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Reductions in qEEG slowing over 1 year and after treatment with Cerebrolysin in patients with moderate–severe traumatic brain injury

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Abstract Changes in quantitative EEG (qEEG) recordings over a 1-year period and the effects of Cerebrolysin (Cere) on qEEG slowing and cognitive performance were investigated in postacute moderate–severe traumatic brain injury (TBI) patients. Time-related changes in qEEG activity frequency bands (increases of alpha and beta, and reductions of theta and delta relative power) and in qEEG slowing (reduction of EEG power ratio) were statistically significant in patients with a disease progress of less than 2 years at baseline, but not in those patients having a longer disease progress time. Slowing of qEEG activity was also found to be significantly reduced in TBI patients after 1 month of treatment with Cere and 3 months later. Therefore, Cere seems to accelerate the time-related reduction of qEEG slowing occurring in untreated patients. The decrease of qEEG slowing induced by Cere correlated with the improvement of attention and working memory. Results of this exploratory study suggest that Cere might improve the functional recovery after brain injury and encourage the conduction of further controlled clinical trials.

Keywords Cerebrolysin · EEG power ratio · Quantitative EEG · qEEG slowing · Cognitive performance · Traumatic brain injury

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Introduction

Electrophysiological abnormalities and changes in growth factors levels have been extensively reported after traumatic brain injury (TBI), and are considered as relevant events in TBI pathophysiology. Alterations in auditory and visual processing (Thornton 2003; Boly et al. 2004; Lew et al. 2004), alpha activity (Vespa et al. 2002; Angelakis et al. 2004; Roche et al. 2004), sleep organization pattern (Valente et al. 2002) and quantitative electroencephalographic (qEEG) parameters (Kane et al. 1998; Thatcher et al. 2001; Wallace et al. 2001; Alvarez et al. 2003; Thornton 2003) have been found in TBI patients. Some of the qEEG changes reported in TBI cases correlate with trauma severity, cognitive performance and clinical outcome measures (Alvarez et al. 2003; Thornton 2003). In a previous study (Alvarez et al. 2003), we observed that EEG power ratio (PR), an index obtained by calculating the ratio of relative power between slow (delta + theta) and fast (alpha + beta) EEG frequency bands, correlated positively with TBI severity (GOS score) and negatively with cognitive performance. However, changes over time in qEEG activity, PR scores and its correlation with TBI severity have not been previously evaluated in postacute TBI patients.

Increased levels of several neurotrophic factors, protein S100B, pro-inflammatory cytokines and amyloid proteins

were detected in the acute phase of TBI (Csuka et al. 1999; Emmerling et al. 2000; Leclerq et al. 2001; Berger et al. 2002; Magnoni et al. 2003; Savola et al. 2004; Winter et al. 2004). The overexpression of inflammation mediators is believed to be detrimental for neuronal survival in TBI; whereas elevations of neurotrophic factors might represent an endogenous attempt to protect against neurodegeneration, which constitutes the rationale for the use of neuroprotective–neurotrophic treatment strategies in TBI (Faden 2002; Chiaretti et al. 2003; Kazanis et al. 2004; Longhi et al. 2004). Although most of the neuroprotection trials in human TBI have failed to show a real therapeutic benefit (Faden 2002), there are two recent experimental studies with IGF-I and NGF supporting the use of compounds with neurotrophic activity to treat TBI patients (Kazanis et al. 2004; Longhi et al. 2004). In fact, we have previously reported (Alvarez et al. 2003) positive effects on brain bioelectrical activity, cognitive performance and clinical outcome after treatment with the neurotrophic compound Cere in patients with postacute TBI. Since the improvement of cognition induced by Cere was accompanied by an EEG-activating effect in the same patients, it is suggested that changes in EEG activity might reflect the neuroprotective action of this compound. In addition, serial qEEG recordings might be useful to monitor the effects of neuroprotective drugs able to limit neuronal damage and to enhance recovery after brain injury.

Cere is a peptide preparation obtained by biotechnological methods, using a standardised enzymatic breakdown of purified porcine brain proteins. It consists of 25% low-molecular weight biologically active peptides and free amino acids. A measure of 1 ml of Cere contains 9 mg of peptides and the consistent qualitative and quantitative composition of the compound is ensured by rigorous quality control procedures, including amino acid analysis and HPLC peptide mapping. Cere has a neurotrophic factor-like mode of action as demonstrated both in vivo and in vitro. It enhances neuronal survival and sprouting in culture (Albrecht et al. 1993) and exerts a neurotrophic activity similar to NGF on dorsal root ganglia neurons (Satou et al. 1994). Akai et al (1992) demonstrated the rescue of medial septal cholinergic neurons in a model of fimbria fornix transection after peripheral injection of Cere indicating that the small molecules are able to penetrate blood–brain barrier in pharmacodynamically significant amounts. Experimental data are also indicating that Cere protects against neurodegeneration induced by hypoxia, ischemia, glutamate and Aβ toxicity (Hutter-Paier et al. 1996, 1998; Schwab et al. 1998; Alvarez et al. 1999a); exerts pro-cognitive effects in rats with Aβ implants into the hippocampus (Álvarez et al. 2000a, b), in ApoE knock-out mice (Masliah et al. 1999) and in aged rats (Gschanes and Windisch 1998; Reinprecht et al. 1999); reduces Aβ1–42 levels and synaptic pathology in hAPP transgenic mice (Rockenstein et al. 2002); increases neurogenesis in the rat hippocampus (Tatebayashi et al. 2003); and reduces microglial activation and the overexpression of IL-1ß induced by stimulation with lipopolysaccharide in vitro and in vivo (Alvarez et al. 1999a, 2000b; Lombardi et al. 1999). All these results suggest a potential positive action of Cere on molecular, morphological and behavioural alterations shared by neurodegenerative conditions like Alzheimer's disease (AD) and TBI. In human studies, it has been demonstrated that Cere enhances cognitive performance and EEG alpha activity in elderly controls (Alvarez et al. 2000a), reduces cognitive deficits and qEEG slowing in TBI patients (Alvarez et al. 2003), and improves cognition and global clinical functioning, exerting a potential disease-stabilizing effect in AD patients (Bae et al. 2000; Ruether et al. 2001, 2002; Panisset et al. 2002; Alvarez et al. 2006). Therefore, Cere seems to have an appropriate pharmacological profile to be used as a treatment to improve functional recovery in postacute TBI patients.

In the present study, we have investigated in postacute TBI patients: (1) the changes over 1-year period in qEEG activity; (2) the potential effects of the administration of Cere (20 i.v. infusions; 30 ml/infusion) on brain bioelectrical activity changes; and (3) the correlations between cognitive performance and qEEG scores.

Material and methods

Subjects and characteristics of the samples

Changes over a 1-year period in qEEG activity were investigated in 20 patients without Cere treatment (6 women and 14 men; age 29.6 ± 2.02 years, range 19–50 years). Initial Glasgow coma scale (GCS) average score (Teadsdale and Jennett 1974) was 5.5 (range 3–15 points), including 3 mild and 17 severe brain injury cases. The time course since brain trauma occurred was 23 months on average. According to the Glasgow outcome scale (GOS, version of five categories) scoring (Jennett and Bond 1975) at baseline (average 3.25; range 2–5), five patients were in a vegetative state (score 2), six cases had severe disability (score 3), eight cases showed moderate disability (score 4) and one case presented mild disability (score 5).

Thirty-nine patients with postacute TBI (13 women and 26 men; age 30.84 ± 1.82 years, range 18–52 years) were treated with Cere. GCS score after trauma was 5.39 on average (range 3–15 points), including 4 mild, 3 moderate and 32 severe brain injury cases. The time course since brain trauma occurred was 21 months on average. Based on the GOS scoring (average 3.05; range 2–5), at baseline 10 patients were in a vegetative state (score 2), 17 cases

had severe disability (score 3), 10 cases showed moderate disability (score 4) and 1 case presented mild disability (score 5). A multipoint EEG evaluation, including an initial EEG recording before the administration of Cere, baseline and postCere EEG recordings and a final EEG recording 3 months after ending Cere treatment, was done in a subgroup of 20 patients (5 women and 15 men; age 31.6 ± 2.24 years, range 19–51 years). Initial GCS score was 5.25 (range 3–15 points) for this sample, including 2 mild, 1 moderate and 17 severe brain injury cases. Average progress time since brain trauma occurred was 23 months. GOS score at baseline was 3.25 (range 2–5), including five patients with a vegetative state (score 2), six cases with severe disability (score 3) and nine having moderate disability (score 4).

General procedures and concomitant therapy

Medical examination, qEEG, ECG and laboratory analysis were performed in all subjects before inclusion. All patients were treated with stable doses of the neuroprotective compounds citicoline (500 mg/day, p.o.) and piracetam (2.4–3.2 g/day, p.o.) and subjected to a long-term standardized neurorehabilitation program before and during the entire evaluation periods. Written informed consent was obtained from all participants, being signed by the patient or the caregiver. The study was approved by the internal review board and conducted according to Good Clinical Practice guidelines.

Study design and treatment regimen

This is an open exploratory study aimed to evaluate the following issues in postacute TBI patients: (1) changes in qEEG activity occurring over a 1-year follow-up period; (2) the potential effects of the i.v. administration of Cere (30 ml/day; 20 infusions/4 weeks) on brain bioelectrical activity; and (3) the time course of qEEG changes before and after the administration of Cere. We also investigated the correlations between cognitive performance, evaluated with the Syndrome Kurztest (SKT) (Overall and Schaltenbrand 1992), and qEEG activity at baseline, as well as the correlations between scores of change from baseline in SKT and qEEG parameters.

The qEEG activity changes occurring in postacute TBI patients over time were evaluated by means of qEEG recordings performed at baseline and 12 months later. Twenty TBI cases were subjected to qEEG follow-up without treatment with Cere.

The duration of the study of Cere-treated patients was 30 days, including: (1) a baseline evaluation, performed before starting the administration of Cere; (2) 4 weeks of treatment with Cere; and (3) a posttreatment evaluation. Thirty-nine participants received 20 i.v. infusions of the Cere solution (30 ml/infusion) from Monday to Friday over 4 weeks on a compassionate use basis.

Since all the patients were receiving treatment with stable doses of neuroprotective compounds (citicoline and piracetam) able to induce changes in brain bioelectrical activity recordings, in those patients having one qEEG recording into the 6 months (2 months on average) before the administration of Cere we performed a multipoint evaluation of qEEG activity in order to: (1) exclude the interference of concomitant medication with the effects of Cere; and (2) to assess the potential influence of Cere treatment on brain bioelectrical activity changes over time in postacute TBI patients. This multipoint qEEG evaluation included an initial qEEG recording 2 months before administering Cere (prestudy), baseline and posttreatment qEEG recordings, and a final qEEG recording 3 months after ending Cere treatment (poststudy), and was done in 20 patients.

Evaluation procedures

Brain bioelectrical activity was assessed with computerized EEG spectral analysis and topographic brain mapping (Alvarez et al. 2003). The qEEG recordings were performed in resting conditions and with eyes closed by using 19 scalp electrodes fixed in an elastic cap (ECI-Electro cap, Eaton, Ohio, USA), located according to the international 10–20 system. The electrode impedance was below 3 kΩ and all electrodes were referred to the right ear. The EEG signal was analog-filtered with a band pass of 0.5–25 Hz in a Microscribe Electroencephalograph (SLE, Croydon Surrey, UK), and digitalized and stored on magnetic disks for further analysis. EEG was visually inspected and free-artifact epochs of two seconds were selected for spectral analysis, performed with a Fast Fourier Transform using a Brain Atlas v2.345 (BioLogic System Corp., Mundelein, IL, USA). Data were normalized and relative power (%) was used as the reference parameter. The following frequency bands were studied: delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (12–16 Hz). Average relative power scores of each frequency band and the EEG power ratio (PR = (delta + theta)/(alpha + beta) relative power) were calculated for each recording and used in the statistical analysis. An increase in the PR indicates EEG slowing, whereas a PR decrease reflects an EEG desynchronization/acceleration (Funke et al. 1998; Nagata et al. 1989).

Cognitive performance was evaluated by using the SKT test, a brief neuropsychological test battery consisting of nine performance subtests (naming objects, immediate

Table 1 Changes in qEEG activity over a 1-year period in postacute TBI patients

Group	EEG band	*N*	EEG recording	
			Baseline relative power (%)	After 1 year relative power (%)
TBI	Delta	20	23.52 ± 2.70%	19.53 ± 2.02*
	Theta	20	26.59 ± 1.89%	24.62 ± 1.83
	Alpha	20	22.94 ± 2.43%	24.79 ± 2.42
	Beta	20	9.94 ± 1.11%	11.28 ± 1.12
TBI-2	Delta	12	26.86 ± 3.42%	20.13 ± 2.88**
	Theta	12	29.77 ± 2.65%	25.70 ± 2.78*
	Alpha	12	19.91 ± 3.06%	22.89 ± 3.37*
	Beta	12	8.808 ± 1.27%	10.96 ± 1.43*

TBI group: all TBI cases. TBI-2 group: TBI cases with a disease progress time of less than 2 years at baseline. *$P < 0.05$ and **$P < 0.01$ versus baseline

recall, naming numerals, arranging blocks, replacing blocks, counting symbols, reversal naming, delayed recall and recognition memory) that define two independent factors of memory and attention deficit. This test has been extensively used in dementia patients, but also to evaluate neuropsychological impairment in other medical conditions including brain trauma (Alvarez et al. 2003). Results can be expressed as raw scores and as normalized values. Total score in the SKT test corresponds to the sum of normalized values in the different items. Higher SKT scores indicate a worse cognitive performance.

Vital signs (systolic and diastolic blood pressure, mean heart rate, body temperature), ECG and laboratory parameters were controlled before and after Cere treatment.

Statistics

The nonparametric Wilcoxon test was used to compare paired data obtained before and after time/treatment for each measure. The correlations of qEEG parameters with baseline TBI progress time (months) and severity (GOS score), as well as the correlations between EEG power ratio and cognitive performance were evaluated with the Pearson's linear correlation test. Partial correlation analysis was employed to evaluate changes in PR scores from baseline as a function of the TBI progress time at baseline after controlling for the influence of TBI severity. Results are presented as mean ± SE. Probability values lower than 0.05 were considered significant.

Results

Changes in brain bioelectrical activity over time

A significant reduction in the slow EEG delta activity frequency band was observed in postacute TBI patients after a 1-year follow-up period ($P < 0.05$); whereas changes in theta, alpha and beta frequencies were not statistically significant (Table 1). Significant decreases in delta ($P < 0.01$) and theta frequencies ($P < 0.05$) and significant increases in alpha ($P < 0.05$) and beta frequency bands ($P < 0.05$) at 1-year follow-up were observed, however, when patients with a TBI progress time lower than 2 years (TBI-2) at baseline were analysed separately (Table 1; Fig. 1).

After a 1-year follow-up period, a significant reduction of PR scores with respect to baseline (1.51 ± 0.24 vs. 1.90 ± 0.30; $P < 0.05$) was found in TBI patients. This reduction represents a 20% decrease in PR values over 1 year. Changes in PR scores correlated significantly with the time (months) elapsed since brain trauma occurred ($r = 0.586$; $P < 0.01$), being decreases of PR values after 1 year more pronounced in patients with a shorter TBI progress time at baseline (Fig. 2). Partial correlation analysis demonstrated a significant relationship between changes in PR scores and TBI progress time ($r = 0.565$; $P < 0.02$) after controlling for the influence of TBI severity. These results are indicating that the time of disease progress at baseline influences the degree of PR reduction over time in postacute TBI patients, apparently with independence of the disease severity.

Cere-induced changes in brain bioelectrical activity

In comparison with baseline EEG recordings, postacute TBI patients showed a significant decrease in the average percentage of slow brain bioelectrical activity frequencies delta (23.5 ± 1.7% vs. 20.4 ± 1.6%, $P < 0.05$) and theta (26.2 ± 1.5% vs. 23.3 ± 1.2%, $P < 0.01$), and significantly enhanced relative alpha (22.5 ± 1.9% vs. 25.1 ± 2.0%, $P < 0.05$) and beta activity power scores (10.0 ± 0.6% vs. 11.9 ± 0.6%, $P < 0.01$) after the administration of Cere. As a consequence, Cere induced a significant reduction of PR scores with respect to baseline values

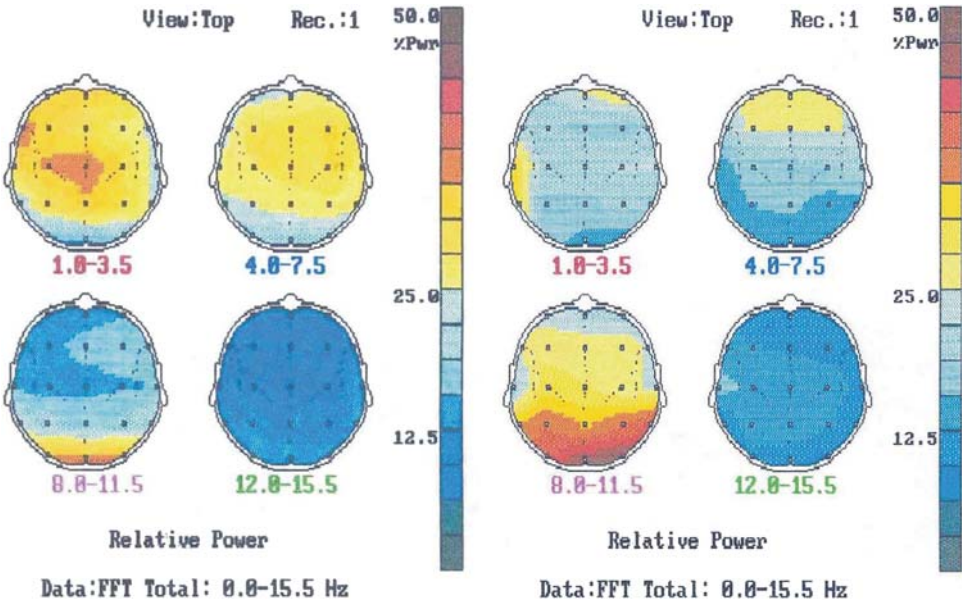

Fig. 1 Topographic brain maps obtained from a TBI patient at baseline (*left*) and after a 1-year follow-up period (*right*). A decrease in slow (delta and theta) activity and an increase in fast (alpha and beta) frequencies can be observed

(1.37 ± 0.12 vs. 1.81 ± 0.17, $P < 0.001$) in postacute TBI patients. Changes in PR scores induced by Cere didn't correlate neither with TBI severity (GOS scores) at baseline ($r = -0.071$, $P =$ ns) nor with the time (months) of TBI progress at baseline ($r = 0.119$, $P =$ ns; Fig. 2).

Neither severe adverse events nor significant changes in lab or ECG parameters were observed in TBI patients after treatment with Cere.

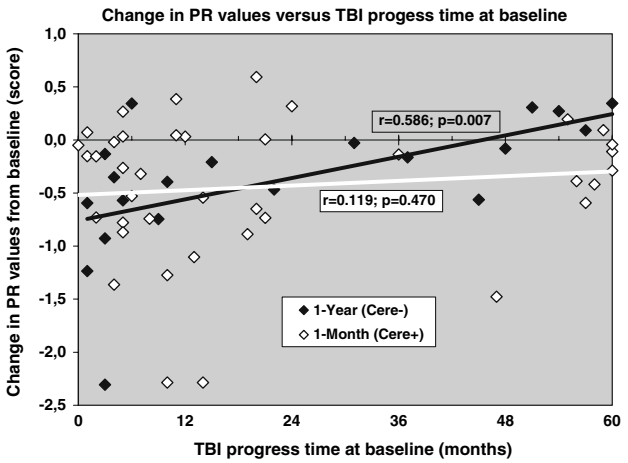

Fig. 2 Changes in EEG power ratio (PR) scores from baseline according to the TBI progress time at baseline after a 1-year follow-up period without Cere treatment (Cere−: *black rhombus*) and after a 1-month treatment with Cere (Cere+: *white rhombus*) in moderate-severe postacute TBI patients. Pearson's correlation lines are presented for each subgroup (Cere−: *black line*; Cere+: *white line*). Note that changes in PR scores were measured after 1-year and 1-month periods, respectively, for Cere− and Cere+ subgroups

Multipoint qEEG evaluation

In patients with a multipoint qEEG evaluation, it was found that PR scores were almost similar 2 months before the study (prestudy 1.73 ± 0.30) and at baseline (1.67 ± 0.24), decreased significantly after the administration of Cere (posttreatment 1.25 ± 0.16, $P < 0.05$ vs. baseline), and remained still reduced 3 months after stopping Cere infusions, but significantly less than at the posttreatment time point (poststudy 1.38 ± 0.20, $P < 0.05$ vs. baseline and posttreatment; Fig. 3). These results indicate that PR values decrease by a 20% over a 6-month period (from pre- to poststudy evaluations) in Cere-treated patients.

Cognitive performance

When the 19 cases undergoing cognitive evaluation (Table 2) were analysed together, it has been found that baseline PR scores correlated positively with SKT total score ($r = 0.549$; $P < 0.05$) and with raw scores of the SKT items arranging blocks ($r = 0.743$; $P < 0.01$) and reversal naming ($r = 0.660$; $P < 0.01$).

A significant ($P < 0.05$) reduction in the time needed to complete the SKT tasks replacing blocks and reversal naming was observed in TBI patients after a 1-year follow-up period without treatment (Table 2). No other significant changes in cognitive performance were found over time.

Patients treated with Cere showed significant improvements in total SKT score and in direct scores of the SKT items consisting of naming objects, naming numerals, replacing blocks, counting symbols and reversal naming (Table 2). Changes induced by Cere in PR values correlated

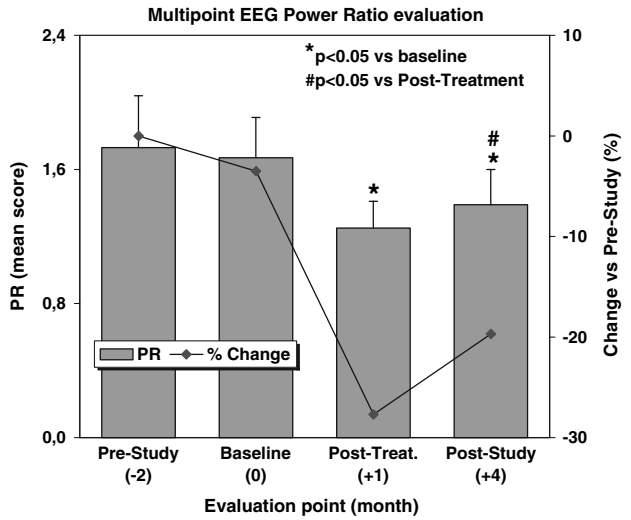

Fig. 3 Effects of the administration of Cere (30 ml/day, i.v.; 20 infusions over a 4-week period) on EEG power ratio (PR) scores in postacute TBI patients: comparison with recordings obtained 2 months before (prestudy) and 3 months after (poststudy) the active treatment period. Mean PR scores and the percent change from prestudy are represented. Results: mean ± SE

positively with those produced in raw scores of the SKT items arranging blocks ($r = 0.702$; $P < 0.05$), counting symbols ($r = 0.813$; $P < 0.01$) and reversal naming ($r = 0.708$; $P < 0.05$). According to these correlations, the acceleration of the EEG activity pattern (PR reduction) is accompanied by an improvement of the cognitive performance (reduction of SKT scores) after treatment.

Discussion

Results of the qEEG follow-up study show that patients with postacute TBI underwent changes in brain bioelectrical activity over a 1-year period consisting of a reduction in slow (delta and theta) and an increase in fast (alpha and beta) frequency bands' relative power (Table 1; Fig. 1). These variations, as reflected by the significant decrease in EEG power ratio scores (Fig. 2), involve an acceleration of the EEG recordings; which, in turn, appears to represent a less pathologic pattern of brain bioelectrical activity. In fact, PR values were approximately twice in severe than in moderate TBI cases (2.4 vs. 1.2) and correlated positively with TBI severity at baseline (negative correlation with GOS scores: $r = -0.474$; $P < 0.05$). The positive correlations between PR scores and cognitive impairment measures (SKT scores) observed at baseline, showing an association of cognitive deterioration with increased EEG slowing, are also suggesting that reductions in PR values might be related with a better brain functioning. Although EEG slowing is commonly recognized as a nonspecific finding associated with the severity of brain damage after TBI (Alvarez et al. 2003; Wallace et al. 2001), there are almost no previous studies assessing long-term qEEG changes in postacute TBI patients. This situation might be owing to the fact that it has been paid much more attention to the investigation of brain damage during the acute phase than during the postacute recovery period. Anyhow, the reduced levels of alpha and beta relative power observed in this study are consistent with the deficits in alpha desynchronization, peak alpha frequency, alpha variability and beta connectivity patterns reported after brain injury by other authors (Vespa et al. 2002; Thornton 2003; Angelakis et al. 2004; Roche et al. 2004).

According to results of the cognitive assessment shown in Table 2, TBI patients improved cognitive performance in attention- and memory-related tasks like replacing blocks and reversal naming, but not in specific memory items after a 1-year follow-up period. These findings seem

Table 2 Changes in cognitive performance after a 1-year period without treatment and after 1 month of treatment with Cere in TBI patients

Group	1-year (Cere−)		1-Month (Cere+)	
	Baseline	After 1 year	Baseline	After 1 month
Parameter				
N	7	7	12	12
SKT: total score	13.8 ± 2.3	12.7 ± 2.1	16.1 ± 1.8	12.8 ± 1.7**
SKT				
Naming objects	27.1 ± 9.1 s	22.8 ± 3.9 s	26.8 ± 4.9 s	18.3 ± 2.5** s
Immediate recall	6.5 ± 1.0 om	6.8 ± 0.5 om	6.8 ± 0.8 om	5.8 ± 0.5 om
Naming numerals	27.8 ± 14.3 s	21.2 ± 7.8 s	26.5 ± 8.3 s	17.3 ± 4.2* s
Arranging blocks	55.0 ± 10.7 s	47.7 ± 8.9 s	64.3 ± 13.6 s	44.6 ± 6.8 s
Replacing blocks	41.8 ± 11.8 s	32.7 ± 7.7* s	44.9 ± 7.9 s	35.6 ± 5.8* s
Counting symbols	52.0 ± 10.7 s	41.8 ± 8.5 s	64.0 ± 10.2 s	43.2 ± 5.6** s
Reversal naming	52.8 ± 11.0 s	34.8 ± 4.5* s	55.7 ± 11.4 s	38.9 ± 5.4* s
Delayed recall	8.2 ± 0.9 om	7.3 ± 1.2 om	7.9 ± 0.9 om	7.6 ± 0.8 om
Recognition memory	3.8 ± 1.0 om	4.2 ± 1.2 om	4.1 ± 0.8 om	3.4 ± 0.9 om

SKT Syndrome Kurztest; *s* seconds; *om* omissions. *$P < 0.05$ and **$P < 0.01$ versus baseline

to indicate a progressive recovery of attention and working memory, which might contribute to enhance execution and processing speed, the most affected functions in TBI patients (Axelrod et al. 2001; Kersel et al. 2001). Although the low number of patients evaluated does not allow us to get general conclusions, our results are in line with previous investigations on the evolution of cognitive deficits in postacute TBI patients (Kersel et al. 2001; Rapoport et al. 2002).

Changes in EEG recordings over time were evident in TBI patients with less than 2 years of disease progress at baseline (Table 1), but not in those with a longer duration of the disease. According to changes in PR scores over time (Fig. 2), it seems that EEG slowing decreases progressively at least during the first 3 years after brain trauma (less than 2 years at baseline), being variations in EEG power ratio less pronounced or even absent thereafter in clinically stable patients with moderate–severe TBI. Since inter- and intra-subject variability is very high for EGG recordings of particular frequency bands, the EEG power ratio constitutes a more reliable index to monitor global brain bioelectrical activity changes over time in medical conditions characterized by the presence of EEG slowing, like moderate–severe TBI. In addition, and taking into account that similar time-related changes in qEEG were observed for both moderate and severe TBI patients, it is suggested that variations in brain bioelectrical activity might reflect the repair-regeneration process of synaptic plasticity occurring postinjury (Ray et al. 2002) regardless of TBI severity. On other hand, since PR scores correlate negatively with cerebral blood flow and oxygen metabolism parameters (Coles et al. 2004; Nagata et al. 1989), the reduction of PR values over time might also be linked to a recovery of the brain metabolic functioning in TBI patients. Synaptic and metabolic changes might also account for the time-related improvement of cognitive performance observed in the same patients.

Considering that all the TBI patients were treated with nootropic compounds (citicoline and piracetam) capable of inducing an EEG-activating effect (Saletu et al. 1995; Cacabelos et al. 1996; Alvarez et al. 1999b) similar to the one reported here, we must be cautious with the interpretation of the present results and especially with their extrapolation to untreated patients. In any case, however, our results are indicating that EEG slowing tends to decrease and that cognitive performance might improve during the first 3 years after brain trauma, at least in patients receiving neuroprotective treatment. This finding is highly relevant because until very recently it was considered that the time period for a successful therapeutic intervention in TBI patients was limited to the first 6–12 months after brain injury. Therefore, according to the present EEG results it seems appropriate to maintain neuroprotection for at least 3 years after brain trauma in TBI patients.

The EEG results obtained in Cere-treated patients, even taking into account the limitations to interpret it in the absence of a parallel placebo group, indicate that the i.v. administration of Cere might increase fast (beta and alpha) frequencies and reduce relative activity power for slow waves (delta and theta) in postacute TBI patients. Our results are also suggesting that Cere accelerates the brain bioelectrical activity pattern as it is clearly inferred from the significant reductions observed in power ratio values, with apparent independence of TBI time course (Fig. 2) and severity at baseline (lack of correlation with GOS scores). According to data of the multipoint EEG evaluation showing that the reduction of PR scores is significantly more marked after Cere treatment than 3 months later, changes in brain bioelectrical activity observed in postacute TBI patients after the administration of Cere seem to be specific and not induced by the concomitant treatment with potentially EEG-activating compounds like citicoline and piracetam (Saletu et al. 1995; Cacabelos et al. 1996; Alvarez et al. 1999b) nor by the time-related EEG changes occurring in TBI. The present results are in agreement with those previously reported by us in a smaller sample of TBI patients (Alvarez et al. 2003), as well as with results of other studies demonstrating that Cere enhances alpha activity and reduces delta frequencies in elderly controls (Alvarez et al. 2000a) and reduces EEG power ratio under hypoxia conditions in young control subjects (Funke et al. 1998).

A significant improvement of cognition was observed in TBI patients after a 1-month treatment with Cere (Table 2). The positive effects of Cere on the time needed to complete the SKT tasks consisting of naming objects, naming numerals, replacing blocks, counting symbols and reversal naming are similar to those previously reported by us in a smaller sample of TBI patients (Alvarez et al. 2003). These cognition-enhancing effects of Cere are suggesting an improvement of attention and working memory might be related to the acceleration of the EGG pattern produced by the compound as indicated by the significant positive correlations between Cere-induced changes in cognitive and qEEG parameters reported here for the first time for TBI patients. According to these correlations, patients presenting the bigger reductions in qEEG slowing (PR scores) show higher improvements in cognitive performance after treatment with Cere. This finding is in line with data reported by other authors on the associations of cognitive impairment in TBI with trauma severity and the level of brain bioelectrical activity (Rapoport et al. 2002; Thatcher et al. 2001). The significant increase of beta activity observed after treatment is also consistent with a potential enhancement of arousal–attention mechanisms mediated

by Cere. The present results are in agreement with previous reports on the cognition-enhancing and EEG-activating effects of Cerebrolysin in brain trauma patients (Alvarez et al. 2003), in Alzheimer's disease patients (Alvarez et al. 2006; Bae et al. 2000; Panisset et al. 2002; Ruether et al. 2001, 2002), as well as in healthy young volunteers (Funke et al. 1998) and in elderly control subjects (Álvarez et al. 2000a). In any case, controlled clinical trials with larger samples of patients are needed to confirm the procognitive effects of Cere reported in this paper, and the exact relationship between cognitive and EEG changes produced by this compound in TBI patients.

Variations in qEEG activity and PR scores (Figs. 2, 3) observed after 1-month treatment with Cere were of similar or even higher magnitude than those obtained after a 1-year follow-up period in postacute TBI patients not treated with the compound (Fig. 2). As depicted in Fig. 3, at the end of the 1-month Cere-treatment period and 3 months later PR values were found to be decreased by approximately 28 and 20%, respectively, with respect to prestudy scores. Therefore, the degree of change in PR values obtained over a 6-month period in patients treated with Cere was similar to that observed after a 1-year follow-up without Cere treatment. This finding suggests that Cere might accelerate the time-related reduction of PR scores naturally occurring in postacute TBI patients, probably by improving the recovery of traumatic brain damage through neuroprotective and/or neurotrophic actions similar to those demonstrated in experimental conditions (Akay et al. 1992; Albretch et al. 1993; Satou et al. 1994; Alvarez et al. 1999a, 2000b; Rockenstein et al. 2002; Tatebayashi et al. 2003). In addition, since Cere upregulates the expression of the blood–brain barrier GLUT1 glucose transporter (Boado et al. 1999), it is also possible that an increase of the brain glucose supply mediated by Cere might contribute to improve brain metabolism and to reduce PR scores in TBI patients. Future studies must evaluate qEEG changes for up to 1 year after treatment with Cere administered as a single course of 20 infusions like in this study, as well as after repeated courses of Cere treatment.

The acceleration of the EEG activity pattern in postacute TBI patients might reflect a certain degree of functional recovery as indicated by the positive correlations of PR values with TBI severity (negative correlation with GOS scores: $r = -0.518$; $P < 0.01$) and cognitive impairment (positive correlations with SKT scores) found in this study, as well as by previous studies reporting that qEEG alterations and PR scores correlate with trauma severity, cognitive performance and outcome measures in TBI patients (Kane et al. 1998; Thatcher et al. 2001; Alvarez et al. 2003). Our clinical observations during the follow-up after treatment with Cere are also consistent with positive changes in executive functions, mental processing speed and interaction with the environment, especially in severe TBI and in minimally conscious state cases. Thus, the EEG-activating action of Cere together with its positive effects on cognition and clinical outcome are supporting the neuroprotective–neurotrophic activity of this compound in brain injury and its potential use for improving the functional recovery of TBI patients. However, further controlled clinical trials are needed to confirm these promising results in postacute TBI patients.

According to the results of present study with postacute moderate–severe TBI patients, it is concluded that EEG slowing seems to decrease during the first 3 years after brain injury regardless of the TBI severity; and that treatment with the neurotrophic compound Cere might induce an EEG-activating effect, independently of the TBI severity and progress time, leading to a faster reduction of the EEG slowing in these patients. Improvements of cognitive performance in attention- and memory-related tasks, which correlated positively with reductions of qEEG slowing, were also found over 1 year without treatment and after the administration of Cere for 1 month. Results of this exploratory study encourage the conduction of controlled clinical trials to evaluate the effects of Cere on functional recovery in patients with traumatic brain damage.

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