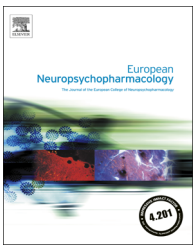
European Neuropsychopharmacology (2013) 23, 1199–1207



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Electroconvulsive therapy exerts mainly acute molecular changes in serum of major depressive disorder patients

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Received 12 June 2012; received in revised form 16 August 2012; accepted 24 October 2012

KEYWORDS
Major depressive disorder;
Electroconvulsive therapy;
Antidepressant drugs;
Multiplexed serum profiling;
Mass spectrometry

Abstract
Electroconvulsive therapy (ECT) is mainly used to treat medication resistant major depressive disorder (MDD) patients, with a remission rate of up to 90%. However, little is known about the serum molecular changes induced by this treatment. Understanding the mechanisms of action of ECT at the molecular level could lead to identification of response markers and potential new drug targets for more effective antidepressant treatments. We have carried out a pilot study which analysed serum samples of MDD patients who received a series of ECT treatments over 4 weeks. Patients received only ECT treatments over the first two weeks and a combination of ECT and antidepressant drugs (AD) over the subsequent two weeks. Blood serum analyses were carried out using a combination of multiplex Human MAP® immunoassay and liquid-chromatography mass spectrometry (LC-MS^E) profiling. This showed that ECT had a predominant acute effect on the levels of serum proteins and small molecules, with changes at the beginning of ECT treatment and after administration of the ECT+AD combination treatment. This suggested a positive interaction between the two types of treatment. Changed molecules included BDNF, CD40L, IL-8, IL-13, EGF, IGF-1, pancreatic polypeptide, SCF, sortilin-1 and others which have already been implicated in MDD pathophysiology. We conclude that ECT appears to exert mainly acute effects on serum molecules.

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http://dx.doi.org/10.1016/j.euroneuro.2012.10.012

1. Introduction

Electroconvulsive therapy (ECT) is usually administered to patients with severe and medication-resistant major depression (refractory depression), mania, catatonia and acute schizophrenia (Mathew, 2005). In the short-term, ECT results in remission rates of 70–90% (Petrides et al., 2001; Sackeim et al., 1993), which is higher than standard antidepressant treatments. Further advantages of ECT include faster improvement and better symptomatic remission. However, ECT has limitations including cognitive side effects and high relapse rates (Mathew, 2005), although some patients receive continuation of ECT for relapse prevention (Kellner et al., 2006). However, most patients treated with ECT receive antidepressant drug (AD) medication as maintenance therapy which reduces the relapse rate (reviewed in Bourgon and Kellner, 2000).

The primary therapeutic effect of ECT is a seizure induced by electrical stimulus. Little is known about the molecular mechanism of action or whether ECT and AD treatments have similar effects. Molecules in several brain areas are affected by ECT, including neurotransmitters, neuropeptides and neurotrophic factors (Wahlund and von Rosen, 2003). Consistent with effects on growth factors, pre-clinical studies have shown that electroconvulsive shock leads to increased hippocampal neurogenesis (Scott et al., 2000), angiogenesis (Newton et al., 2006) and glial proliferation in frontal cortex (Ongur et al., 2007). Also, ECT has been found to increase sympathetic vagal activity (Bar et al., 2010), marked by increased levels of pancreatic polypeptide (PPP). PPP levels have therefore been used as a biomarker for estimating the degree of ECT-stimulated vagal activity.

Here, we have carried out a pilot study to investigate the molecular changes in MDD patients after acute and chronic ECT, and by combined ECT and AD treatment. Sera were subjected to molecular profiling analyses using a combination of multiplex immunoassay and liquid-chromatography mass spectrometry (LC-MS^E) approaches. This multiplex profiling approach was used to eliminate variability across individual measurements, thereby allowing reliable identification of molecules which are co-regulated within and across molecular pathways.

2. Experimental procedures

2.1. Study participants

The study included 12 in-patients of the Department of Psychiatry, Muenster University Hospital, Germany, meeting the Diagnostic and Statistical Manual of Mental Disorders version IV Text Revision criteria for MDD. All patients were in an acute stage of MDD, resistant to ADs and had been drug free for at least 2 weeks before the study. Patients were free of infections and other diseases. All subjects gave informed written consent. Clinical investigations were conducted according to the Declaration of Helsinki, and the University of Muenster's ethical committee approved the study. None of the subjects had previously received ECT treatments. Patients were monitored using the Hamilton Depression rating scale in the beginning (HAMD1), after 6 (HAMD6) and after 12 (HAMD12) ECT treatments. Samples (6 in common) were analyzed using multiplex immunoassay (n=8) and LC-MS^E (n=10) platforms (Table 1).

2.2. ECT treatment

ECT was applied as described (Michael et al., 2003). Briefly, methothecital (0.75–1.5 mg/kg) and succinylcholine (0.5–1.0 mg/kg) were given and a customized Thymatron IV brief-pulse device (Somatics; Lake Bluff, IL, USA) was used for stimulus titration in the first session and continuing to a 2.5-fold stimulus dose. Motor and electro-encephalogram seizure duration were monitored and stimulus intensity adjusted accordingly.

Three ECT sessions/week were performed over 4 weeks. During the first 6 sessions, patients were unmedicated. Thereafter, patients received ADs [clomipramine (n=4), maprotiline (n=2), mirtazapine (n=2), tranylcypromine (n=3), venlafaxine (n=3) and lithium (n=4)] in addition to ECT as was common practice in the facility. However, a special study design was incorporated to facilitate investigation of the molecular changes induced by acute (6 h) and chronic (6 treatments/2 weeks) ECT ± AD treatment.

Blood was collected from subjects at the indicated time points (Figure 1) into S-Monovette tubes (Sarstedt; Numbrecht, Germany) and serum prepared by placing samples at room temperature for 2 h for coagulation and centrifuging at 4000g for 5 min. The supernatants were stored at −80 °C. A quality control (QC) sample was prepared from a pool of spare sera and split into 9 aliquots to evaluate LC-MS^E reproducibility.

2.3. Multiplexed immunoassay profiling

A total of 190 molecules were measured in sera (200–250 μL) using the *Human*MAP® platform in a CLIA-certified laboratory (Myriad-RBM; Austin, TX, USA) (Bertenshaw et al., 2008). This panel of assays targets inflammatory, metabolic, hormonal and neurotrophic pathways and has been applied successfully in studies of psychiatric disorders such as schizophrenia and MDD (Domenici et al., 2010; Schwarz et al., 2010).

2.4. LC-MS^E analysis

Sera were processed in random order to avoid sequential bias and were depleted of the 14 most abundant proteins using MARS14 (Agilent; Santa Clara, CA, USA) on an ÄKTA™ purifier UPC 10 chromatography system (GE Healthcare; Little Chalfont, Bucks, UK) as reported previously (Levin et al., 2010). LC-MS^E profiling was carried out in expression mode using a Waters quadrupole time-of-flight (QToF) Premier mass spectrometer, as described previously (Levin et al., 2007). Resulting data were processed using the ProteinLynx Global Server (PLGS) v.2.3 (Waters) and Rosetta Inpharmatics Biosoftware Elucidator v3.3 (Seattle, WA, USA) (Krishnamurthy et al., 2011). The human Swiss-Prot database (v57, 20,332 entries) search was performed using PLGS with the ion accounting algorithm described previously (Li et al., 2009). The criteria for protein identification were set to ≥3 fragment ions/peptide, and ≥7 fragment ions and 2 peptides/protein. The maximum false identification rate was 4% using a randomized version of the database. Only peptides detected in 2 out of 3 replicates and 60% of samples were included for biological reproducibility. Search results were imported into Elucidator for annotation of aligned features, resulting in a matrix that included intensities for each sample and peptide.

2.5. Apolipoprotein C2 (ApoC2) enzyme-linked immunosorbent assay (ELISA)

ApoC2 levels were measured in whole serum using the human ApoC2 ELISA kit (USCN Life Science; Cambridge, UK). Samples were diluted 1:1500 and assays performed using the manufacturer's instructions.