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Cytokines plasma levels during antidepressant treatment with sertraline and transcranial direct current stimulation (tDCS): results from a factorial, randomized, controlled trial

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Abstract

Rationale—The inflammatory hypothesis of depression states that increased levels of pro-inflammatory cytokines triggered by external and internal stressors are correlated to the acute depressive state. This hypothesis also suggests that pharmacotherapy partly acts in depression through anti-inflammatory effects. Transcranial direct current stimulation (tDCS) is a novel, promising, non-invasive somatic treatment for depression, although its antidepressant mechanisms are only partly understood.

Objectives—We explored the effects of tDCS and sertraline over the immune system during an antidepressant treatment trial.

Methods—In a 6-week, double-blind, placebo-controlled trial, 73 antidepressant-free patients with unipolar depression were randomized to active/sham tDCS and sertraline/placebo (2×2 design). Plasma levels of several cytokines (IL-2, IL-4, IL-6, IL-10, IL-17a, IFN-γ, and TNF-α) were determined to investigate the effects of the interventions and of clinical response on them.

Results—All cytokines, except TNF-α, decreased over time, these effects being similar across the different intervention-groups and in responders vs. non-responders.

Conclusions—tDCS and sertraline (separately and combined) acute antidepressant effects might not specifically involve normalization of the immune system. In addition, being one of the first placebo-controlled trials measuring cytokines over an antidepressant treatment course, our study showed that the decrease in cytokine levels during the acute depressive episode could involve a placebo effect, highlighting the need of further placebo-controlled trials and observational studies examining cytokine changes during depression treatment and also after remission of the acute depressive episode.

Keywords

Major depressive disorder; Transcranial direct current stimulation; Sertraline; Cytokines; Interleukins; Randomized; Controlled trial; Placebo response; Placebo effect

Introduction

Major depressive disorder (MDD) is a chronic, disabling medical illness characterized by depressed mood, lethargy, loss of appetite, reduced motivation, anhedonia, concentration difficulties, sleep disturbances, and apathy. The frontocingulate dysfunction model, in which hypoactivity of the prefrontal cortex and hyperactivity of profound cortical and subcortical areas are described (Pizzagalli 2011), is an interesting framework to explain the heightened

stress response in depression, as subcortical hyperactivity leads to downstream activation of the sympathoadrenomedullar (SAM) axis. Their over-activation leads to subsequently increased sympathetic/ decreased parasympathetic response and is in line with the inflammatory hypothesis of depression (Maes et al. 2009). This is corroborated by findings of increased blood levels of inflammatory cytokines, such as TNF-α and IL-6 (Hiles et al. 2012; Liu et al. 2012) in depressed vs. healthy subjects, even in the absence of medical illness. In fact, acute infections, which are accompanied by intense immunological response and increased levels of inflammatory cytokines, also present psychological symptoms that resemble depression; and acute increasing of some cytokines can trigger depressive episodes (DellaGioia and Hannestad 2010), suggesting a bidirectional relationship between increased cytokine levels and depressive episodes and, therefore, posing the question to whether the former is the cause or the consequence of the latter.

Notwithstanding, in spite of the relatively large number of cross-sectional studies comparing cytokine levels between depression and controls, few placebo-controlled trials have prospectively assessed the impact of antidepressant treatment on cytokine levels and, to the best of our knowledge, this issue has not been addressed yet to non-invasive somatic therapies, particularly transcranial direct current stimulation (tDCS). This technique is based on the application of a weak, direct electric current over the scalp through two electrodes: the anode—which locally increases cortical excitability, and the cathode, which has opposite effects (Brunoni et al. 2012b; Nitsche et al. 2008). When applied repeatedly for several days, active vs. sham tDCS has been found to be an effective treatment for depression (Brunoni et al. 2013b; Loo et al. 2012). Due to its own nature of non-invasiveness and presenting therapeutic effects via direct electric stimulation in targeted brain areas, tDCS have no direct action on peripheral cells. This is important because antidepressant drugs might also display anti-inflammatory, direct effects on the immune cell systems, at least in animal models (Roumestan et al. 2007; Yirmiya et al. 2001); thus suggesting that antidepressant drugs could also ameliorate depression through their anti-inflammatory activity.

Considering these issues, we explored the impact of tDCS on a set of a series of cytokines using data from the Sertraline vs. Electric Current Therapy for Treating Depression Clinical Study (SELECT-TDCS), which was a factorial, sham-controlled trial in which 120 participants with depression were randomized using a 1:1:1:1 permuted block randomization method into four treatment groups: (1) sham-tDCS/placebo-pill (further referred as *placebo*); (2) sham-tDCS/sertraline-pill (*sertraline-only*); (3) active-tDCS/ placebo-pill (tDCS-only); and (4) active-tDCS/sertraline-pill (*combined treatment*).

Our aim was to compare the plasma levels of several cytokines across all groups to determine whether such levels change over time and according to the clinical intervention. We also explored whether (1) baseline cytokine levels are predictors of response and (2) they are surrogate outcomes of response, i.e., their endpoint levels correlate with depression endpoint scores.

Methods

Study design

SELECT-TDCS was conducted at University Hospital, University of São Paulo, Brazil and is registered in clinicaltrials.gov (NCT01033084), being approved by the Local (HU-USP 873/08 and 991/10) and National Ethics Committee (SISNEP CAAE 0083.0.198.000-08), with all participants providing written informed consent. The methodological aspects and main results of this study are described elsewhere (Brunoni et al. 2011b; 2013b). Briefly, the trial was 6 weeks long; encompassing an acute treatment period when ten consecutive daily active/sham tDCS sessions were performed (except on weekends), followed by two tDCS sessions delivered every fortnight. Sertraline (or placebo), a selective serotonin reuptake inhibitor (SSRI), was used in a fixed dose of 50 mg/day and was started and ended simultaneously with tDCS. Therefore, sertraline treatment duration was 6 weeks. In that study, the primary outcome was to evaluate the score changes of the Portuguese-validated version of the Montgomery-Åsberg Depression rating scale (MADRS) (Gorenstein et al. 2000). As a secondary outcome, the score changes of the Portuguese-adapted version of the 17-item Hamilton Depression Rating Scale (HDRS17) were also evaluated.

The main findings of the SELECT-TDCS were (Brunoni et al. 2013b), for both MADRS and HDRS17 scales: (1) at baseline, all groups presented similar depression scores; (2) tDCS-only was statistically superior to placebo at week 6; (3) tDCS-only and sertraline-only presented similar efficacy at all time points; and (4) the combined treatment was superior to all other groups at all time points.

Subjects

Participants with unipolar depressive disorder without psychotic features currently experiencing an acute depressive episode were enrolled. Diagnosed were established by board-certified psychiatrists and by the Portuguese-validated version of the Mini International Neuropsychiatric Interview (Amorim 2000). Subjects were required to have a HDRS17 of ≥17. Comorbid anxiety disorders were permitted. Subjects with other psychiatric disorders, personality disorders, and neurological conditions were excluded.

Subjects underwent a physical exam, psychiatric and medical history, routine laboratory analyses, electrocardiogram, and assessment of vital signs. Subjects were excluded if they were not in good physical health or had any medical disorders such as uncontrolled diabetes, hypertension, seizures, or acute or chronic inflammatory conditions. Other exclusion criteria included pregnancy or breastfeeding, history of substance abuse within the past 2 years, any history of psychotic disorder, bipolar disorder, current suicidal ideation, previous non-response to sertraline, or sertraline treatment in the current major depressive episode.

Subjects were gradually tapered of any psychotropic medications except for those previously taking a benzodiazepine; in such individuals, the dose remained constant throughout the entire study. Subjects then remained free of psychotropic medications for five half-lives of the medication(s) they had been taking (other than benzodiazepines) and for the rest of the 6-week study ((Brunoni et al. 2011b; 2013b) for further details).

Procedures

Standard, commercial tDCS devices (Chattanooga Ionto™ Dual Channel Devices, Chattanooga Group, Hixson, TN 37343 USA) were used in our study. We applied a bifrontal setup, as first used by Ferrucci et al. (2009) and also in other depression studies (Brunoni et al. 2011a, 2012a; Dell'Osso et al. 2009), which consists in placing the anode and the cathode over the left and the right dorsolateral prefrontal cortex (F3 and F4 areas, respectively, according to the 10/20 EEG system). This montage maybe theoretically more advantageous (compared to cathode placement over the right supraorbital area) considering the prefrontal asymmetry observed in depression, i.e., hypoactivity of the left and relative hyperactivity of the right prefrontal cortex (Mayberg et al. 2000). The brain areas were localized using standard procedures (Brunoni et al. 2012b; DaSilva et al. 2011). We used a current density of 0.8 A/m^2 (2 mA/25 cm^2) per 30 min/ day. For the sham condition, the same tDCS montage was used, although the device was turned on for only 1 min and then remained turned off for the lasting 29 min. This method is based on the study of Gandiga et al. (2006) and aims to mimic skin side effects (tingling, itching, local discomfort) usually reported immediately after stimulation onset, although—with only 1 min of active stimulation—without inducing neuromodulatory effects per se (Nitsche and Paulus 2000). Two trained nurses applied all tDCS sessions. They were trained to turn off the device without patient awareness and to not interact with them in any other aspect of the trial. All raters were blind to the procedure being administered (tDCS/sham) and raters and nurses were blind to study medication.

Blood samples were collected by venipuncture immediately before (2–4 p.m.) the first and the last tDCS session—i.e., at the baseline and the endpoint of our study. Within 30 min of sample collection, they were then spun at 3,000 rpm for 15 min at 5 °C and thereafter, plasma aliquots were gently collected and stored at −80 °C until analysis. Blood cytokines (IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, and TNF-α) were simultaneously measured by flow cytometry using the Cytometric Bead Array Human Th1/Th2/Th17 Kit (BD Biosciences, San Jose, CA). Acquisition was performed with a FACSCanto II flow cytometer (BD Biosciences, San Jose, CA). The instrument has been checked for sensitivity and overall performance with Cytometer Setup and Tracking beads (BD Biosciences, San Jose, CA) prior to data acquisition. Quantitative results were generated using FCAP Array v1.0.1 software (BD Biosciences San Jose, CA, USA). The method used detects concentrations as low as 0.274 pg/mL. No concentration fell below this range. Analyses of blood samples were performed blind to group assignment.

Statistical analysis

We used Stata 12 (StataCorp, College Station, TX, USA) for all analyses, with two-sided significance tests at the 5 % significance level. For descriptive data, clinical and demographic variables were compared across groups using one-way analysis of variance (ANOVA), χ^2 tests or Fisher's exact tests, when necessary.

For the primary outcome, we carried out repeated-measures ANOVAs—for each one; the cytokine plasma level was the dependent variable. Few participants (<0.5 % of the sample) presented extreme values (up to 100 times of the sample mean) in some cytokine levels; to

handle with these extreme outliers, we trimmed these values to the mean+3 SD of the variable. In addition, all dependent variables underwent natural logarithmic to achieve Gaussian distribution. In our model, the within-subjects factor was time (two levels: first and second collections) and the between-subjects factors were treatment group (four levels: placebo, sertraline-only, tDCS-only, and combined therapy) and clinical response, defined as ≥50 % MADRS improvement from baseline to endpoint (two levels: responders and non-responders). Therefore, we analyzed the effects of treatment group and clinical response on cytokine levels by verifying whether the main effects of time, group, response, and their interactions were significant. To further explore our findings, we performed additional analyses of covariance (ANCOVAs) in which cytokine changes (pre–post scores) were the dependent variable, group was the independent variable and baseline MADRS was the covariate. Also, as benzodiazepines have peripheral activity and might also have anti-inflammatory properties (Fruscella et al. 2001; Taupin et al. 1993) we performed sensitivity analyses to assess whether removal of patients on this drug class would affect the observed results. Finally, we carried out additional analyses to examine whether other characteristics such as age, gender, and menopausal status influenced the outcome as well as melancholic depression, atypical depression, and obesity (defined as a body mass index ≥30 kg/m^2) as described in literature (Lamers et al. 2013). These variables were explored in separate models.

To explore whether baseline cytokine levels were predictors of response, we used unpaired *t* tests to compare responders vs. non-responders. If significant differences were observed, additional analyses would be performed to explore the influence of treatment. Finally, Pearson's correlations were performed to explore the association of cytokine levels with depression scores.

Results

Overview

Of the 120 participants enrolled, 103 completed the original study of which 73 (71 %) had their baseline and endpoint immunologic profile analyzed. The remaining samples were not collected because of either patient refusal or technical reasons. Their clinical and demographic characteristics did not differ from the completers of the original study, nor across the groups from the present study. Importantly, the main results observed in SELECT-TDCS were replicated in this subsample, i.e., increased clinical improvement of the combined treatment over the other groups, increased improvement of tDCS-only and sertraline-only over placebo and similar effects of tDCS-only compared to sertraline-only (Table 1).

Cytokine plasma levels over time

Table 2 displays the plasma levels of the cytokines at baseline and endpoint (Table 2). The repeated-measures ANOVAs showed main effects of time for all cytokines (except for TNF-α) and no interaction effects of time with group, with response, and with group and response, showing that the values of these variables decreased over time for all groups irrespectively of the treatment condition and clinical response (Table 2). In other words, the

plasma levels of all cytokines (except TNF-α) decreased during the treatment and such decrease occurred for all interventions, regardless of treatment response (Table 3). These findings were further confirmed in the ANCOVAs models in which no effect of group and no effect of baseline MADRS was observed for the changes of the plasma levels of any cytokine (respectively for group and MADRS: F =1.03, p =0.38 and F =0.36, p =0.54 for IL-2; F =1.12, p =0.34 and F =2.93, p =0.1 for IL-4; F =0.4, p =0.75 and F =1.66, p =0.2 for IL-6; F =0.93, p =0.43 and F =0.3, p =0.58 for IL-10; F =0.61, p =0.6 and F =1.74, p =0.19 for IL-17A; F =0.3, p =0.82 and F =1.41, p =0.23 for INF- γ; F =0.28, p =0.83; and F =0.01, p =0.97 for TNF-α).

Sensitivity analyses were performed in benzodiazepine-free patients to explore whether these drugs impacted in the outcome. In this subsample (n =63), we found similar results as presented in Table 3, use of benzodiazepines did not influence the outcome.

Finally, similar results were obtained when evaluating the influence of atypical depression (*ps* >0.66), melancholic depression (*ps* >0.2), obesity (*ps* >0.22), age (*ps* >0.35), gender (*ps* >0.25), and menopausal status (*ps* >0.31) on the outcome.

Cytokine plasma levels and depression scores

At baseline, we found no significant correlations between cytokine levels with HDRS17 and MADRS scores (*ps* >0.15) and that most cytokine levels were significantly correlated with each other (Pearson's r >0.45, *ps* <0.001).

We also analyzed the correlation between score changing (i.e., baseline minus endpoint) of these variables, finding that depression changes were not associated with cytokine changes (*ps* >0.5). At endpoint, we also observed no correlation between HDRS17 and MADRS score with cytokine plasma levels (*ps* >0.1).

Cytokines as predictors of antidepressant response

We found no significant results (*ps* >0.19) in the unpaired t tests performed to compare cytokine plasma levels at baseline according to clinical response. In other words, baseline plasma levels of these cytokines did not predict response after 6 weeks of treatment.

Discussion

Our main finding was that the plasma levels of several cytokines (IL-2, IL-4, IL-6, IL-10, IL-17a, IFN-γ, although not TNF-α) decreased over time in depressed patients during an antidepressant trial, which is in line with literature; although this effect was not specific to the intervention, as such decrease was also observed in the placebo group. In addition, this effect was also not correlated with clinical response. Two recent meta-analyses are relevant to our findings. Hiles et al. (2012) examined the changes of IL-6 and IL-10 in. respectively, 14 and 3 studies. The pooled effect sizes showed a significant decrease of IL-6 but not IL-10. Importantly, most of the studies were non controlled, i.e., the study design consisted of a single group of patients receiving antidepressant treatment and having their interleukin levels measured before and after treatment—in some cases, a control condition of healthy participants was included, who had their cytokine levels measured at a single time point. Of

these previous studies, only two were placebo-controlled. One of them showed a significant reduction of IL-6 levels after 20-weeks of sertraline, but not placebo treatment (Pizzi et al. 2009). The other study showed no significant changes of IL-10 after placebo and active treatment groups (Song et al. 2009). The meta-analysis of Hannestad et al. (2011) explored the changes of TNF-α and IL-6 only in studies that used an antidepressant drug treatment. Similarly to our study findings, the authors did not find significant changes in TNF-α plasma levels despite depression improvement. Also, the authors observed that patients receiving SSRI presented a decrease in IL-6 plasma levels (with improvement of depressive symptoms), although other antidepressant drug classes had no significant effects on IL-6 plasma levels. Nonetheless, as observed in the meta-analysis of Hiles et al. (2012), almost all studies used a single-group design, measuring cytokine changes over treatment, and therefore not disentangling time effects from specific pharmacotherapy effects, since no control group was used.

Regarding the other cytokines examined in our study, most studies investigating changes in IL-2 levels observed no increase after treatment (Eller et al. 2009), whereas one placebo-controlled study with 95 depressed outpatients showed that electroacupuncture, but not fluoxetine, decreased the ratio of IFN-γ/IL-4 (but not IL-4 alone) towards the levels of healthy volunteers (Song et al. 2009). IL-17A is a cytokine related to Th17 profile with strong inflammatory properties (Nwe et al. 2013). Chen et al. (2011) observed higher IL-17 levels in depressed vs. healthy individuals; no study has yet investigated IL-17A as a biomarker of antidepressant treatment. Regarding IFN-γ, some clinical studies described that pharmacotherapy decreased IFN-γ levels after antidepressant treatment (Mohr et al. 2001; Myint et al. 2005; Seidel et al. 1995). Therefore, considering the limited evidence from prior pharmacological trials, our study also observed that cytokine levels decreased over an antidepressant treatment course. However, the use of a placebo arm showed that these changes might not be specific to the intervention, and other hypotheses for cytokine levels decreasing over time in antidepressant trials should be considered. Another possibility is that the inflammatory state observed in depression persists for weeks even after symptom improvement, and therefore, the post-treatment cytokine level measurement time period should be extended, such as in the study of Pizzi et al. (2009) that observed specific decrease of IL-6 for sertraline vs. placebo only after 20 weeks of treatment. Along these lines, studies using N-acetylcysteine—a compound with anti-oxidant and anti-inflammatory properties—observed an improvement in symptoms in patients with bipolar disorder and schizophrenia only after 20 weeks of treatment (Berk et al. 2013), suggesting that inflammatory changes might be more relevant to neuropsychiatric disorders on the maintenance phase—rather than the acute phase—of the illness.

On the field of non-invasive somatic treatments, our results are novel as no repetitive transcranial magnetic stimulation (rTMS)/tDCS clinical trials in depression investigated changes in cytokine levels (Fidalgo et al. 2013) in depression and, in fact, in healthy or unhealthy volunteers. There are only a few rTMS/tDCS studies in experimental animals that measured the expression of some cytokines, with mixed results (Okada et al. 2002; Spezia Adachi et al. 2012). Nonetheless, the effects of rTMS and tDCS on the HPA and SAM axes, which are directly linked to the neuroimmunological response, have been evaluated in healthy volunteers and neuropsychiatric disorders. On this matter, two recent reviews

(Sampaio et al. 2012; Schestatsky et al. 2013) found mixed evidence regarding possible neuromodulatory effects on these systems, with positive results obtained from studies generally employing designs in which participants were submitted to physiological or psychological stress (e.g., (Brunoni et al. 2013c)). Using data from SELECT-TDCS, we observed that tDCS-only and combined with sertraline did not modulate heart rate variability, a marker of sympathetic/parasympathetic activity, throughout the trial (Brunoni et al. 2013a) and also, that these therapies had no specific antidepressant effects on vegetative symptoms (Brunoni et al., in press), as similarly observed in another tDCS trial (Alonzo et al. 2013). This evidence points towards the absence of specific tDCS effects on immune biomarkers of MDD. Nonetheless, computational model studies (for a review see (Bai et al. 2013)) showed that tDCS applied over the scalp has neuromodulatory effects in subcortical structures, the thalamus, and the brainstem. Hence, further tDCS trials are warranted to investigate in which contexts tDCS modulates these peripheral biomarkers. It should be noted, for instance, that other markers of inflammation, such as acute phase proteins, or markers of oxidative stress were not examined in the present study.

One important characteristic of our study is the use of a placebo group, in which a decrease in cytokine levels was observed. Placebo effects over the immune system are in fact described in preclinical and clinical studies, and mainly involve the neuropsychological mechanisms of expectation of benefit and associative learning (Enck et al. 2008). In one clinical study, for instance, an immunosuppressive drug was first paired with a gustatory stimulus and, after that, mere re-exposition of the gustatory stimulus impaired Th1 cytokine expression (Goebel et al. 2002). In major depression, placebo response is associated with cortical activation and subcortical de-activation (Mayberg et al. 2002). As decrease in activity of subcortical areas is associated with downregulation of the pro-inflammatory state (Kupfer et al. 2012; Nemeroff and Goldschmidt-Clermont 2012), such mechanism could explain the non-specific antidepressant (pharmacological and non-pharmacological) effects over the immune system, at least during the acute depressive episode.

Some methodological considerations should be underscored. First, sertraline in a higher dose than employed in the original study (50 mg/day) could have induced different effects on cytokine levels due to greater efficacy in depression improvement, since Yoshimura et al. (2013) found that IL-6 decreased in treatment responders but not non-responders. However, despite using 50 mg/day of sertraline, we did see clinical improvement and this was without correlative changes in cytokines plasma levels. This supports that the changes in immunoinflammatory markers are not necessary to acute changes in depressive symptoms. Conversely, other studies (Lanquillon et al. 2000; Sluzewska et al. 1996) and ours observed a significant decrease in cytokines in both responders and non-responders. Moreover, no clinical trial examined the effects of different sertraline doses on inflammatory cytokines and Baharav et al. (2012), in an animal model of rheumatoid arthritis, observed that all employed doses of sertraline had a significant anti-inflammatory effect.

Second, we ran several analyses of variance, thus increasing the probability of type I error. We did not correct for multiple analyses since this was a primarily exploratory study. In fact, even using the Bonferroni method (which would have set the p threshold for significance at 0.007) the decrease in the plasma levels of IL-2, IL-4, IL-6, IL-17A, and IFN-γ would have

remained statistically significant. Moreover, the changing in cytokine levels was robustly correlated, decreasing the probability of our findings being spurious.

Conclusion

In one of the first placebo-controlled trials examining cytokine changes throughout an antidepressant trial, we found that the plasma levels of cytokines IL-2, IL-4, IL-6, IL-10, IL-17A, and IFN-γ decreased after a 6-week clinical trial in major depression, although these changes were neither specific to the type of intervention (pharmacological vs. non-pharmacological), as they were observed in the placebo group; not associated with clinical response. Nonetheless, taking into account that we did not exhaust the evaluation of other relevant cytokines, the relatively short time period between the first and the second measurements, and this being the first trial evaluating the impact of a non-invasive brain stimulation technique on the immune system in depressed patients, further studies in this topic are warranted. Particularly, future studies should be placebo-controlled to evaluate whether there are specific effects of antidepressant interventions on cytokine plasma levels, or that the decrease observed in most trials thus so far is, perhaps, a byproduct of a non-specific, placebo effect observed in such trials.

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Psychopharmacology (Berl). Author manuscript; available in PMC 2015 April 01.

Table 1

Clinical and demographic characteristics of the sample of the present study at baseline

	Placebo	Sertraline-only	tDCS-only	Combined treatment	*p* Value	Total	Total from the original study	*p* Value
Clinical characteristics								
Sample size	19	18	15	21	0.79	73	120	–
Age, years (SD)	50 (12)	41 (1)	41 (12)	41 (13)	0.1	41 (12)	42 (12)	0.58
Women, *n* (%)	12 (63)	11 (61)	10 (66)	18 (85)	0.3	51 (70)	82 (68)	0.77
Using benzodiazepines (%)	2 (10)	2 (11)	1 (7)	5 (23)	0.53	10 (14)	23 (20)	0.3
BMI, kg/m^2 (SD)	26 (6)	25 (3)	25 (6)	27 (5)	0.54	26 (5)	26 (5)	0.92
Depression characteristics at baseline, *n* (%) or mean (SD)								
Refractory depression	7 (37)	9 (50)	6 (40)	7 (33)	0.75	29 (40)	50 (42)	0.78
Severe depression	12 (63)	11 (61)	11 (73)	12 (57)	0.8	46 (63)	70 (58)	0.49
MADRS	31.5 (6)	31 (7)	32 (6)	31 (6)	0.92	31 (6)	31 (6)	0.5
HDRS17	22 (4)	22 (4)	22(4)	22 (4)	0.99	22(4)	22(4)	0.75
Depression endpoint scores, mean (SD) and response, *n* (%)								
MADRS	24 (9)	19 (13)	19 (12)	10 (6)	<0.01	18 (11)	19 (11)	0.38
HDRS17	17 (7)	14 (8)	13 (7)	9 (5)	0.01	14 (8)	15 (7)	0.34
Response	4 (21)	7 (39)	7 (46)	16 (76)	<0.01	34 (46)	47 (39)	0.52

Significant *p* values (<0.05) are highlighted in bold

tDCS transcranial direct current stimulation; *MADRS* Montgomery-Asberg depression rating scale; *HDRS17* Hamilton Depression Rating Scale, 17-items; BMI body mass index; SD standard deviation. *Refractory depression* patients who had therapeutic failure of two or more antidepressants in the current major depressive disorder. *Severe Depression* MADRS >30

Psychopharmacology (Berl). Author manuscript; available in PMC 2015 April 01.

Table 2

Cytokine plasma levels at baseline and endpoint according to the treatment group

		Placebo	Sertraline-only	tDCS-only	Combined treatment
IL-2 (pg/mL)	Baseline	1.57 (0.46)	1.67 (0.29)	1.57 (0.11)	1.51 (0.23)
	Endpoint	1.05 (0.2)	1 (0.41)	1.08 (0.38)	0.97 (0.36)
IL-4 (pg/mL)	Baseline	1.23 (0.14)	1.23 (0.14)	1.23 (0.25)	1.23 (0.18)
	Endpoint	0.91 (0.53)	0.89 (0.27)	0.83 (0.27)	0.97 (0.24)
IL-6 (pg/mL)	Baseline	1.74 (0.18)	1.74 (0.1)	1.78 (0.1)	1.79 (0.1)
	Endpoint	1.43 (0.7)	1.35 (0.35)	1.35 (0.28)	1.4 (0.15)
IL-10 (pg/mL)	Baseline	1.64 (0.38)	1.44 (0.16)	1.43 (0.3)	1.43 (0.37)
	Endpoint	1.43 (0.7)	1.35 (0.35)	1.35 (0.29)	1.41 (0.15)
IL-17a (pg/mL)	Baseline	1.9 (0.2)	1.86 (0.1)	1.86 (0.14)	1.9 (0.07)
	Endpoint	0.83 (0.36)	0.82 (0.2)	0.84 (0.14)	0.8 (0.19)
IFN-γ (pg/mL)	Baseline	3.54 (0.17)	3.54 (0.08)	3.54 (0.17)	3.5 (0.17)
	Endpoint	3.02 (0.35)	3.06 (0.27)	3.02 (0.15)	3.02 (0.2)
TNF-α (pg/mL)	Baseline	0.28 (0.27)	0.25 (0.12)	0.37 (0.3)	0.3 (0.3)
	Endpoint	0.43 (0.86)	0.23 (0.17)	0.33 (0.6)	0.32 (0.19)

The table displays the median and interquartile range (IQR) values of several interleukins (pg/mL) at baseline and endpoint (6 weeks) of the clinical trial

Table 3

Statistical values of the repeated-measures analyses of variance of cytokine plasma levels

	Time			Time × group			Time × response			Time × group × response		
	F	**d.f.**	***p***	***F***	**d.f.**	***p***	***F***	**d.f.**	***p***	***F***	**d.f.**	***p***
IL-2	69.5	145.1	<0.01	0.3	145.3	0.8	0.63	145.1	0.42	1.05	145.3	0.37
IL-4	10.5	145.1	<0.01	0.18	145.3	0.9	0.22	145.1	0.64	0.58	145.3	0.63
IL-6	8.77	145.1	<0.01	0.31	145.3	0.8	0.27	145.1	0.6	0.43	145.3	0.72
IL-10	6.81	145.1	0.01	0.17	145.3	0.91	0.91	145.1	0.35	0.27	145.3	0.84
IL-17a	151	145.1	<0.01	0.15	145.3	0.92	0.03	145.1	0.85	0.35	145.3	0.78
IFN-γ	19.1	145.1	<0.01	0.17	145.3	0.91	0.01	145.1	0.92	0.47	145.3	0.7
TNF-α	0.83	145.1	0.94	0.13	145.3	0.94	0.18	145.1	0.67	0.08	145.3	0.97

The table displays the results from the repeated-measures analyses of variance performed in our study. Significant *p* values (<0.05) are highlighted in bold *F F* values, *d.f.* degrees of freedom