Investigación clínica

5-aminolevulinic acid and neuronavigation in high-grade glioma surgery: results of a combined approach

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ABSTRACT

In high-grade glioma surgery, several techniques are used to achieve the maximum cytoreductive treatment preserving neurological functions. However, the effectiveness of all the methods used alone is reduced by specific limitations of each. We assessed the reliability of a multimodal strategy based on 5-aminolevulinic acid (5-ALA) and neuronavigation. We prospectively studied 18 patients with suspected, non eloquent-area malignant gliomas amenable for complete resection. Conventional illumination was used until the excision appeared complete. The cavity was then systematically inspected in violet-blue light to identify any residual tumour. Multiple biopsies of both fluorescent and non-fluorescent tissue were performed in all cases. Each specimen was labelled according to the sampling location (inside or outside the boundary set by the neuronavigator). The samples were analysed by a neuropathologist blinded to the intraoperative classification. We reviewed the results of both methods, either singly or in combination. Individual analysis showed higher 5-ALA reliability compared to neuronavigation. However, several false-negative fluorescent specimens were detected. With the combined use of fluorescence and neuroimaging, only 1 sample (negative for both 5-ALA and navigation) was tumoral tissue. In our experience, the combined approach showed the best sensitivity and it is recommended in cases of lesions involving non-eloquent areas.

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Palabras clave:
Resección total
Cirugía de gliomas de alto grado

Ácido 5-aminolevulínico y neuronavegación en la cirugía de los gliomas de alto grado: resultados de la técnica combinada

RESUMEN

En la cirugía de gliomas de alto grado se utilizan diversas técnicas para lograr el máximo tratamiento citoreductivo y la conservación de las funciones neurológicas. Sin embargo, la efectividad de todos los métodos utilizados en solitario queda reducida por las limitaciones específicas de cada uno de estos métodos. Evaluamos la fiabilidad de una estrategia...
Neuronavigation
Ácido 5-aminolevulínico

multimodal basada en el ácido 5-aminolevulínico (5-ALA) y la neuronavegación. Estudiábamos de modo prospectivo a 18 pacientes con una zona sospechosa y no elocuente de gliomas malignos, susceptibles de resección completa. Se utilizó iluminación convencional hasta que la extracción nos pareció completa. Se inspeccionó entonces sistemáticamente la cavidad con luz azul violeta para identificar cualquier tumor residual. En todos los casos se realizaron biopsias múltiples tanto del tejido fluorescente como del no fluorescente. Se etiquetó cada muestra conforme al emplazamiento de cada una de ellas (interior o exterior a los límites del neuronavegador). Las muestras fueron analizadas por un neuropatólogo, quien se atuvo a la clasificación intraoperatoria. Revisamos los resultados de ambos métodos, tanto de manera individual como combinada. El análisis individual mostró una mayor fiabilidad del 5-ALA en comparación con la neuronavegación. Sin embargo, se detectaron diversas muestras fluorescentes falso-negativas. Con el uso combinado de la fluorescencia y la neuroimagen, únicamente 1 muestra (negativa para el 5-ALA y la navegación) constituía tejido tumoral. En nuestra experiencia, la técnica combinada mostró una mejor sensibilidad, recomendándose para los casos de lesiones que impliquen zonas no elocuentes.

Introduction

Gross total resection (GTR) in high grade glioma surgery acts as an independent prognostic factor on survival. Lacroix showed a significant median survival advantage from 8.8 to 13 months associated with resection of 98% or more of the tumor volume. The intratumoral heterogeneity and the migratory behaviour of glioma cells along axonal and basal membrane-like structures make GTR very challenging.

Several methods have been introduced to help achieve the maximum cytoreductive treatment, such as intraoperative MRI, neuronavigation, and ultrasonography. Recently, 5-aminolevulinic acid (5-ALA) has been shown to help visualize tumor tissue intraoperatively. A randomized controlled phase III trial showed a clinical benefit, in terms of completeness of tumor removal and progression-free survival by use of 5-ALA-induced fluorescence guidance. Surgical radicality was achieved in 65% of treated cases instead of 36% of controls.

Surgical excision based solely on fluorescence, however, has several limitations. The presence of false positive fluorescent samples, particularly in eloquent cortical areas, could decrease the effectiveness of 5-ALA. The specificity is reduced especially in cases of recurrent gliomas. Therefore, the site and the recurrence of a lesion influence both the surgical strategy and the techniques used to maximize the resection respecting neurological functions.

Feigl has recently proposed a new multimodal approach combining 5-ALA and intraoperative monitoring for resections of gliomas in eloquent areas. Gross total resection was achieved in many cases with preservation of functional areas.

We report the experience of high grade gliomas in non eloquent areas resected using a multimodal strategy based on fluorescence and neuronavigation. The accuracy of both techniques in detecting pathological tissue was evaluated. Advantages, limitations and possible indications to use a combined approach are discussed.

Materials and methods

Protocol of study

We designed a prospective cohort study of 18 patients consecutively operated on in our Neurosurgical Division in the period November 2008-October 2009. The project received approval from the ethics committee and all patients signed the informed consent form.

Patients

Eighteen individuals aged 43-76 years with suspected, newly diagnosed, untreated malignant glioma and who were amenable to complete resection of contrast-enhancing tumor. Exclusion criteria were: more than one contrast-enhancing lesion suggestive for widespread disease; tumors of the midline, basal ganglia, cerebellum, brain stem or involving eloquent areas; substantial, non-contrast-enhancing tumor areas suggesting a low-grade glioma with malignant transformation; and patients with a Karnofsky Performance Scale score of less than 70%. Informed consent was obtained from all patients.

Imaging protocol

Our preoperative workup consisted of thin-layer, contrast-enhanced Computed Tomography (CT) images, T1-weighted with and without contrast enhancement sequences, T2-weighted sequences, diffusion-weighted images, and fluid-attenuated inversion recovery sequences Magnetic Resonance Imaging (MRI). Imaging data were merged and loaded into a neuronavigational system, and used intraoperatively.

Treatment

Patients orally received 5-ALA (20 mg/kg body-weight), diluted in 50 mL of drinking water 3 hours (range 2-4)
before induction of anaesthesia. Pretreatment with 4 mg dexamethasone three times a day was mandatory for at least 2 days before operation. Surgery was performed by use of a modified neurosurgical microscope (OPMI Pentero, Carl Zeiss), equipped with a fluorescence kit. A single surgeon, trained in the use of 5-ALA, was designated for all the operations. Microsurgical removal was started using a conventional illumination. At the end of resection, the surgeon switched the white xenon light to the violet-blue excitation light. The cavity was systematically inspected in the violet-blue light and with the neuronavigation system to identify any residual tumor. All fluorescent tissue areas were compared with the neuronavigation data.

**Multiple sampling procedure**

Multiple biopsies were sampled. Specimens were labeled according to the 5-ALA fluorescence status, positive or negative, and the neuronavigation data, inside or outside the limits tracked.

For each patient, we biopsied a sample of tissue from each of the following areas (Table 1):

A. Fluorescent specimen inside the limits marked by the neuronavigator.
B. Fluorescent specimen outside the limits marked by the neuronavigator;
C. Non-fluorescent specimen inside the limits navigated.
D. Non-fluorescent specimen outside the limits navigated.

**Neuropathology**

Histopathological tumor diagnosis was established according to the World Health Organization 2007 diagnostic consensus criteria. Tumor cell proliferation was assessed immunohistochemically with the MIB-1 antibody (anti-Ki-67). The designated neuropathologist was blinded to the intraoperative 5-ALA fluorescence status and the neuronavigation data.

**Data analysis**

The pathologist defined as positive all samples consistent with the following histological criteria: presence of anaplastic foci and diffused atypies, increased mitotic activity (Ki-67>5%), microvascular proliferation and pallissading necrosis. Non-pathological specimens were defined as negative. Sensitivity and specificity of both 5-ALA and neuronavigation were estimated to assess the accuracy of the techniques in the detection of tumor tissue. Finally, we evaluated the accuracy of the combined use of neuroimaging and fluorescence imaging. Therefore, we compared the samples positive for at least one technique (non-fluorescent samples inside the navigated limits and fluorescent specimens within or outside the boundaries set by the neuronavigation.) with the specimens negative for both methods.

**Results**

It was possible to perform a conventional surgical excision in all cases. Fluorescent tumor tissues were well visualized intraoperatively. In all patients, fluorescent areas were observed both inside (Fig. 1) and outside (Fig. 2) the limits of the lesions showed by the navigator. Moreover, 5-ALA negative areas were observed within the limits of the neuronavigation data (Fig. 3).

No newly diagnosed permanent deficit was observed. The 5-ALA was well tolerated. No adverse effects were observed. There were no postoperative complications.

All patients harbored glioblastomas multiforme (GBM). In the group A all samples resulted pathological. In the group B, 14/18 (77,8%) samples proved to be tumoral tissue. Nonspecific reactive gliosis was observed in 4 samples (22,2%). In the group C, 16/18 (88,9%) samples proved to be normal white matter and 2/18 specimens (11,1%) resulted pathological. In the last group, only 1 sample showed pathological features (Table 2).

Fluorescence-guided resection showed a sensitivity of 91,4% and a specificity of 89,2% (Table 3). Four false positive cases were detected. The histopathological analysis showed an increased reactive mitotic activity associated with the peri-tumoral inflammation. On the other hand, we observed a low cellularity in the false negative samples.

The use of an image-guided system alone showed a high rate of both false negative and positive (Table 4). Sensitivity and specificity were less than 60% (57,1% and 56,8%, respectively).

The sensitivity of the combined methods was 97,2% (Table 5). On the other hand, the specificity is greatly reduced (45,9%).
Discussion

Many authors reported the impact of neuronavigation or 5-ALA on the cytoreductive treatment of solitary contrast-enhancing malignant gliomas\(^2\)\(^,\)\(^6\)\(^,\)\(^7\). Several reports showed a significant impact of neuronavigation to achieve GTR and to improve survival time\(^6\)\(^,\)\(^7\). Nevertheless, the studies bear the disadvantages of retrospective analyses and limited cohorts. A subsequent prospective randomized study showed that the extent of resection and consequent prolongation of patient survival is not enhanced by the use of a frameless stereotactic system\(^8\). The effectiveness of neuronavigation is biased by the brain shift phenomenon that occurs after CSF aspiration, brain retraction, and tumor removal. Therefore, the extent of a lesion can be underestimated due to the shift and GTR could be even more difficult to achieve\(^9\).

Intraoperative MRI and ultrasonography could be used to update the navigation system during surgery\(^10\). Nevertheless, several limitations should be considered. MRI is costly and not widely available\(^11\). Ultrasonography is highly accurate before excision, but shows an overestimation of tumor tissue during resection. Moreover, tumor remnants in surgical cavity may be undetected\(^12\). Therefore, alternative techniques for intraoperative visualization of residual tumor tissue have been sought.

5-ALA-induced fluorescence showed a high sensitivity for the assessment of malignant glioma. Nonetheless, its application could be hampered by low specificity due to high false-positive rate. Inflammatory cells and reactive astrocytes may in fact appear fluorescent, especially in recurrent glioma\(^3\). It is also still debated whether the vague positive 5-ALA fluorescence can be evaluated as tumor tissue or not. Heterogeneity of gliomas, invasion beyond the resection cavity and intercell heterogeneity of porphyrin IX fluorescence can significantly change the intensity of fluorescence and thus reduce the sensitivity of 5-ALA\(^13\).
Table 2 – Correlation between the fluorescence of the samples (F), neuronavigation (N) and histopathology

<table>
<thead>
<tr>
<th></th>
<th>Histolopathology (+)</th>
<th>Histolopathology (−)</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Group A (F+ N+)</td>
<td>18 (100%)</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Group B (F+ N−)</td>
<td>14 (77.8%)</td>
<td>4 (22.2%)</td>
<td>18</td>
</tr>
<tr>
<td>Group C (F− N+)</td>
<td>2 (11,1%)</td>
<td>16 (88,9%)</td>
<td>18</td>
</tr>
<tr>
<td>Group D (F− N−)</td>
<td>1 (5,6%)</td>
<td>17 (94,4%)</td>
<td>18</td>
</tr>
</tbody>
</table>

+: tumoral tissue; −: non pathological tissue.

Table 3 – Histopathology of 5-ALA positive and negative specimens

<table>
<thead>
<tr>
<th></th>
<th>Histolopathology (+)</th>
<th>Histolopathology (−)</th>
<th>Total</th>
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<tbody>
<tr>
<td>F+</td>
<td>32</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>F−</td>
<td>3</td>
<td>33</td>
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<tr>
<td>Total</td>
<td>35</td>
<td>37</td>
<td>72</td>
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</table>

Table 4 – Histopathology of the specimens within and outside the limits marked by the neuronavigation

<table>
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<tr>
<th></th>
<th>Histolopathology (+)</th>
<th>Histolopathology (−)</th>
<th>Total</th>
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<tbody>
<tr>
<td>N+</td>
<td>20</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td>N−</td>
<td>15</td>
<td>21</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>37</td>
<td>72</td>
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</table>

Table 5 – Histopathology of the specimens collected according to the combined approach (5-ALA and neuronavigation)

<table>
<thead>
<tr>
<th></th>
<th>Histolopathology (+)</th>
<th>Histolopathology (−)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>F+ N+/F− N−</td>
<td>34</td>
<td>20</td>
<td>54</td>
</tr>
<tr>
<td>F− N−</td>
<td>1</td>
<td>17</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>37</td>
<td>72</td>
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In our study neuronavigation was used in all fluorescence-guided high grade glioma resections. We hypothesized that a combined approach may correct the reduced reliability of both techniques when used separately.

In all patients, surgical excision was performed initially with a conventional illumination. When a total resection seemed to be achieved, the surgeon switched to the violet-blue light. Additional tissue samples were collected according to the protocol of the study (Table 1).

Sensitivity and specificity of both techniques were analyzed individually. Fluorescence-guided resection showed an accuracy consistent with the literature\(^\text{13}\). Hefti observed vague fluorescence and reduced sensitivity in heterogeneous tumors with lower grade elements and satellite lesions\(^\text{13}\). In our series, we observed 3 false negative samples with a low cellularity. We suppose that a low number of cells could not be able to generate a detectable signal. On the other hand, peri-tumoral inflammatory state and increased reactive mitotic activity, as suggested by Utsuki\(^\text{3}\), could explain the false positivity observed in 4 cases.

The accuracy of the image-guided system alone was very low. The poor results could be due to the brain shift phenomenon.

The combined use of neuroimaging and 5-ALA allowed to slightly improving sensitivity by adding to samples underdiagnosed by fluorescence. As a matter of fact, only a positive specimen (2,8%) was both non-fluorescent and outside the marked area. However, if we assume as positive all samples showed as pathological by at least one of the two methods, the specificity decreases significantly (Table 5).

In our experience, 5-ALA alone allowed to increase the extent of resection obtained with a conventional illumination. It was possible to extend the surgical excision in all cases of our series. The image-guide system improves the sensitivity of the fluorescence, but reduces its specificity. Therefore, considering gliomas not involving eloquent areas, a combined
use of fluorescence and navigation appears to be a reliable technique in order to achieve a complete surgical excision.

Conclusions

5-ALA has been proven safe, reliable and highly sensitive. Nevertheless, the presence of false negative tissue could be detected. The fluorescence-guided sensitivity can be improved with the use of an image-guided system. On the other hand, the specificity of 5-ALA merged to the neuronavigation is reduced. In cases involving non-eloquent areas, the combined use of fluorescence and neuroimaging appears to be the best way to achieve a complete resection.

References