilepsy

More and more studies, point that loss of regulation of metal homeostasis in the central nervous system contributes to pathophysiological processes in various neurodegenerative diseases. For example: it is hypothesized that iron loading and beta-amyloid plaque pathology are synergistic events in Alzheimer's disease (AD) [5,6]; perturbations in iron and copper metabolism contribute to the pathology of Parkinson's disease (PD) [7,8]; mutations in copper-transporter protein ATP7A in Menkes disease result in severe neurodevelopmental deficiencies and epileptic seizures as a result of copper deficiency in

the brain [9]; extensive research on zinc signaling in epileptic seizures indicate that interplay between intracellular and extracellular concentrations of this metal may contribute to harmful or protective effects [10]. Therefore, targeting the key players of metal homeostasis could be helpful in treatment of neurodegenerative diseases. However, it is very demanding to identify precise targets responsible in maintaining balance of essential metals in healthy or diseased brain due to complexity of interactions among the metals themselves and the plethora of metalloproteins. Nevertheless, field of metal biology is gaining its place under the spotlight of neurosciences.

Perturbations in metal homeostasis are possible cofactor in the onset and/or progress of epilepsy. Meta-analysis performed by Saghazadeh and co-workers indicate differences in concentrations of trace elements in sera of the epilepsy patients (on or without therapy), and control subjects [11]. However, what happens in relation of various metals concentrations in the brain itself exposed to epileptic seizures, remained to be determined and systematically analyzed. Therefore, the goal of our investigations is to contribute to the field of metal biology in epilepsy by determining total concentrations and regional distribution of some essential trace elements in sclerotic hippocampus resected from drug resistant mTLE-HS patents as surgical therapeutic approach. We have used two powerful techniques: inductively coupled plasma optical emission spectrometry (ICP-OES), to measure total concentrations of metals; and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) imaging, in order to provide detailed metal maps of epilepsy related sclerotic hippocampus. In our research we have investigated HS ILAE type 1 (HS1) and type 2 (HS2). HS1 is the most common among all mTLE patients that undergo surgical intervention, and represent severe lost of neurons in all segments of cornu amonis (CA1 – CA4), as well as in gyrus dentatus (GD). HS2 is the rare type seen in 5-10% of surgical cases of mTLE and affects exclusively CA1 region. Our strategy was to: compare epilepsy-induced HS with control tissue; compare regions with neuronal degeneration with preserved ones.

Measurements of total concentrations of metals gained by ICP-OES can be observed in table 1 (adapted from [12]). The profile of sodium, potassium, copper, and manganese in hippocampi of patients with mTLE-HS differed considerably from the control tissue. Disturbances in levels of main extracellular and intracellular electrolyte, *i.e.* increased sodium and lowered potassium concentration point out to the neuronal cell loss and changed ratio between intracellular and extracellular volume of sclerotic hippocampus. Further, hippocampus of mTLE patients is marked by significant copper and manganese deficiency [12]. Spatial distribution of copper and manganese in hippocampi of patients with mTLE-HS acquired by LA-ICP-MS shown in figure 1, revealed that these metals mostly concentrate in areas reach in neuronal somata, which are the following regions of hippocampus: subiculum (SUB), CA4, GD, and stratum pyramidale (SPy) in CA1 and CA2; while alveus and other regions with axons and dendrites generally showed lower levels of Cu, and Mn [13]. What is important is that Cu concentrations were significantly lower in areas of hippocampus affected with neuronal loss (SPy of CA1) compared to preserved regions (SUB). Furthermore, Cu and Mn concentrations positively correlated

with neuron somata density in the SPy of CA1 [13]. This is in good agreement with Cu/Mn deficiency registered in sclerotic hippocampi compared to controls [12].

Table 1. Hippocampal metals and electrolytes in patients with mTLE-HS and controls (data from [12]).

	Hippocampi	
Metals 1μg/g of tissue	Controls (n = 17)	HS (n = 24)
Na	**1040 ± 25, p = 0.010**	**1131 ± 22**
K	**2322 ± 6, p < 0.001**	**2001 ± 59**
Ca	104.6 ± 14.1, n.s.	101.0 ± 14.6
Mg	118.2 ± 4.4, n.s.	106.8 ± 3.5
Zn	10.97 ± 1.03, n.s.	13.97 ± 1.51
Fe	61.9 ± 10.7, n.s.	62.2 ± 5.1
Cu	**3.57 ± 0.33, p < 0.001**	**2.34 ± 0.12**
Mn	**0.41 ± 0.06, p = 0.004**	**0.205 ± 0.030**

What is the meaning of altered copper levels in hippocampus of mTLE patients? Copper deficiency in epilepsy-related sclerotic hippocampus may result in multiple functional impairment of this structure. Namely, copper is known to be modulator of synaptic activity, since it is released in synaptic cleft after neuronal depolarization [14]. Copper is considered to act as negative regulator of N-methyl-D-aspartate (NMDA) receptor, which play important role in hippocampal neuronal excitability and impairment in Cu-homeostasis has been associated with epileptic seizures in Menkes disease [15,16]. Furthermore, proper functioning of some important metalloprotins, such as Cu-Zn superoxide dismutase (CuZnSOD), dopamine β-hydroxylase, cytochrome c oxidase etc., critically rely on copper. However, our study on hippocampal antioxidative system in mTLE showed that the activity/level of CuZnSOD were not significantly different from those in the control hippocampi, implying that that changes in cytosolic production of superoxide are not implicated in generation of oxidative stress [17]. On the other hand, it seems that reduced availability of copper affects neither levels nor activity of CuZnSOD [12,17]. Similar situation was observed in substantia nigra (SN) of PD patients, *i.e.* SN is the area defined with high neuronal loss, prominent reduction in total copper level, and the activity/level of CuZnSOD comparable to control [7]. However, other areas of the brain of PD patients characterized with limited cell loss, had normal Cu level, but then increased activity of CuZnSOD [7]. Therefore, it will be useful to determine and compare total Cu concentrations and activity/level of CuZnSOD in two subgroups of mTLE patients, the one with high degree of hippocampal sclerosis (HS1) and the other with low degree (HS2), and to estimate how better structural preservation influences Cu levels and functioning of metalloenzyme CuZnSOD. In addition, since role of copper-transporting ATPase was shown to be required for hippocampal neuronal activation, we hypothesize that there is Achilles' heel of copper turnover in mTLE-HS that causes copper deficiency and in thus way contribute to pathology of epileptic seizures.

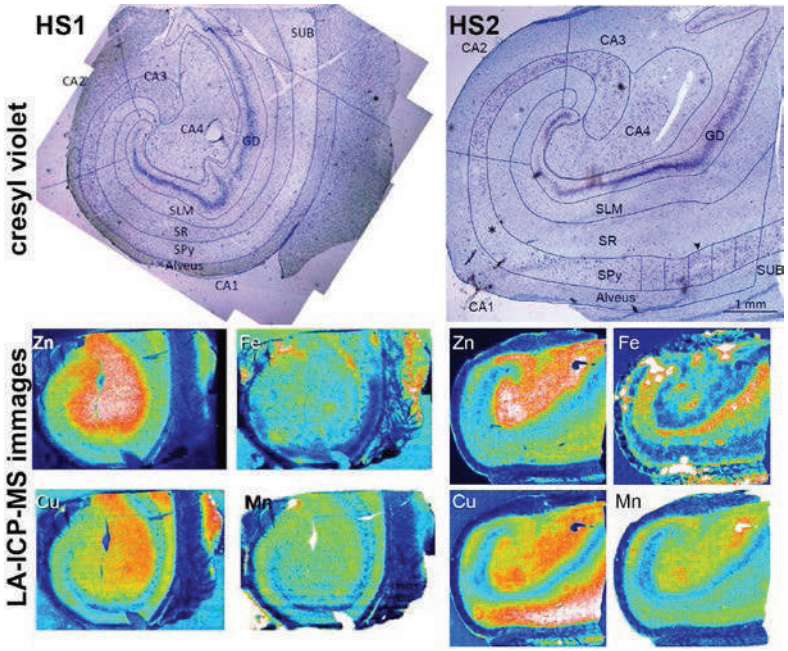

Figure 1. Micrograph of the coronal cresyl violet stained section of a sclerotic hippocampus HS1 and HS2, with delineated anatomical regions. LA-ICP-MS images of the subsequent coronal section of the same patient, presenting zinc, iron, copper, and manganese maps. GD, gyrus dentatus (demarcated together with the stratum moleculare, stratum granulosum and polymorphic layer); SUB, subiculum; SPy, stratum pyramidale; SR, stratum radiatum; SLM, strata lacunosum and moleculare. Straight lines demarcate different fields of the cornu ammonis (CA1–CA3).

Manganese deficiency registered in human HS could contribute to the pathology of mTLE by affecting: main manganese metalloprotein and astrocytic enzyme - glutamine synthetase (GS); and/or member of mitochondrial antioxidative system - manganese superoxide dismutase (MnSOD). Indeed, down-regulation of GS has been reported previously in animal model of epilepsy, as well as in the hippocampus of mTLE patients [18-21]. Resent study reported the almost exclusive astrocytic GS expression in the neuronal somatic layers of hippocampus of mTLE patients and that GS down-regulation positively correlated with the degree of neuronal loss [21]. This is in line with our data that Mn concentrations correlated positively with neuron density in the neuronal somatic layer of CA1, region highly affected with neuronal loss [13]. Since it was shown that manganese deficiency in the brain is accompanied with seizures in animal model of epilepsy, we hypothesize that reduced concentration of manganese in human epilepsy-related HS could interfere with GS level/activity, which would result in augmented levels of glutamate leading to neuronal hyper-excitability and excitotoxicity [22]. Furthermore, our data show that neurons with on-going degeneration were immunopositive for MnSOD [17]. These degenerated neurons were present in all neuronal layers, and could not be detected in areas

of total neuronal loss, and in regions with no neuron somata, such as alveus, which is again in good agreement with Mn spatial organization that positively correlates with density of neuronal bodies [13,17].

Our results show that iron accumulation is not characteristic of sclerotic hippocampus of mTLE patients [12,13]. Comparable iron concentrations of mTLE and control hippocampi support this statement. However, metal maps showed that iron mainly follows the paths of hippocampal blood vessels. It was hard to draw any conclusions regarding local iron accumulation and/or deficiency, since high amounts of this metal was resent in the vasculature. Although, the phenomenon of hippocampal iron accumulation is the feature associated with other neurodegenerative diseases, such as AD and PD, it is accepted that epilepsy- and neurodegeneration-related pathological changes of hippocampus are markedly different mostly due to preserved neurogenic capacity of epileptogenic hippocampus [3,23].

Considering the role of metals in epilepsy, zinc signalling is the most extensively investigated. However, owing to the complex relationship between generation of seizures and zinc signalling, the data about the role of intracellular/extracellular zinc are often conflicting [10]. Therefore, information considering total concentration, as well as providing data about regional distribution of this metal would help to resolve in which direction zinc will act. Our results show that the total level of zinc in human hippocampi obtained from patients with drug resistant epilepsy does not differ significantly from the levels of zinc in control hippocampi [12]. Analysis of zinc regional distribution revealed that Zn is mainly located in mossy fiber reach regions: GD, CA4, and CA3. Rank of order in zinc levels in pyramidal layer of cornu amonis were as follows: CA4 > CA3 > CA2 = CA1 [13]. Except validating previous findings on Zn hippocampal distribution, gained with histochemical staining, our results also imply that we need to go beyond regional distribution, and to map Zn at cellular resolution, using synchrotron-based x-ray fluorescence microscopy for example. That way may provide information on fine tuning of Zn levels inside and outside of the cell, which may give the deeper insights at the role of Zn signalling in mTLE [24-27].

Conclusion

Determining the total concentrations, as well as spatial distribution of essential metals in crime scene of mTLE, which is sclerotic hippocampus, contribute to overall knowledge about pathology of epilepsy and point to the much needed non-invasive therapeutic directions. Among the examined metals, we confirmed the deficiency of copper in sclerotic hippocampus on two levels: (i) in whole structure (ii) and locally in the areas of neuronal loss, with significant correlation between copper concentration and neuron density. Therefore, our findings identify members of copper turnover, including proteins that serve as copper transporters, carriers and chaperons, as crucial targets of future investigation.

Acknowledgements

This study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant numbers III41014 and OI173014, and by Slovenian Research Agency (ARRS), Project numbers P3-0171 and P1-0034.

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