Biomarkers for Antidepressant Efficacy of Electroconvulsive Therapy: An Exploratory Cerebrospinal Fluid Study

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Abstract

IN PREPARATION

Introduction

Electroconvulsive therapy (ECT) is an effective and safe treatment option for specific forms of depression, such as treatment-resistant depression, psychotic depression or melancholia in both, uni- and bipolar affective disorder [1, 2], with response rates usually ranging around 70%. Despite this remarkable efficacy even in highly treatment-resistant depression, there is still a considerable group of non-responders to ECT. Therefore, it is of high clinical interest to identify factors or markers that could provide information prior to treatment on whether the individual patient will respond to ECT or not.

Previous studies have provided a large body of evidence about potentially prognostic demographic or clinical factors such as age, bipolarity, family history of depression or comorbid axis II disorder that might contribute to the risk of non-response to ECT [3, 4]. Others tried to elucidate whether a set of ictal parameters might predict the clinical outcome [5, 6]. More recently, several imaging markers were successfully used to predict the outcome to ECT in major depression, for example the baseline level of higher BOLD signal fluctuations of the subcallosal cingulate cortex [7], the subgenual cingulate volume [8] and several gray matter regions [9].

A few blood-based candidate biomarkers for ECT treatment response have been identified, including vascular endothelial growth factor [10] and homovanillic acid [11]. In contrast to markers from blood that might not be central nervous system (CNS)-specific, markers measured in the cerebrospinal fluid (CSF) could reflect cerebral processes much more directly. However, biomarkers in the CSF that could predict outcome to ECT in patients with major depression have not been identified yet.

We recently ran a study in which CSF samples before and after a course of ECT in patients with severe depression were collected. Thus, we had the opportunity to analyse several markers from different areas, such as lipids (endocannabinoids and sphingolipids), cytokines, elements from the kynurenine pathway and different markers of neurodegeneration. In this

study, we analysed these biomarkers in patients with depression to examine if there were correlations of their baseline CSF levels before ECT with the clinical outcome of ECT.

Methods

Patients

Our prospective study was approved by the appropriate ethics committee and was performed in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). Written informed consent was obtained from all participants before enrolment. The study took place from 2012 to 2014 at the Department of Psychiatry at the Central Institute of Mental Health in Mannheim, Germany. Inclusion criteria were a present treatment resistant depressive episode (defined as failure to achieve response or remission to at least two proven antidepressant trial with adequate dosing and duration [12]) within the context of a diagnosis of either major depressive disorder or bipolar disorder according to DSM-IV, age above 18 years and the clinical decision for an ECT treatment. Exclusion criteria were substance-related disorders including tobacco and alcohol, the lifetime diagnosis of schizophrenia, any signs of an on-going inflammation process such as leucocytosis, elevated acute phase proteins or fever, the intake of any medication with known immunomodulatory properties such as non-steroidal anti-inflammatory drugs or glucocorticoids and the reported use of Δ^9-tetrahydrocannabinol (THC) twelve months before the study or detection of THC in urine drug screen. The patients continue their prior psychotropic medication during the ECT treatment. The Hamilton Depression Rating Scale (HDRS; 21 items version) was used to assess the severity of depression before and after the ECT in each patient. Response was defined as a reduction of at least 50% in symptoms measured by the HDRS. The Mini-Mental-State Examination (MMSE) was used to assess the gross cognitive performance prior to treatment.

ECT treatment

Right unilateral brief pulse ECT was performed with a Thymatron IV device (Somatics, LLC. Lake Bluff, IL, USA), s-ketamine was used as the anaesthetic substance (~1.0 mg/kg) [13, 14]

and succinylcholine for muscle relaxation (~1.0 mg/kg). Seizure threshold in all patients was titrated at the initial session and dosing at subsequent treatments was given at > 2.5 times threshold. The energy was subsequently increased if patients did not respond clinically or if seizures were insufficient during the ECT course. Two or three ECT sessions per week were performed. All patients started with unilateral stimulation, in the case of non-response, it was possible to switch to bilateral stimulation as a decision of the ECT supervisor.

Sampling

Before the first ECT session and between one and seven days after the last ECT session, all CSF samples were drawn at around 9:00 am. The patients were in a non-fasting status. They had a light breakfast at 8:00 am. Lumbar punctures were performed in the sitting position according to standardized procedure. All samples were taken, aliquoted and transferred at -80°C within 30 min either in glass vials (Hycultec, Beutelsbach, Germany) for the analyses of the lipids, in polypropylene tubes for the analyses of the markers of neurodegeneration or in protein LoBind tubes (Eppendorf, Germany) for all other substances. The samples were stored at -80°C and were never thawed or refrozen prior to the presented analyses.

CSF and serum laboratory analyses

Baseline and follow-up samples were analyzed on one occasion on the same plates using the same batch of reagents.

Tau proteins and β-amyloids

All samples were measured at the National TSE (Transmissible Spongiform Encephalopathies) Reference Centre (Göttingen, Germany). CSF levels of $A\beta_{1-40}$, $A\beta_{1-42}$, total tau and its phosphorylated isoform were determined with commercially available enzyme-linked immunosorbent assay (ELISA) kits, described elsewhere [15].

Neurogranin

All samples were measured at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital. CSF Ng concentration was measured by enzyme-linked immunosorbent assay (ELISA), essentially as described [16], using the anti-Ng antibody pair Ng2 and Ng22. Intra-assay coefficients of variation were below 10%.

Endocannabinoids

All samples were measured at the Lipidomics/Mass Spectrometry Facility at the Institute for Physiological Chemistry at the University Medical Center Mainz. Endocannabinoids were qualitatively and quantitatively analyzed using liquid chromatography/ multiple reaction monitoring (LC/MRM) according to the procedures described elsewhere [17]. Levels of both AEA and 2-AG were measured, as well as levels of arachidonic acid (AA), from which both endocannabinoids are derived.

Elements of the innate immune system

CSF concentrations were measured as instructed by the manufacturer, using sandwich ELISA kits for interleukin-6 (IL-6), neopterin, soluble CD14 (sCD14), soluble CD163 (sCD163), macrophage migration inhibitory factor (MIF) and monocyte chemotactic protein 1 (MCP-1) as described elsewhere [18].

Metabolites of the Kynurenine Pathway

CSF levels of kynurenine (KYN), kynurenic acid (KYNA), picolinic acid (PIC), quinolinic acid (QUIN), tryptophan (TRY), 3-hydroxykynurenine (3-HK) were determined using a high-performance liquid chromatography kit. For further details see Supplementary Methods. From these values, the kynurenine-to-tryptophan ratio (KYN/TRY) was calculated.

Sphingolipids

BESCHREIBUNG AUS HEIDELBERG FEHLT NOCH

sKlotho

All samples were measured at the Department of Pediatric Kidney, Liver and Metabolic Diseases, Hannover Medical School, Hannover, Germany. The concentration of Klotho in CSF was quantified using a human Klotho enzyme-linked immunosorbent assay (ELISA) kit measuring the entire soluble α-Klotho protein secreted in the systemic circulation after its release from the cell membrane via shedding (cleaved Klotho) or alternative splicing (secreted Klotho) with an assay sensitivity of 6.15 pg/mL (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan).

Statistical analyses

Statistics were performed using STATA® (StataCorp, Texas 77845, USA, version 11) at a significance level ≤ 0.05. Normality was examined using the Shapiro-Wilk and data were log-transformed when necessary. No correction for multiple testing was performed to avoid type II errors in this study with its small sample size. In line with that, results with significance levels between 0.05 and 0.1 were additionally reported as trend observations. Spearman's correlation (r_s) was used to determine the strength and direction of the relationship between the concentration of the different markers and either the extent of depressive symptoms at baseline or the reduction of depressive symptoms during ECT or possible moderators. Linear regression was used to estimate which single marker could predict the reduction of the HDRS score during ECT and multiple regression analysis with stepwise inclusion was used to identify models, in which more than one marker could add significantly to the prediction. Group differences between responder and non-responder were examined with the Wilcoxon rank-sum test. Logistic regression (responder vs. non-responder) was not be performed due to the small sample size.

Results

We included 12 patients into the study, who underwent a completed course of ECT and lumbar puncture before and thereafter. In general, ECT was effective with a reduction of the mean initial HDRS from 29.9 ±6.6 to 9.0 ±5.2 after the final session ($p<0.001$). Ten patients (83.3%) could be classified as treatment responder, whereas two patients (16.7%) were considered as

non-responders to ECT. Three patients (two females, one male, mean age: 75.7 ±6.4) had the clinical diagnosis of an Alzheimer's disease (AD) additional to their current severe depressive episode. Demographic and clinical features are shown in Table 1.

Absolute reduction of depressive symptoms

CSF baseline levels of AEA (r_s=0.65; p=0.029), $A\beta_{1-40}$ (r_s=0.64; p=0.025), tau protein (r_s=0.68; p=0.015) and its phosphorylated isoform (r_s=0.58; p=0.049), Ng (r_s=0.64; p=0.024), sCD14 (r_s=0.82; p=0.0021), KYN (r_s=0.64; p=0.036) and KYN/TRY (r_s=0.63; p=0.038) positively correlated with the absolute reduction of depressive symptoms during ECT. At trend level, we found this correlation also for CSF levels of IL-6 (r_s=0.56; p=0.072), neopterin (r_s=0.54; p=0.087), sCD163 (rs=0.54; p=0.084), PIC (r_s=0.68; p=0.062), QUIN (r_s=0.67; p=0.070), phosphatidylcholines (r_s=0.58; p=0.080) and the total amount of sphingolipids (r_s=0.58; p=0.080) (Figure 1-4). Age (p=0.79), MMSE at baseline (p=0.39), clinical AD diagnosis (p=0.12) and sex (p=0.44) were not associated with reduction of depressive symptoms during ECT. A summary of the significant findings is presented in Table 2.

Linear regression models established that all, sCD14 (F(1, 9)=18.91; p=0.002) , tau (F(1, 10)=9.3; p=0.013) and its phosphorylated isoform (F(1, 10)=9.49; p=0.012) (Figure x) could statistically significantly predict the absolute reduction of depressive symptoms. However, no multiple regression model could be found that predicted this absolute reduction better than any single marker in the linear regression analyses.

Relative reduction of depressive symptoms

Correlations with the relative reduction of depressive symptoms during ECT were found for sCD14 (r_s=0.65; p=0.032), PIC (r_s=0.79; p=0.021) and KYN (r_s=0.61; p=0.047) and additional for KYNA (r_s=0.54; p=0.086) and the kynurenine-to-tryptophan ratio (r_s=0.56; p=0.076) at a statistical trend level of significance. We found no correlation with potential confounders such as age (p=0.51), MMSE at baseline (p=0.49) or the clinical AD diagnosis (p=0.33). However, at trend level, females had a higher relative HDRS reduction by ECT than men (p=0.057).

A linear regression model established that PIC ($F(1, 6)=6.56$; $p=0.043$) could statistically significantly predict the absolute reduction of depressive symptoms. Additionally, combining PIC and KYN in a multiple regression model predicted the relative reduction of depressive symptoms with both variables added statistically significantly to the prediction ($F(1, 9)=27.8$; $p=0.0020$).

Responders vs. Non-Responder

We found a difference in the baseline CSF levels of KYN ($z=2.1$; $p=0.034$) in responders compared to those patients, who did not show a response to the treatment. At trend level, we found this correlation also for CSF levels of neurogranin ($z=2.0$; $p=0.052$), tau protein ($z=1.7$; $p=0.086$) and its abnormal phosphorylated isoform ($z=2.0$; $p=0.052$), sCD163 ($z=1.7$; $p=0.099$), kynurenic acid ($z=1.7$; $p=0.096$) and PIC ($z=1.7$; $p=0.096$). Age ($p=0.39$), MMSE at baseline ($p=1.00$), the clinical AD diagnosis ($p=0.42$) or sex ($p=0.33$) were not associated with the outcome parameter “response to ECT”, defined as a reduction of at least 50% in symptoms measured by the HDRS.

Discussion

This is the first study that estimated the antidepressant therapy-outcome of ECT based on biomarker profiles in CSF at baseline, which could be a very interesting approach for future applications of biomarkers. We found a variety of markers from different areas that were correlated with either absolute or relative reduction of the HDRS that could be observed during a course of ECT or with the status responders vs. non-responders after ECT.

Markers of neurodegeneration, the innate immune system and metabolites of the kynurenine pathway were those that correlated the most with the antidepressant efficacy. In terms of neurodegeneration tau protein, its abnormal phosphorylated isoform, $A\beta_{1-40}$ and neurogranin, all known to be highly correlated or associated with each other [19] were correlated with any form of therapeutic effect, independent of confounders such as age or the presence of a clinical diagnosis of AD. A similar relationship between those markers and response to any

antidepressant efficacy has not been reported yet and should be investigated in more detail in future studies. The baseline CSF concentrations of the metabolites of the kynurenine pathway, KYN, KYNA, the kynurenine-to-tryptophan ratio, PIC and QUIN were all positively correlated with the antidepressant effect of the ECT in those severely depressed patients. These findings are in general in line with the proposed involvement of the kynurenine pathway in depression [20-22]. Concerning the response to ECT, two recent studies that analysed the possible correlation to tryptophan metabolites in plasma did not observe such a correlation [23, 24], but no such studies has been carried out with analysis of the CSF, yet. Based on the relative abundance on the relationship between the immune system and different aspects of depression [25-27], it is not surprising that the baseline CSF levels of sCD14 and to a lesser extent of IL-6, neopterin and sCD163 - all involved primarily in the innate immune response [18] were positively correlated with the antidepressant outcome of ECT. Interestingly those three complex systems are tightly associated with each other: Changes of (p-)tau protein and Aβ within Alzheimer's disease (AD) are known to be associated with alterations in the metabolites of the kynurenine pathway [28] and inflammation [29], whereas the kynurenine pathway links inflammation and the immune system to the pathophysiology of depression [21, 30, 31] and inflammation is seen in both, depression and AD with dysfunctional Aβ and tau [32]. Our findings point towards an involvement of these complex systems in the antidepressant mechanism of ECT. To complement these modes to the established pathomechanisms of ECT, this involvement has to be explored in a more detailed way.

For lipids, the initial CSF levels of the endocannabinoid AEA and of elements of the sphingolipids were associated with antidepressant efficacy during ECT. Those markers have not been investigated concerning the issue of antidepressant outcome prediction yet, but at least our group recently reported that CSF AEA levels are increased by ECT and that those levels were positively correlated with the number of ECT session that were performed in each individual patient [33]. This finding corroborates a growing body of - primarily preclinical - evidence that suggests an involvement of the endocannabinoid system in the pathophysiology of depression [34] and asks especially for more studies about the relationship between

depression/antidepressant action and the endocannabinoid system in human. Sphingolipids, especially ceramides have already been linked with depression [35, 36], thus the results at a trend level significance could be cautiously integrated into this concept.

Limitations

The small sample size is probably the major limitation of our study. The samples were taken from a study, in which the patients were asked to consent to lumbar puncture twice - before and after ECT, therefore the recruitment was extremely difficult. Nonetheless, the sample size was large enough to identify larger effects and at least suggesting more moderate effects as well. Thus, these positive results from our small sample are promising to be replicated in future studies. Surely, we cannot exclude that with a larger sample size, smaller effects of other markers could have been unveiled. A further limitation in respect to the small sample size is that no adjustment for possible confounders such as age, sex or BMI could be performed. Based on the clinical reality, the study was only possible to conduct with our patients continuing some of their psychotropic medication that consisted of antidepressants, antipsychotics, benzodiazepines and lithium during the ECT treatment. In our cases, the medication in each patient was at least kept constant during the ECT course. Due to the design of our study, it is not possible to unmask any causal relationship between ECT and the identified CSF markers that correlated with the antidepressant efficacy. However, it is at least tempting to assume that the most efficient known antidepressant biological method, namely ECT works mostly efficient, when biological markers that might reflect any part of the pathway of depression are abnormal.

In summary, our CSF study was primarily meant to identify markers that indicate whether the therapy option of ECT in patients with severe depression might be successful. Based on these markers, further research regarding the mechanism of ECT and personalized antidepressant therapy is suggested, but of course, treatment decisions for the individual must not be made based on our preliminary findings.

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Table

Number of included subjects	12
Age (mean) ± SD in years (min - max)	59.0 ± 21.9 (21 - 83)
Sex female/male n/n (in %)	7/5 (58.3/41.7)
Type of depression: unipolar/bipolar n/n (in %)	9/3 (75.0/25.0)
Duration of current depressive episode in months, mean ± SD (min - max)	18.7 ± 21.9 (1 - 60)
Age of onset (mean) ± SD in years (min - max)	49.2 ± 21.8 (12 - 80)
Duration of illness in years, mean ± SD (min - max)	9.9 ± 9.1 (0.5 - 26)
Body mass index, mean ± SD (min - max)	23.5 ± 3.5 (20 - 31)
HDRS, sum score	
Baseline HDRS, mean ± SD (min - max)	29.9 ± 6.6 (21 - 41)
Final HDRS, mean (min - max)	9.0 ± 5.2 (3 - 16)
Mean change ± SD (min - max)	20.9 (-6 - 36) (p<0.001)
Numbers of ECT sessions, mean ± SD (min - max)	10.6 ±5.0 (4 - 19)
Days between the two CSF samples, mean ± SD (min - max)	35.7 ±15.3 (17 - 59)
Days between last ECT and 2nd sample, mean ± SD (min - max)	4.6 ±2.4 (2 - 7)
Switch to bilateral stimulation n (in %)	3 (25)
Remitters/Responders/Non-responders* n/n/n (in %)	5/10/2 (41.7/83.4/16.7)

Table 1: Demographic and clinical features of the patients.

	Absolute HDRS reduction	Relative HDRS reduction	Responder vs. Non-Responder	No correlation with outcome
Markers of neurodegeneration	Aβ1-40 tau protein p-tau protein Ng	-	tau protein* p-tau protein* Ng*	Aβ1-42
Endocannabinoids	AEA	-	-	2-AG AA
Elements of the innate immune system	sCD14 IL-6* Neopterin* sCD163*	sCD14	sCD163*	MIF MCP-1
Metabolies of the kynurenin pathway	KYN KYN/TRY PIC* QUIN*	PIC KYN KYNA* KYN/TRY*	KYN KYNA* PIC*	TRY 3-HK
Spingholipids	Total amount of sphingolipids* Phosphatidylcholines*	-	-	Ceramide Sphingomyelin
sKlotho	-	-	-	sKlotho

Table 2: Summary of the results for each category.

*=Statistical significance at trend level (0.1>p>0.05)

Figures

IN PREPARATION