Cortical biometals: Changed levels in suicide and with mood disorders

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Abstract

Background: Changes in levels of metals have been suggested to contribute to the pathophysiologies of several neurodegenerative disorders but to our knowledge this is the first metallomic study in CNS from patients with mood disorders. The focus of this study was on cortical regions affected by the pathophysiologies of bipolar disorders and major depressive disorders.

Methods: Levels of metals were measured using inductively coupled plasma mass spectrometry in Brodmann’s areas (BA) 6, 10 and 17 from patients with major depressive disorders (n = 13), bipolar disorders (n = 12) and age / sex matched controls (n = 13).

Results: There were lower levels of cortical strontium (BA 6 & 10), ruthenium (BA 6 & 17) and cadmium (BA 10) from patients with major depressive disorder as well as lower levels of strontium in BA 10 from patients with bipolar disorders. Unexpectedly, there were changes in levels of 16 metals in the cortex, mainly BA 6, from suicide completers compared to those who died of other causes.

Limitations: Cohort sizes were relatively small but comparable to the majority of studies using human postmortem CNS. Like all studies on non-treatment naïve patients, drug treatment was a potential confound in our experiments.

Conclusions: Our exploratory study suggests there are changes in levels of metals in bipolar disorders and major depressive disorders which could be affecting cortical may have implications with regards to the oxidative balance in the cortex of patients with mood disorders. Our data raises the possibility that measuring levels of also suggest exploring specific biometals in the blood could be useful as biomarkers for increased risk of suicide.
Keywords: cortex, metallocimetallomics, bipolar disorders, major depressive disorders, cobalt, cadmium.
**Introduction**

Genetic studies indicate common risk genes that contribute to an increased susceptibility to several psychiatric disorders (Consortium, 2013), suggesting there may be some common pathophysiological pathways affected across such disorders. Neuroimaging studies report mutual and differential changes in CNS structures in patients with the bipolar disorders and major depressive disorders (Konarski et al., 2008). Importantly, whilst both bipolar disorders and major depressive disorders are defined by the presence of depressive symptoms, bipolar disorders are ultimately defined by the presentation of manic or hypomanic episodes (American Psychiatric Association, 2013). This hypothesis of both common and disparate biologies associated with bipolar disorders and major depressive disorders is also supported by studies showing similar and disparate changes in the blood biochemistry in patients with these disorders (Goldsmith et al., 2016).

The treatment of bipolar disorders changed radically upon the discovery of lithium having mood stabilising properties (Geddes et al., 2004) and its capacity to reduce suicidality (Cipriani et al., 2009) in patients with bipolar disorders. Whilst these outcomes showed that the biometal lithium could be used to treat bipolar disorders, they also suggested biometals such as lithium could be involved in the pathophysiology of the disorder. Such a hypothesis seems feasible as metals have long been known as important neurotransmitter regulators, in particular the uptake of noradrenaline and serotonin (Komulainen and Tuomisto, 1981; Lai et al., 1982). These latter data are of significance because the serotonin transporter has been implicated in major depressive disorders and bipolar disorders (Haase and Brown, 2015; Miller and Teitler, 1992) and noradrenaline has been implicated in major depressive disorders (Moriguchi et al., 2017). Hence, it has been hypothesised that metals, though their ability to affect many CNS functions (Duce and Bush, 2010), are
important in both the pathophysiology and treatment of bipolar disorders and major depressive disorders (Menon et al., 2016).

The notion that changes in levels of metals could contribute to the pathophysiologies of neurodegenerative disorders has been primarily explored by measuring levels of metals in postmortem CNS tissue, both at the levels of individual metals (Religa et al., 2006) and at the level of metallomics (Szabo et al., 2016). By contrast, there appears to be a dearth of information on levels of metals in CNS from patients with mood disorders, despite evidence to implicate a disturbance of biometal homeostasis in their pathophysiologies. Hence, we decided to measure levels of a number of biometals in the frontal pole, premotor cortex and the primary visual cortex of patients with mood disorders as these regions are all suggested to be affected by the pathophysiologies of mood-those disorders (Dean et al., 2016; Keedwell et al.; Krüger et al.). The level of lithium was purposely not measured in CNS tissue in order to avoid the major confound of this biometal being used in the treatment of bipolar disorders.
Methods

Human Tissue Collection and Processing

Human postmortem CNS tissue was collected after obtaining approval from the Ethics Committee of the Victorian Institute of Forensic Medicine; all tissue was collected at that Institute after gaining written consent from the nearest next-of-kin. Following tissue collection, case history reviews were conducted using the Diagnostic Instrument for Brain Studies (Hill et al., 1996; Roberts et al., 1998), a post-mortem assessment tool which enables a diagnostic consensus to be reached using DSM-IV criteria (American Psychiatric Association, 1994). Using data from the Diagnostic Instrument for Brain Studies, duration of illness was calculated as the time from first hospitalisation to death, the final recorded dose of relevant psychotropic drugs were converted to a standardized drug dose and post-mortem interval was calculated as the time from death to autopsy. Where death was not witnessed, tissue was only collected from patients who had been seen alive up to 5 hours prior to being found dead. In these instances, the post-mortem interval was taken as the midpoint between the person being found dead and last seen alive.

Important to this study was that all cadavers from which tissue was collected were refrigerated within 5 hours of being found as the first step to minimising autolysis (Ferrer et al., 2007). The left hemisphere was removed at autopsy, rapidly processed and frozen to -70°C using a standardised procedure (Dean et al., 1999) by the same individual in a way designed to further minimise autolytic effects (Ferrer et al., 2007). The pH of the brain tissue was measured as described previously (Kingsbury et al., 1995) as this provides a good measure of overall tissue preservation (Stan et al., 2006).
For this study, tissue was collected from Brodmann’s area 10 (frontal pole: the rostral portions of the superior frontal gyrus and the middle frontal gyrus that is bounded ventrally by the superior rostral sulcus but not including the cingulate sulcus), Brodmann’s area 6 (premotor cortex: the caudal portion of the superior frontal gyrus and the middle frontal gyrus extending from the cingulate sulcus on the medial surface to the lateral sulcus on the lateral surface) and Brodmann’s area 17 (visual cortex: the area of the cortex in and around the calcarine fissure in the occipital lobe) from 15 patients with major depressive disorders, 15 subjects with bipolar disorders and 15 controls. However, after removing individuals with outliers in their biometal data the study was completed using cortical tissue from 13 patients with major depressive disorders, 12 patients with bipolar disorders and 13 controls (Table 1).

Biometal analysis by inductively coupled plasma mass spectrometry (ICPMS)

Tissue of known wet weight was homogenized in Tris buffered saline (pH 7.4) containing EDTA-free protease inhibitor cocktail (1:50, Roche, Dee Why, NSW, Australia) and centrifuged 100 000 g at 4°C for 30 min. The protein concentration of the resultant supernatant was measured using the BCA protein assay (Pierce, Mt Waverley, VIC, Australia). Subsequently, 50µl of tissue supernatant of known protein concentration was lyophilized by freeze-drying and then suspended in 69% nitric acid (ultraclean grade, Aristar, BDH) overnight. Each sample was heated for 20 min at 90°C, and an equivalent volume of hydrogen peroxide (30% Aristar, BDH) added followed by a further incubation at 70°C for 15 min. The average volume left after this process was determined to be 35 µL to which 1% HNO3 (800 µL) was added to each tube to give a final sample volume of 835 µL. The metals in each sample were then measured using an Agilent 7700 series ICPMS under routine multi-element operating conditions and a Helium Reaction Gas Cell. The instrument was calibrated using 0, 5, 10, 50, 100 and 500 ppb of certified multi-element ICPMS standard calibration solutions (ICP-MS-CAL2-1, ICP-MS-CAL-3 and ICP-MS-CAL-4, Accustandard) for a range of elements.
Each sample was measured in triplicate with the concentrations determined from the standard curve of each biometal and normalized to protein concentration.

Statistical analyses:

Unless stated otherwise all statistical analyses was completed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA). Demographic, pharmacological and CNS collection data were compared across the three diagnostic groups using one-way ANOVA and a post hoc Tukey's multiple comparisons test. Data relevant only to cases with psychiatric disorders was compared using Student t-test. χ² test was used to identify any variance in frequency of gender and suicide completion between diagnoses.

Cases where levels of a biometal were below the sensitivity of our assays were treated as missing data in subsequent analyses. Outliers in the experimental data were identified with the boxplot function in Minitab® Statistical Software and individuals with outlying data were removed from further analyses. Data from the remaining individuals were overwhelmingly distributed normally and hence to minimise type 1 errors parametric analyses was utilised for subsequent analyses. With either diagnosis and region or suicide status and region as the experimental models, two-way ANOVAs with post hoc Bonferroni tests identified the source of any variance. Pearson product-moment correlations were generated to identify relationships between experimental data and case demographics, pharmacological or tissue collection data. Given the cohort sizes in this study, only strong correlations ($r^2>0.49$, $p<0.05$ (Cook and Weisberg, 1999)) were taken as indicating a potential confound and required their inclusion in our analyses as a covariate. When necessary, analysis of co-variance (ANCOVA) with the appropriate parameters as co-variates was carried out.
with post hoc Bonferroni tests to identify the source of any variance (Minitab Version 18, Minitab Inc., NSW, Australia).

Results

Demographic, pharmacological and CNS collection:

There were no significant differences in gender frequency, age, post-mortem interval, brain weight or duration of illness across the diagnostic groups (Table 1: Supplementary Table 1). However, as CNS pH was significantly higher in patients with major depressive disorders compared to bipolar disorders and controls, all subsequent analyses of variance included CNS pH as a covariant. There was also a significant variation in the rates of suicide completion across diagnostic cohorts with a very strong trend to significant variation in the frequency of suicide completion between bipolar disorders and major depressive disorders. Due to the binary nature of this variable and its high incidence in patients with major depressive disorder, it was decided to assess the impact on suicide completion on cortical metals as secondary analyses were completed using two-way ANOVAs and post hoc Bonferroni tests, with suicide status and region as the experimental model.

A significant variation in levels of cobalt, rubidium, molybdenum and ruthenium was observed in some cortical regions with gender (Supplementary Table 2). This was due to higher levels of cobalt and molybdenum in Brodmann’s area 6, higher levels of ruthenium in Brodmann’s areas 6 and 17 and higher levels of rubidium in Brodmann’s area 10 from females. Hence, in further analyses of these metals, gender was included as a covariate.
Cortical Analyses of Mood Disorders:

There was significant variance in metals other than cadmium across all three cortical regions studied (Supplementary Table 3).

Including CNS pH and, where appropriate, gender as covariates, there were significant omnibus variations in levels of cortical cobalt, strontium, ruthenium and cadmium with diagnoses (Figure 1A-D; Supplementary Table 3). Levels of cobalt in tissue from patients with bipolar disorders or major depressive disorders did not differ significantly from controls in any cortical region (Figure 1A). By contrast, some of the variations in levels of strontium were due to lower levels of this biometal in Brodmann’s areas 6 and 10 from subjects—patients with major depressive disorders and lower levels in Brodmann’s area 10 from patients with bipolar disorders (Figure 1B). Lower levels of ruthenium were present in Brodmann’s areas 6 and 17 from subjects—patients with major depressive disorders (Figure 1C). Very low levels of ruthenium in Brodmann’s area 10 hindered comparative analyses in that region. Finally, some of the variation in levels of cadmium was due to lower levels of the biometal in Brodmann’s area 10 from patients with major depressive disorders.

Cortical Analyses of Suicide:

There was significant variation in levels of boron, sodium, magnesium, aluminium, phosphate, calcium, chromium, manganese, nickel, copper, zinc, selenium, strontium, molybdenum, ruthenium and cadmium in the cortex of suicide completers compared to those who did not commit suicide had died from other causes (Figure 2). This variance was due, at least in part, to lower levels of boron, sodium, magnesium, chromium, manganese, nickel, copper, zinc, selenium, strontium, molybdenum, ruthenium and cadmium as well as higher levels of calcium in Brodmann’s area 6 from suicide completers (Figure 2). In addition, there were lower levels of strontium and
molybdenum in Brodmann’s area 10 from suicide completers. Finally, levels of strontium, molybdenum and ruthenium were lower in Brodmann’s area 17 from those who had completed suicide.

*Analyses of Potential Confounds:*

Whilst some linear regression lines describing the relationship between experimental data with demographic, disease related or CNS related data deviated from the horizontal, none of these relationships were of sufficient strength to suggest they were of sufficient strength to warrant being included as a covariant when analysing experimental data (Supplementary Table 4).
Discussion

Here we report omnibus changes in levels of cobalt, strontium, ruthenium and cadmium in the cortex of patients with bipolar disorders and major depressive disorders. The changes in cobalt seemed to be mainly due to higher levels of that biometal across the cortex from patients with major depressive disorders, although differences in levels of this biometal did not reach significance in any single cortical region studied. Changes in cortical cobalt in major depressive disorders could be relevant as it has previously been reported that this biometal can affect iron transportation in the CNS (Chikh et al., 2008), the degradation of neurotransmitters (Sadiq et al., 2012) and the regulation of glutamatergic neurotransmission (Chung et al., 2005; Nagy et al., 1994); processes that have all been implicated in the pathophysiology or treatment of bipolar disorders (Berk et al., 2011; Bymaster and Felder, 2002; Jun et al., 2014).

We found lower levels of strontium in Brodmann’s areas 6 and 10 from subjects patients with major depressive disorders and Brodmann’s area 10 from subjects patients with bipolar disorder. It has been suggested that strontium can act as a calcium-like ion in modulating neurotransmission (Xu-Friedman and Regehr, 2000) in which case the altered levels of cortical strontium in mood disorders could have a widespread impact on different neurotransmitter systems involving ion channels. Most of the literature on ruthenium reports on its action as an anti-cancer agent but provides little if any information of the role of this biometal in CNS functioning. Finally, our findings of lower cadmium levels in Brodmann’s area 10 from patients with bipolar disorders is in contrast to a prior study reporting higher levels of this biometal in urine and blood of patients with the disorder (González-Estecha et al., 2011). However, combining our data with these peripheral data supports an argument that low levels of cadmium in CNS are caused by excess excretion of the biometal in patients with bipolar disorders and could be relevant given its role in controlling cortical apoptosis.
(Mahdavi et al., 2017), the actions of estrogen (Aquino et al., 2012) and the functioning of calcium channels (Hinkle et al., 1992), all of which are reported as being altered in patients with the disorder (Fountoulakis and Vieta, 2008; Group, 2011; Kim et al., 2010).

Our study appears to be the first metabolomic study in mood disorders. One study has reported that, compared to controls, levels of copper, aluminium and iron are higher whilst levels of zinc are lower in DNA extracted from the frontal cortex of bipolar disorder patients who were all suicide completers (Mustak et al., 2010). The authors argue that changes in levels of redox metals could affect DNA stability in the bipolar disorder cortex, an outcome that could also result from changes in the metals that we report in patients with mood disorders. Notably, studies have reported higher levels of iron and lower levels of arsenic and cadmium in the frontal cortex of patients with Alzheimer’s disease (Szabo et al., 2016). These data argue that the changes in metals we report in mood disorders have some diagnostic specificity.

Variations in the levels of 16 metals in the cortex from suicide completers are also reported in our study. These were at least in part due to lower levels of boron, sodium, magnesium, chromium, manganese, nickel, copper, zinc, selenium, strontium, molybdenum, ruthenium and cadmium as well as higher levels of calcium in Brodmann’s area 6 from the left hemisphere of suicide completers. Biometals are potent regulators of oxidative balance, metabolism and cellular signaling (Pokusa and Trancikova, 2017) and disruption of these pathways by altered biometal levels could in part be responsible for the development of suicidal ideation. Past suicidal behaviour has been associated with higher activation in Brodmann’s area 6 in the left hemisphere of patients with bipolar disorders (Minzenberg et al., 2015) and changes in the level of the metals observed in this study may be a contributing factor in the altered activity within this region. In Brodmann’s area 10, another region of the cortex reported to display increased activity in suicide attempters (Jollant et
al., 2008), we found lower levels of strontium and molybdenum. Finally, we have found lower levels of strontium, molybdenum and ruthenium in Brodmann’s area 17 from suicide completers, a region of the cortex that at present does not seem to have been implicated in suicide ideation, attempts or completion.

 Whilst we are not aware of any studies correlating cortical metals with suicide incidence, the lower levels of zinc in the cortex of suicide completers may have relevance to a study describing changes in zinc transporter expression in suicide completers (Rafalo-Ulinska et al., 2016). Such changes in zinc transporter expression are likely to perturb zinc homeostasis in the CNS of suicide completers. Our data on zinc also may be of significance to the reported antidepressant properties of zinc (Manosso et al., 2015).

Limitations

Despite this study being the first metallomic study in mood disorders and suicide it has a number of limitations. Whilst diagnostic cohort sizes are typical of postmortem studies in psychiatric disorders, the cohort sizes are relatively small and premortem treatments are possible confounds. Other potential confounders that may limit the outcomes from metal analysis in post-mortem tissue include patient age and nutrient intake as both have previously been observed to influence a range of metals. Notably, age was not a confounder in our cohort of patients and data provided no strong evidence that treatment with either mood stabilisers or antidepressant drugs influenced the levels of metals in the cortex. In addition, whilst we could find no evidence to suggest that lithium directly affects levels of other cortical biometals, it is notable that some of the patients with bipolar disorders (n = 3) were recorded as receiving lithium close to death. It is therefore possible that
ingesting pharmacological levels of lithium could be affecting levels of other cortical biometals in three patients in this study.

Clinical Implications

We have shown that elements termed biometals are altered in the cortex of patients with mood disorders and suicide completers. These biometals are increasingly recognised as being central to maintaining oxidative balance in the CNS (Pokusa and Trancikova, 2017), a mechanism considered particularly important in the pathophysiology of mood disorders (Balmus et al., 2016; Berk et al., 2011) and open to therapeutic intervention (Ng et al., 2008) through biometal homeostatic restoration (Robert et al., 2015). By contrast, the pathways by which metals are involved in suicide remain to be elucidated and our findings indicate that further exploration into the utility of blood biometal levels, as well as other biomarkers of suicidal ideation (Le-Niculescu et al., 2013), would be worthwhile.
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Figure 1. Metal levels significantly altered in post mortem cortical regions of patients with mood disorder. Levels (mean ± SEM % mean controls) of cobalt, strontium, ruthenium and cadmium in Brodmann’s areas 6, 10 and 17 from patients with bipolar disorders or major depressive disorders.
Figure 2. Metal levels are significantly altered in post mortem cortical regions of suicide completers. Levels (mean ± SEM % mean non-suicide deaths) of cobalt, sodium, magnesium, aluminium, phosphate, potassium, calcium, chromium, manganese, iron, cobalt, nickel, copper, zinc, selenium, rubidium, strontium, molybdenum and cadmium in Brodmann’s areas 6, 10 and 17 from suicide completers. a: p < 0.05, b: p < 0.01, c: p < 0.001.
Table 1: A summary (mean ± SEM) of the demographic, pharmacological and CNS collection data for cases from which cortical tissue was obtained from patients with bipolar disorders (BD), major depressive disorders (MDD) and controls (Cont).

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>pH (hr)</th>
<th>PMI (hr)</th>
<th>brain Wht (gms)</th>
<th>DI (yr)</th>
<th>Sui</th>
<th>Y / N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>7 / 8</td>
<td>61 ± 4.0</td>
<td>6.29 ± 0.07</td>
<td>47 ± 4.3</td>
<td>1288 ± 36</td>
<td>0 / 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD</td>
<td>6 / 6</td>
<td>60 ± 3.8</td>
<td>6.26 ± 0.06</td>
<td>38 ± 4.4</td>
<td>1186 ± 146</td>
<td>17 ± 3.1</td>
<td>4 / 8</td>
<td></td>
</tr>
<tr>
<td>MDD</td>
<td>7 / 6</td>
<td>63 ± 3.9</td>
<td>6.57 ± 0.05</td>
<td>45 ± 4.3</td>
<td>1258 ± 67</td>
<td>17 ± 2.6</td>
<td>10 / 3</td>
<td></td>
</tr>
</tbody>
</table>

F or $\chi^2$ 0.14 0.15 8.46 1.09 0.39 18.1
d.f. 2 2,37 2,37 2,37 2,32 23 2
p 0.93 0.86 0.0009 0.35 0.68 0.97 0.0001

Cont vs BD p = n.s.
MDD p = p < 0.01
BD vs MDD = p < 0.01 0.05

Abbreviations: CNS = central nervous system, d.f. = degrees of freedom, DI = duration of illness, F = female, gms = grams, hr = hour, M = Male, N = no, n.s. = not significant, PMI = postmortem interval, Sui = suicide completion, Wht = weight, yr = year and Y = yes.
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