The usefulness of sLORETA in evaluating the effect of high-dose ARA-C on brain connectivity in patients with acute myeloid leukemia: an exploratory study

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Summary

Cytosine arabinoside (Ara-C) is one of the key drugs for treating acute myeloid leukemia (AML). High intravenous doses may produce a number of central nervous system (CNS) toxicities and contribute to modifications in brain functional connectivity. sLORETA is a software used for localizing brain electrical activity and functional connectivity. The aim of this study was to apply sLORETA in the evaluation of possible effects of Ara-C on brain connectivity in patients with AML without CNS involvement. We studied eight patients with AML; four were administered standard doses of Ara-C while the other four received high doses. sLORETA was computed from computerized EEG data before treatment and after six months of treatment. Three regions of interest, corresponding to specific combinations of Brodmann areas, were defined. In the patients receiving high-dose Ara-C, a statistically significant reduction in functional connectivity was observed in the fronto-parietal network, which literature data suggest is involved in attentional processes. Our data highlight the possibility of using novel techniques to study potential CNS toxicity of cancer therapy.

KEY WORDS: acute myeloid leukemia, brain dysfunction, cytosine arabinoside, functional connectivity, neurotoxicity, sLORETA.

Introduction

Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by clonal expansion of myeloid blasts in peripheral blood, bone marrow and/or other tissue. It is the most widespread form of acute leukemia in adults. Cytarabine, or cytosine arabinoside (Ara-C), is the single most effective treatment for AML. It has been estimated that symptomatic remission is achieved in around 75% of patients under the age of 60 years when Ara-C is combined with an anthracycline (Rubin et al., 1992). Thus, an anthracycline/cytarabine-based induction therapy is considered the standard of care for patients aged 18-60 years with a diagnosis of AML (Rowe, 2009). The prevalent therapeutic dose of Ara-C is 100 mg/m²/day administered intravenously although a high-dose regimen, corresponding to a dose ≥ 2 g/m²/day, has also been reported (Rowe, 2009). The literature reports that use of high-dose Ara-C, as a post-remission treatment, results in a survivorship comparable to that associated with allogeneic bone marrow transplantation (Mayer, 1988). In particular, in patients with AML, chemotherapy with Ara-C may prolong survival and potentially improve quality of life. However, high-dose Ara-C can cross the blood-brain barrier (BBB) and may induce therapy-related toxicities of the central nervous system (CNS); these may occur during administration of the high-dose treatment or within a few days after treatment (Peddi et al., 2014). Monitoring for possible neurotoxicity is therefore mandatory, especially with doses capable of crossing the BBB. Indeed, CNS toxicities can cause significant morbidity and result in discontinuation or interruption of potentially effective therapies. The most frequent and widespread symptom is acute or subacute symmetric pancebellar syndrome; in addition, many patients report signs of concomitant brain dysfunction, such as disorientation, cognitive dysfunction, somnolence, memory loss, confusion, psychosis and, rarely, seizures, coma or death, with an incidence of 3-26% (Lazarus et al., 1981; Baker et al., 1991). Although the outcome of this cerebellar syndrome is variable, these neurologic dysfunctions have been reported to resolve within five days of discontinuing the drug (Baker et al., 1991). In the case of Ara-C treatment, electroencephalography (EEG) has often demonstrated...
diffuse, slow wave activity, a finding compatible with the concurrent encephalopathy. Moreover, computed tomography (CT) scans or magnetic resonance imaging (MRI) can detect cerebellar atrophy for several months after the acute phase of cerebellar toxicity (Baker et al., 1991).

With regard to the impact of chemotherapy and possible related brain changes, studies have also begun to examine whole brain functional connectivity (Bruno et al., 2012; Hosseini et al., 2012), which is considered an index of functional integration among neuronal populations (Fingelkurts et al., 2005). Functional connectivity is examined to gain information on the temporal correlation between spatially remote brain regions in order to clarify the way in which brain networks support specific cognitive functions (Dumas et al., 2013). In fact, changes in cognitive functions without clinically evident symptoms represent one type of chemotherapy-induced adverse effect (Koppelmanns et al., 2013).

Moreover, the presence of drug-related cognitive impairment is not always evident to the patient or the physician, and its actual degree does not necessarily match the patient’s subjective complaints (Clemens et al., 2006; Cho et al., 2012). Clinicians therefore need to recognize the effects of cancer therapy on the CNS (Rinne et al., 2012) and to detect potential CNS toxicities before the onset of clinical symptoms. From this perspective, the availability of tools for the early identification of treatment-related CNS toxicities could represent a fundamental clinical advance.

The literature contains no reports on methods for the evaluation of brain/cognitive dysfunctions in AML patients. Indeed, the majority of studies related to structural and functional brain changes in patients receiving chemotherapy have concerned women with breast cancer (Janelinsins et al., 2011; Bruno et al., 2012; Hutchinson et al., 2012). Imaging studies have shown chemotherapy to be related to worse cognitive performance in breast cancer patients, an effect lasting up to several years after treatment (Meyer, 2008; de Ruter et al., 2011; Bruno et al., 2012). Accordingly, the presence of cognitive deficits possibly related to a change in brain functioning has been reported in chemotherapy patients (Dumas et al., 2013; Bruno et al., 2012). A meta-analysis performed by Hodgson et al. (2012) highlights the importance of addressing this potential neurotoxicity in patients with cancer in sites other than the breast, in particular in cancer types such as leukemia and lymphoma.

To date, no standardized approach has been developed for assessing the possible effects, on functional connectivity, of systemic chemotherapy regimens using drugs able to cross the BBB, such as high-dose Ara-C treatment in AML patients (Park et al., 2013). Functional MRI and PET have been the most widely used methods for examining functional connectivity (Canuet et al., 2012), and although both are associated with high spatial resolution, they may not appropriately distinguish between functional excitation and inhibition of neuronal activity (Pascual-Marqui et al., 1999).

Otherwise, EEG has been adopted to visualize synchronization across frequency bands in large-scale functional networks. In particular, quantitative analysis of background EEG (qEEG) frequencies may constitute another simple and objective method of evaluating the effect of chemotherapy on the CNS and its possible impact on cognitive function, since background EEG activity represents the functional state of the brain (Clemens et al., 2006; Cho et al., 2012). Despite these benefits, qEEG is also associated with “the problem of volume conduction and active reference electrodes in the assessment of functional connectivity”, which can result in spurious correlations between time series recorded from neighboring electrodes (Stam et al., 2007). With regard to Ara-C, the effect of this drug on EEG background activity was recently investigated by Maschio et al. (2016) using qEEG.

The past decade has seen the development of more suitable methods for localizing electrical activity and exploring functional brain connectivity, such as “standardized LORETA” (sLORETA), a low-resolution EEG tomography method for evaluating brain electromagnetic activity (Pascual-Marqui et al., 1994). With respect to qEEG, this novel measure of connectivity is resistant to non-physiological artifacts, specifically ones linked to volume conduction and low spatial resolution of input data.

To date sLORETA has been used to study changes in functional connectivity in patients with Alzheimer’s disease and schizophrenia (Pascual-Marqui et al., 1999; Canuet et al., 2012). Assuming that all chemotherapeutic drugs used for non-CNS cancer can induce functional connectivity changes when they cross the BBB, sLORETA may be used to evaluate these changes.

In the present study, we used sLORETA in patients with AML to evaluate the possible effects of Ara-C treatment on functional connectivity after six months of chemotherapy.

Materials and methods

Patients

We studied eight patients with newly diagnosed AML (3 males and 5 females with a median age of 56 years, standard deviation 10 years, range 42-74 years).

All patients were studied at diagnosis with CNS imaging and diagnostic lumbar puncture: in no case was the leukemia found to involve the CNS. Exclusion criteria were preexisting organic or psychiatric disorders or previous treatments with drugs potentially interfering with the CNS (other than Ara-C). Four of the patients were treated with induction chemotherapy containing a standard dose of Ara-C (100 mg/m²/daily in continuous infusion for 10 days), whereas the other four patients received a high-dose treatment (2 g/m²/daily for 5 consecutive days). Two patients were found to meet the exclusion criteria and were not eligible for the study. All patients underwent an EEG evaluation before and after six months of treatment with i.v. Ara-C.

This study was approved by our institute’s ethics committee.

EEG recording

For electroencephalographic evaluation, we used a 25-channel MICROMED BQ2400 Studio ACQDV EEG ma-
chime (MICROMED srl, Mogliano Veneto, Treviso). Nineteen scalp electrodes were placed according to the 10-20 International System. Electrode impedance was maintained below 20 KOhm. Filters were set at 1.6 and 70 Hz, and the signal was notch filtered. All EEG recordings were acquired with a 256-bit sampling rate.

The off-line spectral analysis was performed using the fast Fourier transform technique on 5-10 min of EEG signal, manually segmented into 2-second epochs, after visual elimination of ictal and/or interictal abnormalities, movement artifacts, eye blinking, muscle activity or drowsiness. These epochs were collected for each frequency band: delta [1-3.5] Hz, theta [4-7] Hz, alpha [8-12.5] Hz and beta [13-30] Hz.

The EEG recordings were exported into American Standard Code for Information Interchange (ASCII) files and imported into the sLORETA software.

### Analysis of resting-state EEG functional connectivity

The connectivity analysis was based on the lagged correlation between two or more Brodmann areas (BAs), based on the Talairach brain atlas, across successive 2-s epochs over the investigated sample. For each patient a dataset of 20 epochs was collected for each examination. In particular, the connectivity analysis was performed by computation of lagged phase synchronization (Imperatori et al., 2014). Lagged phase synchronization measures the similarity (a corrected phase synchrony value) between signals in the frequency domain based on normalized (unit module) Fourier transforms (Canuet et al., 2012).

This measure represents the connectivity between two signals after the artifactual component has been excluded. The sLORETA software computes lagged phase synchronization $\rho^2_{xy}(\theta)$ by means of the formula (Pascual-Marqui et al., 2011):

$$\rho^2_{xy}(\theta) = \frac{\text{Im}[\text{Re}(x(\theta))]}{1 - \text{Re}[\text{Re}(x(\theta))]^2}$$

where $\theta$ is the discrete frequency considered, $x$ and $y$ are the EEG sources; $\text{Re}$ and $\text{Im}$ denote the real and the imaginary part of a complex function, respectively; and $x(\theta)$ and $y(\theta)$ indicate the discrete Fourier transforms of the two signals of interest $x$ and $y$ at frequency $\theta$, respectively. The general lagged phase synchronization is defined as the partial coherence between the normalized complex-valued stochastic variables $x(\theta)$ and $y(\theta)$ with the zero-lag effect removed (Pascual-Marqui et al., 2011).

More details on the sLORETA connectivity algorithm are reported in Pascual-Marqui’s studies (Pascual-Marqui et al., 2011).

### Regions of interest

Correlating the activity of LORTEA-localized regions of interest (ROIs) is a useful strategy for correlating quantitative EEG variables measured at scalp electrodes and it offers a deeper understanding of intra-hemispheric cortico-cortical connectivity.

Three ROIs corresponding to specific combinations of BAs were defined in order to evaluate connectivity modifications, if any. In particular, the BAs included in the ROIs were the posterior cingulate cortex (BA 31), the precuneus (BA 7), the inferior parietal cortices, mainly the angular gyrus (BA 39), and the medial prefrontal cortex (BAs 10 and 11) (Raichle et al., 2001). The cortical ROIs investigated by sLORETA are reported in Table I.

In this study, the ‘single voxel’ option was used, i.e. each ROI consisted of a single voxel located at the BA centroid. The sLORETA software computed the lagged phase synchronization values between all these ROIs (total 19×19 = 361 electrodes and 3 ROIs x 3 ROIs = 9 connections).

### Statistical analysis

EEG connectivity data were compared between AML patients before and after treatment and according to dose (standard or high) for each frequency band. Paired t-tests between pre- and post-treatment for the two groups of patients were performed for each frequency band to assess the connectivity of the areas involved. P-values were corrected for multiple comparison tests: the non-parametric randomization procedure available in the sLORETA program package was applied for the correction (Nichols and Holmes, 2002).

T-thresholds, corresponding to statistically significant thresholds ($p < 0.05$ and $p < 0.01$), were calculated using the statistical tool provided by the sLORETA software (Friston et al., 1991).

### Results

The oncological disease and neurological status remained stable in all the enrolled patients over the investigation period.

The EEG intervals selected for the analysis were suitable for all the patients. Visual evaluation of the EEG recordings selected for the analysis showed no relevant artifacts of the background rhythm frequency, focal abnormalities or epileptic discharges. No subject showed evidence of drowsiness or sleep during the recordings. For each couple of ROIs and for each frequency band, an sLORETA connectivity analysis was performed. In the first group of patients (standard dose), no statistically significant differences between baseline (before

### Table I - Cortical ROIs determined by sLoreta with t-thresholds.

<table>
<thead>
<tr>
<th>ROI</th>
<th>BA</th>
<th>t (p&lt;0.01)</th>
<th>t (p&lt;0.05)</th>
<th>t</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROI1</td>
<td>7, 31 vs 10, 11</td>
<td>3.15</td>
<td>2.63</td>
<td>2.91</td>
<td>Yes</td>
</tr>
<tr>
<td>ROI2</td>
<td>10, 11 vs 39</td>
<td>3.18</td>
<td>2.58</td>
<td>2.92</td>
<td>Yes</td>
</tr>
<tr>
<td>ROI3</td>
<td>7, 31 vs 39</td>
<td>3.27</td>
<td>2.63</td>
<td>2.61</td>
<td>No</td>
</tr>
</tbody>
</table>
chemotherapy) and post-treatment (after 6 months of chemotherapy) values were found in any frequency band across all ROIs. The connectivity analysis was also performed for the second group of patients (high dose). In the analysis of BAs 7 and 31 combined with BAs 10 and 11 (ROI1), the thresholds for significance were $T = 2.63$, corresponding to $p < 0.05$, and $T = 3.15$, corresponding to $p < 0.01$. In the analysis of BAs 10 and 11 combined with BA 39 (ROI2), the thresholds for significance were $T = 2.58$, corresponding to $p < 0.05$, and $T = 3.18$: in the analysis of BAs 7 and 31 combined with BA 39 (ROI3) the thresholds for significance were $T=2.63$ and $T=3.27$, corresponding to $p>0.05$ and $p>0.01$, respectively.

In all patients treated with high-dose Ara-C a significant modification was observed in the beta2 band after 6 months of treatment (20.5-30 Hz) (Figures 1, 2). This modification consisted of a decrease in linear lagged phase synchronization between the cortical areas identified by ROI1 (Figure 1). Similarly, a decrease in non-linear lagged phase synchronization was obtained between the cortical areas identified by ROI2 (Figure 2). No significant differences were observed in the other frequency bands either for ROI1 or for ROI2. Similarly, in the analysis of BAs 7 and 31 combined with BA 39 (ROI3), $t$-tests revealed no significant differences for any frequency band. Table I summarizes the results for each ROI.

Discussion

Our findings revealed a statistically significant decrease in connectivity in patients treated with high dose of Ara-C after six months of therapy. Reduction in connectivity was found both for BAs 10 and 11 (medial prefrontal cortex) combined with BA 7 (precuneus) and BA 31 (posterior cingulate cortex) and for BA 39 (inferior parietal cortices) combined with BAs 10 and 11. No modification of connectivity was found for BA 39 combined with BAs 7 and 31.

In the group of patients treated with a high-dose of Ara-C, we found a reduced signal intensity representing the connection between the ROIs and a statistically significant reduction of the lagged connectivity index in the beta2 band for all the investigated ROIs. Conversely, no modifications were observed in the patients treated with a standard dose of Ara-C.

Thus, our findings show that in patients with AML and neurological stability, the high dose of Ara-C (since it crosses the BBB) can decrease EEG functional connectivity, especially in the fronto-parietal network.

Experimental studies in humans and monkeys have shown that the fronto-parietal network is involved in attentional processes (Buschman and Miller, 2007; Ptak, 2012; Miller and Buschman, 2013; Vossel et al., 2014); activity in these brain regions has been found to be correlated with synchronized oscillations at beta band frequencies (Haegens et al., 2012; Miller and Buschman, 2013; Kilavik et al., 2013). Modulations of beta band activity have frequently been described in the context of the preparation and execution of motor responses (Neuper et al., 2006), as well as during somatosensory processing (Kilavik et al., 2013). Recently, beta band activity modulations have also been suggested to play a role in higher cognitive processing (Engel and Fries, 2010; Donner and Siegel, 2011).

Figure 1 - Graphic representation of 3D brain scalp (left panel) and scalp related color scale with setting parameters (right panel) on sLORETA software. The red line represents the statistically significant decrease in connectivity observed between BAs 7 and 31 and BAs 10 and 11 ($\beta_2$ band). Additional information on statistical results and $T$-thresholds is given in Table 1.

Figure 2 - Graphic representation of 3D brain scalp (left panel) and scalp related color scale with setting parameters (right panel) on sLORETA software. The red line represents the statistically significant decrease in connectivity between BA 39 and BAs 10 and 11 ($\beta_2$ band). Additional information on statistical results results and T-thresholds is given in Table 1.
No definitive conclusions can be drawn from our study because of the small sample size and the short follow-up. Nevertheless, sLORETA is a novel approach for the evaluation of chemotherapy-related effects on functional connectivity and electrical brain activity, which represents a step forward with respect to other techniques described in the literature. Indeed, in Maschio et al. (2016), qEEG was used for evaluating Ara-C-induced toxicity, but this approach failed to reveal changes in EEG background activity caused by high-dose Ara-C administration. Thus, sLORETA analysis, providing an accurate measure of electrophysiological connectivity, seems to be more effective in detecting changes in functional connectivity and electrical brain activity. In fact, sLORETA decomposes connectivity into instantaneous and lagged components (Canuet et al., 2012; Pascual-Marqui et al., 2011). Lagged phase synchronization represents the connectivity between two signals after the artificial instantaneous zero-lag contribution has been excluded. This new connectivity measure is resistant to non-physiological artifacts, in particular ones linked to low spatial resolution of input data and volume conduction (Stam et al., 2007), therefore it allows evaluation of ‘true’ connectivity (Canuet et al., 2012).

Using sLORETA, the high temporal resolution of brain electrical data can be fully exploited for functional imaging of brain activities of different qualities, since brain electrical activity can be separately inspected for the different EEG frequency ranges, each of which has specific functional significance (Pascual-Marqui et al., 2011). Thus, our results demonstrate the feasibility of using sLORETA for early evaluation of subclinical effects of drugs that cross the BBB.

Nevertheless, further studies on larger cohorts and longer follow-ups will be needed to extend these findings and provide definitive conclusions on modifications of functional connections and on potential drug-induced cognitive impairments.

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References
