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Brief reports

Soluble neural cell adhesion molecule and behavioural recovery in minimally conscious patients undergoing transcranial direct current stimulation

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ABSTRACT

Background: Transcranial direct current stimulation (tDCS) is used for therapeutic purpose in severely brain-injured patients. The relationship between the recovery after tDCS and potential biomarkers in plasma has been limitedly investigated in patients with minimal conscious state (MCS).
Objective: To investigate soluble neuronal adhesion molecule (sNCAM) plasma levels in relation to tDCS and recovery processes in MCS.
Methods: sNCAM was measured in plasma before (T_{-1},T_0), during (T_1) and after (T_2, T_3) tDCS sessions in eight patients with a post traumatic etiology and at least one year of chronic state.
Results: While sNCAM levels were highly correlated overtime, no significant difference was observed in relation to tDCS. An inverse relation was observed between sNCAM levels at baseline and the tDCS long-lasting effects (T_{-1}, $r = -0.852$, $p = 0.007$; T_0, $r = -0.787$, $p = 0.020$).
Conclusions: This exploratory research suggests the sNCAM levels, potentially associated with tDCS outcomes, as a candidate biomarker of neurobiological after-effects in MCS patients.

1. Introduction

Transcranial direct current stimulation (tDCS) is used for therapeutic purposes in several human conditions to induce brain neuroplasticity through the application of electrical currents [1]. In addition to the modulation of neurotransmitters and sympathetic regulation of vascular tone and blood pressure [2–4], the electric field could favor protein shedding from cell membranes. In turn, this would release molecules of interest in plasma as potential biomarkers of tDCS effects and disease recovery. Differently from the acute phase of traumatic brain injury (TBI), in which some circulating molecules were suggested as promising biomarkers [5,6], for the chronic state, and particularly in relation to tDCS, data are not available in the literature.

Among proteins released by physiopathological stimuli, and potentially by tDCS, we selected neuronal adhesion molecule (NCAM, CD56), present as soluble NCAM (sNCAM) in body fluids, generated by neutrophil proteases and metalloproteases from alternatively spliced membrane-bound isoforms [7]. According to severity of TBI in patients, a decreased number of natural killer cells exposing NCAM has been described [8], and NCAM/sNCAM have been studied in relation to recovery after brain injury as well as to potential for neuronal plasticity [9,10]. In animal models, NCAM could act as an inhibitor of remyelination [9], and NCAM mimetic peptides have been administered (reviewed in [11]) to explore potentially therapeutic approaches of TBI or ischemic brain damage.

Recently, we reported a longitudinal pilot study with an open-label design in severe compromised patients in minimally conscious state (MCS) [12]. Bilateral application of anodal tDCS over the primary motor cortex (M1) improved the consciousness measured withthe Coma Recovery Scale-Revised (CRS-R) total score [13,14]. The present study focuses on sNCAM plasma levels in relation to clinical and functional characteristics of these patients, and particularly to the behavioural recovery after tDCS. Finding association would propose sNCAM as a candidate plasma biomarker for tDCS application, limitedly

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Patient ID	Age	Sex	Time from trauma	Implantable device	Medications	Baseline CRS-R total score	Delta CRS-R total scores
1wp2	35	M	11 y	ITB	-	15	2
2wp2	36	M	8 y, 9 m	VPS	-	10	1
3wp2	47	M	4 y, 7 m	ITB	Levetiracetam	13.5	2.5
4wp2	34	M	19 y	-	-	12.5	1.5
5wp2	24	F	2 y	-	Levetiracetam, amantadin	12	4
6wp2	27	F	7 y, 6 m	ITB	Levetiracetam	10	2
9wp2	63	M	1 y	VPS	Carbamazepine	7	3
10wp2	21	M	1 y, 1 m	VPS	-	9.5	1.5

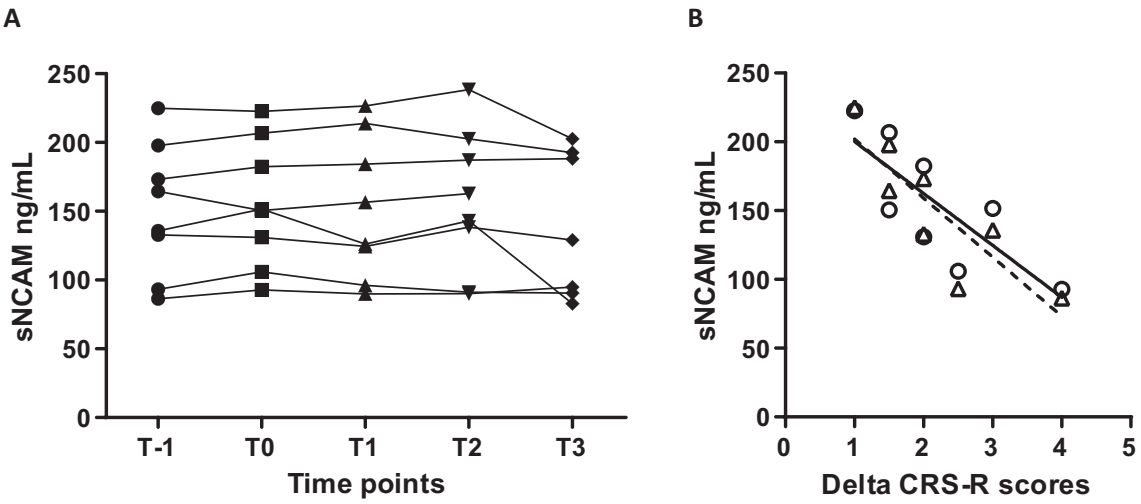

Fig. 1. Upper part: clinical and demographic characteristics of patients. F, female; M, male; y, years; m, months; ITB, intrathecal baclofen; VPS, ventriculoperitoneal shunt; CRS-R, coma recovery scale-revised. Delta of the CRS-R total scores of clinical evaluation between baseline (T_0) and 14 weeks after T_2 (end of 10 tDCS sessions) is reported. Lower part: soluble neural cell adhesion molecule 1 (sNCAM) concentration in plasma of patients with minimally conscious state (n = 8) evaluated at five time-points (A), and (B) in relation to variation (Delta) of the CRS-R scores measured at T_0 and 14 weeks after T_2, at the last follow-up. A) T_{-1} and T_0, before starting the rehabilitative program; T_1, intermediate point after 5 tDCS sessions; T_2, end of treatment after 10 tDCS sessions; T_3, follow-up at 2 weeks post-treatment. B) Empty triangle, values at T_{-1}; empty dots, values at T_0. Dotted line, correlation between T_{-1} and Delta CRS-R (Pearson coefficient, r = −0.852, p = 0 .007); continuous line correlation between T_0 and Delta CRS-R (Pearson coefficient, r = −0.787, p = 0 .020).

investigated so far in patients with MCS.

2. Material and methods

Patients in MCS with a post traumatic etiology and at least one year of chronic statewere enrolled during a multidisciplinary rehabilitation program [12]. Patients with i) metallic implants,ii) skull defects or skull plates and iii) severe cardio-pulmonary, renal, or hepatic diseases were excluded.

The detailed study design (Clinicaltrial.gov database NCT02288533) has been previously reported [12].

The experimental protocol consists of 10 sessions (five sessions/ week for two weeks) of 2 mA bilateral M1 anodal tDCS applied by a a battery-driven constant current stimulator for 40 min each session (Brainstim, EMS, Italy). Two electrodes (anode) were placed on M1 bilaterally and the reference electrode at the nasion. The electrode sponge surface area of 16 cm2 (4 × 4 cm) was soaked in saline solution.

At the end of each session, an adverse event questionnaire related to tDCS was completed and any behavioural changes were recorded by the investigators.

Behavioural changes were assessed by two experienced clinicians using the CRS-R [13,14] two weeks before (T_{-1}) and one day before (T_0) the start of the experimental protocol, halfway through (after five sessions) (T_1), at the end of the ten sessions (T_2), at two weeks after T_2 (T_3) and at 14 weeks after T_2 (T_4).

For eight patients (Fig. 1) repeated plasma samples were available. Blood samples were obtained in citrate tube at five-time points, T_{-1}, T_0, T_1, T_2 and T_3, spanning across 1 month and half.

sNCAM levels were assayed using Luminex Screening Assays magnetic bead kits (Luminex R&D Systems Inc., Minneapolis, MN, USA) as previously reported [15]. Data were acquired using the Luminex® 100 system and analyzed using Bioplex Manager Software version 6.0 (Biorad Laboratories, Hercules, CA). Concentrations were calculated according to the standard curve and expressed as ng/mL. The intra-assay coefficient of variation was 1.74%. All statistical analyses and figures were produced by Graphpad prism version 6.01 (GraphPad Softwere, Inc. La Jolla, CA, USA).

3. Results

Plasma sNCAM levels were measured in eight patients, and in addition in 39 healthy subjects as reference. The median concentration was 150.1 ng/mL (IQR = 103.1–191.7) in patients at T_{-1}, and 127.5 ng/mL (IQR = 97.8–154.7) in healthy subjects ($p = 0.258$, Mann-Whitney U test).

In patients, repeated measures at the five time-points sampling

(Fig. 1A) showed an extremely high correlation of values over time (i.e. T_{-1}-T_0, $r = 0.980$, $p < 0.001$; T_{-1}-T_2, $r = 0.996$, $p < 0.001$) and high coefficients of determination (T_0-T_1-T_2, $R^2 = 0.98$; T_{-1}-T_0-T_1-T_2-T_3, $R^2 = 0.95$). Protein concentration did not correlate with patients' age and showed a trend for correlation with time from trauma (T_{-1}, $r = 0.598$, $p = 0.118$; T_0, $r = 0.662$, $p = 0.074$; T_1, $r = 0.700$, $p = 0.054$; T_2, $r = 0.599$, $p = 0.117$; T_3, $r = 0.825$, $p = 0.022$).

Variation over time of concentration (Fig. 1A), evaluated by ANOVA for repeated measures, was not significant (T_0-T_1-T_2, $p = 0.466$; T_{-1}-T_0-T_1-T_2-T_3, $p = 0.156$).

We investigated the relation of protein levels at baseline with the CRS-R score at baseline, and with long-lasting effects of tDCS, measured as the Delta of the CRS-R total scores between T_0 and the last follow-up [12]. Protein levels at T_{-1} and T_0, which precede the tDCS, did not correlate with the CRS-R score at baseline. Noticeably, an inverse relation was found between the tDCS long-lasting effects and sNCAM concentration before tDCS, both at T_{-1} (Pearson coefficient, $r = -0.852$, $p = 0.007$) and at T_0 (Pearson coefficient, $r = -0.787$, $p = 0.020$, Fig. 1B). The relation between the Delta of CRS-R scores and sNCAM concentration was detectable at all the time-points (T_1, $r = -0.819$, $p = 0.013$; T_2, $r = -0.819$, $p = 0.013$; T_3, $r = -0.826$, $p = 0.022$).

4. Discussion

The present study is aimed at evaluating plasma concentration of a candidate protein in plasma in relation to tDCS, and to recovery processes in chronic patients with MCS, with the limitations of an open-label design and the lack of a sham condition, which might limit tDCS efficacy interpretation.

The plasma protein concentration, measured at five time-points with high correlations, indicates sNCAM as a stable biomarker in individual patients. These novel observations also suggest either that tDCS did not appreciably affect the spontaneous protein release, or that the amounts of released protein were insufficient to modify its circulating levels. Accordingly, variation in sNCAM plasma concentrations would not belong to the group of neurobiological after-effects of non-invasive brain stimulation [2].

The inverse relation of sNCAM plasma concentration with the long-lasting tDCS effects, which might indicate modification of the residual functions, suggests that patients with low levels of sNCAM could experience better outcomes after tDCS stimulation. Worth noting, sNCAM has been reported as marker of peripheral demyelination and its higher levels correlated with the lowest motor conduction velocity in patients with demyelinating polyneuropathies [16]. Our study does not permit to infer whether the circulating levels were directly or inversely related to the trans-membrane NCAM protein isoforms. Further, in light of its size, our study has limitations and cannot evaluate variation of sNCAM, and of its polysialylated isoforms, in relation to medication. Nevertheless, these hypothesis generating findings might support that, despite the chronic state of patients with MCS, some neural plasticity [17] could be inversely reflected by sNCAM plasma concentration.

5. Conclusion

The present exploratory research suggests plasma sNCAM, endowed with a favorable stability overtime, as a candidate biomarker of tDCS response in patients with MCS. Our findings support further studies aimed at evaluating if single assays before tDCS have a predictive value in randomized clinical trials.

Declarations of interest

None.

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