Influence of Valproic Acid on Outcome of High-grade Gliomas in Children

AMIRHADI MASOUDI, MARILY ELOPRE, ELHAM AMINI, MARGARET E NAGEL, JOANN L. ATER, VIDYA GOPALAKRISHNAN and JOHANNES E.A. WOLFF

Division of Pediatrics, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, U.S.A.

Abstract. Background: Chemotherapy has limited effects in the treatment of high-grade gliomas (HGGs). Valproic acid (VPA), a histone deacetylase (HDAC) inhibitor, may sensitize HGGs to radiochemotherapy. As the drug has been given frequently as an antiepileptic drug, a retrospective analysis was conducted to ensure relevant information was not missed before a prospective study was launched. Materials and Methods: An analysis of 66 pediatric patients (range, 1-19 years of age) with glioblastoma multiforme (GBM) (n=40) or anaplastic astrocytoma (AA) (n=26) was retrospectively conducted for predictors of survival and response and for effects of VPA on outcome or toxicity. Results: The overall survival (OS) was better for AA (p=0.0114) and complete resection (p<0.00005) and event-free survival (EFS) was better for complete resection (p=0.0049). Nine patients received VPA for seizure plus further oncological treatment. The most severe adverse effect was a pulmonary embolism (n=1); no other severe side-effects were noted. The response to nonsurgical treatment after 8 weeks was: complete response (CR): 0, partial response (PR): 3, stable disease (SD): 4, progressive disease (PD): 2. Some of the patients with SD responded later resulting in best response: CR:0, PR:5, SD:2, PD:2. Conclusion: Treatment with VPA plus radiochemotherapy is well tolerated with an encouraging response rate.

High-grade gliomas (HGGs), such as anaplastic astrocytomas (AA) and glioblastoma multiforme (GBM), are relatively rare in children (1, 2). HGGs in children are typically treated with a combination of surgery, radiotherapy (RT) and/or chemotherapy (3, 4). Grossly total resection of HGG has been shown to improve survival and this improvement is particularly striking in pediatric patients (5, 6). RT is also known to improve survival (4). Treatment with prednisone, lomustine, and vincristine has also been shown to improve survival in children with HGG (7) and temozolomide improves survival in adults with HGG (8, 9). However, the best choice of agents remains an area of debate (10). Furthermore, despite all therapeutic approaches, the prognosis for patients with HGG is generally dismal (2, 6), emphasizing the need for novel approaches such as epigenetic modulation of gene expression, a powerful therapeutic option for various other malignancies (11, 12).

Valproic acid (VPA) has traditionally been used to treat epilepsy and migraines and for mood stabilization (13). Now it has also been shown to be a histone deacetylase (HDAC) inhibitor (14, 15). HDACs are enzymes involved in the remodeling of chromatin and they have a key role in the epigenetic regulation of gene expression (16). Inhibition of HDAC induces tumor cell differentiation, apoptosis and growth arrest (12, 14). In preclinical studies, VPA inhibited human and rodent glial tumor cell growth and induced a distinct mature glial phenotype (12, 17). Evidence has also been presented that HDAC inhibitors can sensitize malignant cells to RT (18, 19) and chemotherapy (14, 20). Clinical evidence of cellular differentiations and tumor control is rare, however, epileptic patients receiving VPA have significantly enhanced hemoglobin F levels, supporting the hypothesis that nontoxic levels of VPA can induce cellular differentiation (21). Tumor response to valproate sodium has also been described (11).

Prospective studies will be necessary to clarify the possible role of VPA as an adjuvant to chemotherapy in HGG. However, because VPA is given for antiepileptic treatment in brain tumor patients, a retrospective study of HGG patients could offer important insights into VPA effects and help guide prospective study design. Therefore,

Correspondence to: Dr. Johannes E.A. Wolff, Division of Pediatrics, Section of Pediatric Neuro-Oncology, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Unit 87, Houston, TX 77030, U.S.A. Tel: +1 713 794 4963, Fax: +1 713 794 5042, e-mail: jwolff@mdanderson.org

Key Words: Anaplastic astrocytoma, glioblastoma multiforme, high grade glioma, histone deacetylase inhibitor, pediatric, valproic acid.
before launching a prospective study, pediatric patients (children and adolescents <19 years of age) with HGGs were retrospectively analyzed to determine whether VPA influenced survival and response rates or adverse events in these patients.

Patients and Methods

This retrospective chart analysis was reviewed and approved by the Institutional Review Board of The University of Texas M.D. Anderson Cancer Center. The institutional database was used to locate pediatric patients with HGG from 1997 to 2007. The eligibility criteria for the first data extraction included age <19 years and histologically confirmed HGG. These eligibility criteria were validated by clinical records as primary data sources resulting in a smaller, improved database encompassing sex, age at diagnosis, tumor location, histology, surgery type, radiation dose, chemotherapy protocol, adverse events, VPA use, response, follow-up time and status at last follow-up.

Patients’ disease response to either radiation, or chemotherapy or both was classified as continuing complete remission (CCR; complete absence of radiographically identifiable tumor after resection), complete response (CR; complete disappearance of all detectable disease), partial response (PR; a reduction in tumor volume of ≥50% ), stable disease (SD; a decrease in tumor volume of <50% or an increase of ≤25% ), and progressive disease (PD; an increase in tumor volume of >25% ).

The Common Terminology Criteria for Adverse Events Version 3.0 (from the U.S. National Cancer Institute (22)) was used to assess side-effects. These criteria do not include lactic acid dehydrogenase (LDH) level among the defined toxic effects. Therefore, a total LDH level of <270 U/l was defined as normal and a total LDH level of 270-675 U/l, 676-1350 U/l, 1351-5500 U/l and >5500 U/l as grade I, II, III and IV toxic effects, respectively.

In order to determine if the relevant parameters differed in the VPA group from the other patients, the total group of pediatric HGG patients was described and potential predictive factors were analyzed for their effect on event-free survival (EFS) and overall survival (OS) times using the log-rank test, subgroup analyses and Cox regression analyses. To analyze response, the patients who achieved a CCR after complete resection were excluded.

The relevant parameters were then compared between the patients who received VPA and those who did not receive VPA using Chi-square analysis for the qualitative parameters of the database and analysis of variance for the quantitative parameters.

A matched-pair analysis of the effect of receiving VPA versus not receiving VPA was conducted using the predictively relevant factors (tumor grade and resection type) and follow-up time. If two or more patients who did not receive VPA were eligible to match with one patient who did receive VPA, the most recently treated patient was selected. The initial response (8 weeks after the initiation of treatment), the best response, which included both early and late response, as well as EFS and OS were compared between patients who received VPA and those who did not.

Descriptive tools were used to assess the side-effects. For all the analyses, SPSS software, version 12.0 (SPSS Inc., Chicago, Illinois, USA) was used. Because the analyses were descriptive, no level of statistical significance was set. However, \( p < 0.05 \) was considered relevant enough to merit further attention.

Results

Sixty-six patients with histologically proven grade III or IV glioma who were treated at M.D. Anderson Cancer Center between 1997 and 2007 were found. There were 34 males and 32 females, with a median age at diagnosis of 12.5 years (range, 1-19 years). The median follow-up time was 53 weeks. Twenty percent of the patients had undergone a complete resection, 59% had undergone a subtotal or partial resection and 21% had undergone a biopsy without resection. Thirty-nine patients received antiepileptic drugs (AEDs) either because of known epilepsy or to prevent seizure after surgery and 9 of these received VPA. The median RT dose was 56 Gy. The initial response to RT and/or chemotherapy reported 8 weeks after initiation of treatment was evaluated; 17 patients had PD, 16 had SD, 13 achieved a PR and 7 achieved a CCR. No response information was available in 13 patients. The best response achieved was PD in 10 patients, SD in 17, PR in 18 and CCR in 8. No best response information was available in 13 patients. The median OS time of all patients was 1.5 years.

A significant difference in OS (\( p < 0.00005 \)) and EFS (\( p = 0.0049 \)) was detected between patients who had undergone total resection or less than total resection. The patients who had undergone a complete tumor resection achieved the best survival, and those who had undergone biopsy without resection had the worst survival (Figure 1). The patients with AA achieved significantly better OS than patients with GBM (\( p = 0.0114 \), Figure 2). On Cox regression analysis, both resection type and tumor grade were statistically relevant predictors of survival. There were no significant differences in EFS between the groups categorized by sex (\( p = 0.8912 \)), age at diagnosis (\( p = 0.5681 \)), tumor location (\( p = 0.1611 \)) and tumor grade (\( p = 0.0567 \)). There were also no significant differences in OS between groups categorized by sex (\( p = 0.5130 \)), age at diagnosis (\( p = 0.4797 \)) and tumor location (\( p = 0.2062 \)). Subgroup analyses did not reveal any relevant differences either. The patients who achieved at least a PR had better survival times than those with SD or PD regardless of whether their initial response after 8 weeks of treatment (OS \( p = 0.0016 \) and EFS \( p < 0.00005 \); Figure 3) or their best response (OS \( p = 0.0209 \) and EFS \( p < 0.00005 \)) was used for analysis. These comparisons remained significant on Cox regression analysis.

Nine out of the 66 patients received VPA as an AED in addition to further oncological treatment (Table 1). The median age at diagnosis was 11 years. The median duration of VPA administration was 18 weeks and the median follow-up time was 55 weeks. The location of the tumor was supratentorial in seven patients and infratentorial in two. Five patients came to M.D. Anderson at the time of primary diagnosis and four came upon relapse. In eight patients, VPA was prescribed as a monotherapy to control seizures.
However, one patient continued to have seizures, and clonazepam was added to VPA. Valproate plasma levels were between 50 and 173 mg/l, with a median of 62 mg/l. One patient had one episode of VPA toxicity reported, with a valproate blood level of 173 mg/l.

Table II summarizes the adverse events experienced by the nine patients who received VPA. In five of the patients, no grade III/IV hematological events were documented. One patient had grade IV leukopenia, two had grade III thrombocytopenia, and one had both grade III thrombocytopenia and grade III leukopenia. All the patients with grade III or IV thrombocytopenia were treated with platelet transfusions. The incidence of fever and neutropenia was experienced by two patients. The most severe side-effect documented was in a patient who developed deep venous thrombosis with a pulmonary embolism (grade IV cardiovascular toxicity). The patient developed aspiration pneumonia and was intubated and hospitalized for a total of 14 days. No severe hemorrhages were reported, but one patient developed mild gastrointestinal bleeding, and one patient had grade I epistaxis that was controlled with pressure. There were no documented cardiac or endocrine side-effects. Gastrointestinal events were observed in all the patients, including mild (grade I or II) nausea (n=6) and grade II colitis with bloody stools (n=1). However, no severe gastrointestinal events, such as pancreatitis, were reported.

Although hepatic reactions were the main concern monitored, none of the patients experienced hepatic failure or a severe change in hepatic enzyme levels. Dermatological and skin adverse effects were common (n=6), but not severe. The patients complained of mild dry skin, alopecia, and grade I rash, and one patient experienced a grade I injection site reaction that resolved with topical treatments.

Treatment with VPA was discontinued in seven patients because of death resulting from PD. In the remaining two patients, VPA treatment was stopped owing to lack of epileptic activity based on electroencephalography and clinical findings.

All nine patients received further oncological treatment during their treatment with VPA (Table I). The initial (after 8 weeks) response was PD in two, SD in four and PR in three. No CCRs or CRs were observed. Some of the tumors responded later, and the best responses were PD in two patients, SD in two and PR in five.

The patients in the matched-pair analysis who did not receive VPA had the same initial responses and best responses (Table III). There were no significant differences in response.
Table I. Characteristics of patients who received VPA during HGG treatment.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Age at diagnosis (years)</th>
<th>Histology</th>
<th>Treatment received: with VPA</th>
<th>Duration of treatment with VPA (weeks)</th>
<th>Duration of treatment with VPA plus additional treatment (weeks)</th>
<th>Follow-up duration (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>15</td>
<td>AA</td>
<td>RT (54 Gy), then TPCH</td>
<td>41</td>
<td>41</td>
<td>62</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>8</td>
<td>GBM</td>
<td>Etoposide</td>
<td>182</td>
<td>56</td>
<td>380</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>11</td>
<td>GBM</td>
<td>Azacitidine</td>
<td>15</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>15</td>
<td>GBM</td>
<td>Azacitidine</td>
<td>18</td>
<td>18</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>7</td>
<td>AA</td>
<td>Etoposide</td>
<td>18</td>
<td>18</td>
<td>88</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>11</td>
<td>GBM</td>
<td>RT (58 Gy)</td>
<td>15</td>
<td>15</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>17</td>
<td>GBM</td>
<td>TMZ + CCNU + IRI</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>7</td>
<td>GBM</td>
<td>MTX, then EPV, then RT (50 Gy)</td>
<td>18</td>
<td>18</td>
<td>33</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>16</td>
<td>GBM</td>
<td>RT (50 Gy) + TMZ then ITC</td>
<td>76</td>
<td>76</td>
<td>88</td>
</tr>
</tbody>
</table>

AA, anaplastic astrocytoma; CCNU, lomustine; EPV, etoposide, cisplatin, vincristine; F, female; GBM, glioblastoma multiform; IRI, irinotecan; ITC, isotretinoin, temozolomide, and celecoxib; M, male; MTX, methotrexate; RT, radiotherapy; TMZ, temozolomide; TPCH, 6-thioguanine, procarbazine, lomustine, and hydroxyurea; VPA, valproic acid.

Table II. Adverse events associated with VPA plus radiotherapy and/or chemotherapy.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Adverse event (Grade*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood/bone marrow</td>
</tr>
<tr>
<td>1</td>
<td>Leu (IV), Thr (II), HbD (II)</td>
</tr>
<tr>
<td>2</td>
<td>Leu (III), Thr (III), HbD (I)</td>
</tr>
<tr>
<td>3</td>
<td>Leu (I), Thr (II), HbdD (I)</td>
</tr>
<tr>
<td>4</td>
<td>Leu (II), Thr (III), HbD (II)</td>
</tr>
<tr>
<td>5</td>
<td>Leu (I), Thr (III), Hbd (I)</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>Leu (II), Thr (II)</td>
</tr>
<tr>
<td>8</td>
<td>Leu (II), Hbd (II)</td>
</tr>
<tr>
<td>9</td>
<td>Leu (I), Thr (I)</td>
</tr>
</tbody>
</table>

Alk, alkaline phosphatase; ALT, alanine aminotransferase; Ano, anorexia; AST, aspartate aminotransferase; Con, constipation; Epis, epistaxis; FeN, febrile neutropenia; HbD, hemoglobin decrease; HTN, hypertension; IwnN, infection with grade III or IV neutropenia; IwnN, infection with normal absolute neutrophil count or with grade I or II neutropenia; LDH, lactate dehydrogenase; Leu, leukopenia; Nau, nausea; Om, oral cavity mucositis; Thr, thrombocytopenia; Vom, vomiting. *According to Common Toxicity Criteria Version 3.0 from the U.S. National Cancer Institute (22).

(p=0.288), EFS (p=0.0937), or OS (p=0.2254) between the patients who received VPA and those who did not.

Discussion

Nine patients had been treated with VPA for epilepsy or other reasons during treatment for HGG, but it did not seem to have an effect on adverse events or outcomes in these patients. HDAC inhibitors are to be tested in clinical trials because of promising preclinical data and the clinical data presented here provide assurance that such a trial would be safe to start in this patient population.

Recently, HDAC inhibition has emerged as a potential strategy to reverse aberrant epigenetic changes associated with cancer (16). The clinical experience with new HDAC inhibitors, such as suberoylanilide hydroxamic acid and...
Additional toxicity was reported by Raymond when VPA was given in a study that investigated the effects of irinotecan on GBM. Witt et al. modified the dose of VPA while continuing chemotherapy. AEDs. Those hematological side-effects decreased after the chemotherapeutic agents. VPA was the most common non-EI-AED and more frequent in GBM patients treated with standard chemotherapeutic agents. The most severe adverse effect of VPA is hepatotoxicity (grade I-III) which was seen in a fetal case at a median drug level of only 78 mg/l (24). Although no hepatic failure was observed in our study, this risk should not be discounted when treating children with VPA. Other potential, but serious side-effects are pancreatitis, hypothyroidism, and polycystic ovarian syndrome. None of the patients in our study had these side-effects. This retrospective study found that adding VPA to radiochemotherapy did not increase toxicity compared to what is widely known for the chemotherapy protocols given here and it is likely that the adverse effects observed in our patients came from the radiochemotherapy. A prospective study is needed to confirm these findings.

The effects of VPA on brain tumors have been studied in vitro (17) and in vivo (11, 19, 25) since 1998, and VPA has been shown to have a potentially relevant therapeutic benefit. However, the combination of chemotherapeutic agents and AEDs has not been investigated sufficiently (26).

In particular, little is known about the combination of VPA and chemotherapy in pediatric patients with HGG. More is known from adult patients, but the data appear contradictory. Grossman et al. (27) evaluated reactions between 9-aminocamptothecin and AEDs and did not find any grade III or IV myelosuppression, suggesting that VPA did not add to the toxicity of the chemotherapy. In contrast, Bourg et al. (28) found that chemotherapeutic plus VPA in patients with brain tumors resulted in a three-fold higher incidence of reversible thrombocytopenia, neutropenia, or both in comparison with chemotherapy combined with other AEDs. Those hematomatological side-effects decreased after the dose of VPA was modified while continuing chemotherapy. No additional toxicity was reported by Raymond et al. (29) when VPA was given in a study that investigated the effects of irinotecan on GBM. Witt et al. (11) reported drowsiness as the main side-effect in a patient with GBM who received VPA (145 mg/l) plus chemotherapy. Finally, Oberndorfer et al. (26) compared the effects of P450 enzyme-inducing AEDs (EI-AED such as carbamazepine) and non-EI-AEDs in GBM patients treated with standard chemotherapeutic agents. VPA was the most common non-EI-AED and more frequent in GBM patients treated with VPA than in the other patients. To our knowledge, there have been no reports of severe adverse effects of VPA when it is added to chemotherapy for HGG. This adds evidence to our conclusion that it is safe to start combination treatment studies giving VPA with other oncolgical treatments.

Among the 66 patients with HGG in the present study, complete resection of HGG led to better survival outcomes than subtotal/partial resection or biopsy only, patients with AA had a longer survival than patients with GBM and response to nonsurgical treatment was prognostic of survival in children with HGG. These findings may appear intuitively obvious, yet are frequently debated and not necessarily true for all pediatric brain tumors. Our findings support a maximal surgical resection approach and the value of continuing to search for effective nonsurgical treatments, such as the addition of HDAC inhibitors to chemotherapy or radiotherapy in this patient population.

Tumor response to VPA treatment has been described in vitro (12, 20, 30) and in patients (11, 25, 26). Driever et al. (25) reported on a child with a relapsed supratentorial primitive neuroectodermal tumor who received VPA for epilepsy. In contrast to the initial tumor, the recurrent tumor showed glial differentiation and a low proliferation index. Witt et al. (11) reported a CR in a 10-year-old boy with GBM who received VPA. The tumor had not responded to radiochemotherapy (54 Gy; vincristine, cisplatin, etoposide, and ifosfamide for 20 weeks; and then topotecan for 10 weeks) and the patient developed seizures. Magnetic resonance imaging showed a CR after 10 months of VPA. Among the nine patients in this study, no CRs were reported. This might be because the VPA doses received were lower (median 62 mg/l) than that received in the patient in Witt et al.’s study (142 mg/l). However, the responses presented here were still quite good for this patient population and might suggest that VPA increased the efficacy of chemotherapy. This hypothesis is supported by the findings of Oberndorfer et al. (26), who reported significantly better responses in patients with GBM who received VPA plus lomustine than in patients who received other AEDs and lomustine. These data, though encouraging, are limited: a prospective study is warranted.

In summary, this study confirmed that complete resection of HGG resulted in better EPS and OS and patients with AA achieved better survival than patients with GBM. Furthermore, the relevance of treatment response to overall survival was also shown. In addition, adding VPA to radiochemotherapy did not appear to increase toxicity. These results laid the groundwork for further investigations.
for a now ongoing clinical trial combining VPA and etoposide in pediatric patients with recurrent brain tumors and may encourage future studies of VPA as an adjunct to postoperative radiochemotherapy in children with HGG.

Acknowledgements

We thank members of the Department of Biostatistics at The University of Texas M.D. Anderson Cancer Center for their helpful suggestions. Parts of this paper were presented at the 12th Annual Meeting of the Society for Neuro-Oncology on November 15, 2007, in Dallas, TX.

References


