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Exploratory analysis of the serum ionomic profile for bipolar disorder and lithium treatment

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In the present work, a very simple and fast experimental design using ICP-MS was employed to search for differentiations in the levels of free ions present in human blood serum samples obtained from healthy individuals ($n = 25$) and bipolar disorder (BD) patients under different treatments: (i) using lithium ($n = 15$) and (ii) using other drugs than lithium ($n = 10$). Fourteen ions that showed statistically significant alterations in its levels when comparing the studied groups were detected, providing a hint for further studies on BD and lithium treatment biomarker discovery.

1. Introduction

Bipolar disorder (BD) is a chronic and highly disabling psychiatric illness. It is characterized by recurrent mood disturbances, manifesting itself as episodes of mania (or hypomania) and depression separated by periods of normal mood (euthymia) and affects 1–3% of the worldwide population. Nevertheless, the mechanisms at the molecular level of this disorder, as well as of its treatment with lithium, which is the most widely used medication, are still unknown [1–3].

Several biochemical abnormalities that are observed in populations that have behavioral diseases are related to the brain chemistry. As an example, copper is an important factor in the conversion of dopamine to norepinefrine, and zinc is necessary for an efficient synthesis of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter [4].

Metallomics is a scientific area that consists in the study of free or complexed metals or semimetals present in a biological system (cells, fluids, tissues or organisms) [5–8]. When the study considers only the identification and/or quantification of free metal or semimetal ions, as in the case of the present work, it is called ionomics [9].

In the recent literature [10], an ionomic study was performed using serum and blood samples from Alzheimer-diseased patients. The determination of Al, Ba, Be, Bi, Cd, Co, Cr, Hg, Li, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Tl, V, W and Zr was performed using a sector field—inductively coupled plasma mass spectrometer. In relation to healthy individuals, the differences found in serum samples were the highest levels of mercury and tin ($p \leq 0.01$) and in blood samples the differences were the highest levels of cobalt, lithium, manganese and tin as well as the lowest levels of molybdenum ($p < 0.01$).

In a previous work, we used a metalloproteomic strategy employing laser ablation ICP-MS (direct measure and imaging) in order to evaluate the profile of metals and semimetals bound to serum proteins of BD patients treated with lithium or other drugs not including lithium [11]. Some elements bound to proteins could be measured (Ca, Co, Fe, K, Na, Mg, P, Ti and Zn), but lithium was not detected when complexed with proteins.

There are several analytical techniques used for lithium measurement in serum and brain samples from BD patients and/or animal models. Some examples are ^{7}Li magnetic resonance spectroscopy [12], functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) [13]. Herein, a simple and

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multielemental analytical technique, ICP-MS, was chosen in order to determine lithium levels and also to evaluate the ionomic profile of serum samples.

Based on the results of our previous studies where differences in the profiles of metabolites [14], proteins [15], and metalloproteins [11] were found when comparing healthy subjects and BD patients under distinct treatments (including lithium or not), the aim of this work was to perform a comparative ionomics analysis of blood serum from the same samples, in order to search for differential free metal ions that may be further considered for biomarker discovery studies.

2. Materials and methods

2.1. Equipment, reagents and solutions

The quadrupole inductively coupled plasma mass spectrometer Elan DRCe and the multi-element standard solution were obtained from Perkin Elmer (Norwalk, USA). Nitric acid and Triton X-100 were purchased from J.T. Baker (Phillipsburg, USA).

2.2. Samples

The human serum samples used in this work, as well as details concerning collection and storage, are described in our previous publications [11,14,15]. Briefly, 50 serum samples were collected and classified into three groups: (i) control group, consisting of 25 samples of subjects without BD, (ii) BD patients under treatment using Li group, consisting of 15 patients and (iii) BD patients under treatment with other drugs not including Li group, consisting of 10 patients.

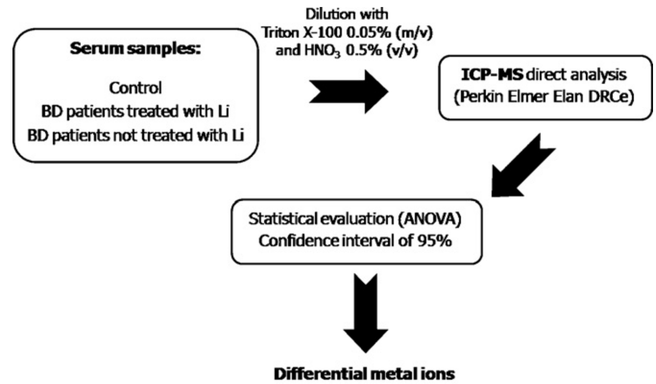

Fig. 1. Schematic analytical workflow used for ionomics analysis.

2.3. Determination of free ions in blood serum by ICP-MS

The serum samples were diluted 10 times with a solution containing 0.05% (m/v) Triton X-100 and 0.5% (v/v) HNO_3 [16] and then analyzed in the ICP-MS Elan DRCe mass spectrometer. Semi-quantitative analysis was performed in triplicate and the evaluated mass-to-charge (m/z) ratio regions were: 6–15, 19–39, 42–210 and 230–240 u. The quadrupole voltage was set to 0.4 V. Nebulizer gas, auxiliary gas, plasma gas and sample injection flow rates were 0.91, 1.2, 15 and 1.5×10^{-3} L min^{-1}, respectively. The lens voltage was between 7.5 and 11 V (as AutoLens mode was used, the lens voltage was adjusted automatically as different mass isotopes were measured) and radiofrequency power was set to 1200 W. After the measurements, data was treated statistically by single factor analysis of variance (ANOVA) at a confidence interval of 95%. A representation of the analytical workflow is shown in Fig. 1.

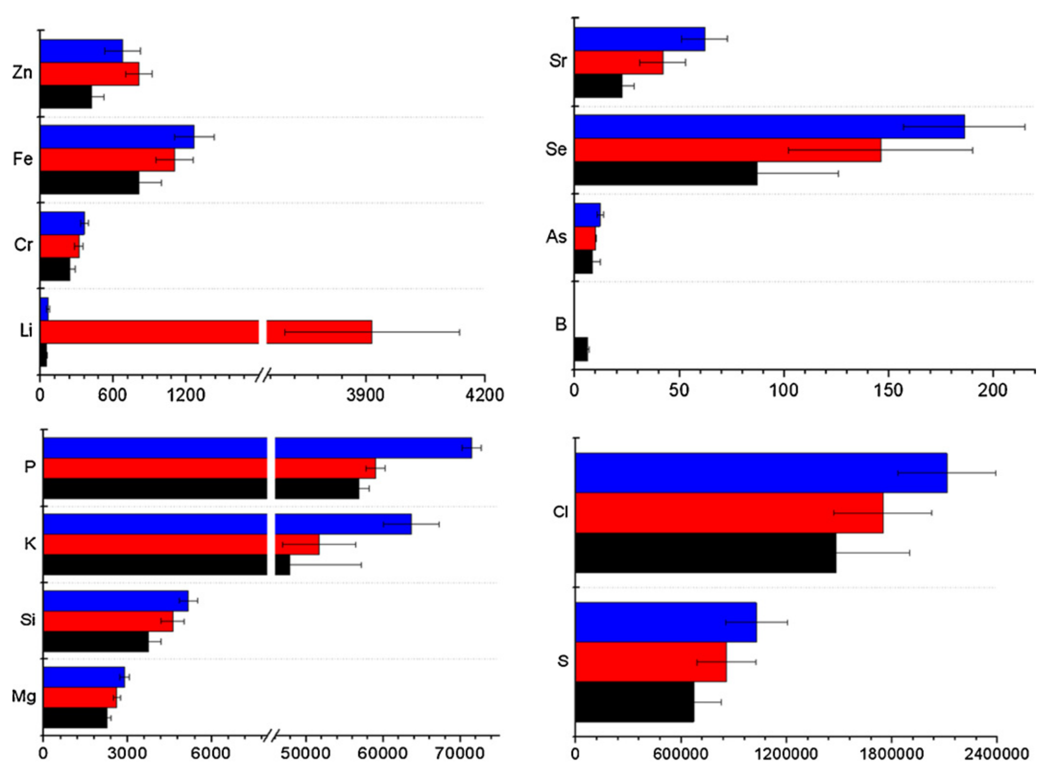

Fig. 2. Graphical illustration of the semiquantitative analysis by ICP-MS of the detected differential ions. The black bars represent control group, while red and blue bars represent the groups of bipolar patients treated with Li or other drugs than Li, respectively. Concentrations (x axis) are expressed in $\mu g\,L^{-1}$.

3. Results and discussion

The simple and fast protocol used in this work allowed the detection of 26 free ions in serum samples. From these 26 ions, 14 showed statistically significant differences between healthy individuals (control group) and BD patients treated with Li or with other drugs not including Li. The differential ions found were: As, B, Cl, Cr, Fe, K, Li, Mg, P, S, Se, Si, Sr and Zn. Although it should be considered that Cl, P and S are usually not finely determined by ICP-MS, they were mentioned herein because of the statistical significance of the result. Fig. 2 illustrates the distinctions in the levels of the differential metal ions when comparing the three studied groups.

All differential ions, except B, were found at higher level in BD patients groups than in controls. Only Li and Zn were found at higher level in BD patients treated with Li than in BD patients not treated with this drug. The Li behavior was already expected in the case of the first group of BD patients, because of the intake of this metal ion as treatment.

Zn is an important modulator of glutamatergic transmission, attenuating NMDA (N-methyl D-aspartic acid) receptor function [17]. Preclinical studies have demonstrated the antidepressant-like effects of Zn administration in animal tests/models of depression and that Zn enhances the efficacy/potency of citalopram or imipramine in preclinical paradigms sensitive to antidepressants [18]. According to studies that demonstrate alterations in Zn concentration induced by antidepressive drugs and electroconvulsive shock treatment (ECT) in rat brain, it is possible to infer that Zn plays an important role in the mechanism of psychiatric therapies. Besides, Zn level alterations in blood (serum and plasma) can be considered a marker for depression, as a lower Zn concentration is found in depressed patients in relation to healthy individuals, and the highest the gravity of the disorder (treatment-resistant patients) the lowest the Zn concentration [19,20]. Nourmohammadi et al. [21] related more recently a hypozincemia in BD I patients, as we found in this work for BD patients not treated with Li. Based on these studies, the higher level of Zn found in serum of BD patients treated with Li is in agreement with the hypothesis that Zn may play a role in the mechanism of therapies for psychiatric disorders [19–21].

The differences found in the levels of some ions in serum show that the ionomic profile is altered in the presence of BD and also allow the distinction between BD patients treated with Li or other drugs excluding Li. Although such alterations cannot be explained by a simple model, some elements can be further studied as potential biomarkers for BD and/or its treatment with Li.

4. Conclusions

The exploratory analysis of serum samples by ICP-MS allowed the observation of distinct ionomic profiles when comparing healthy subjects and BD patients. Besides, the different BD treatments (using Li or not) caused alterations in the ionomic profile of the samples. In this way, the differential ions identified (As, B, Cl, Cr, Fe, K, Li, Mg, P, S, Se, Si, Sr and Zn) can be evaluated in future works as potential markers for BD and/or its treatment using Li.

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