Cerebrospinal Fluid Polyamines in Patients with Glioblastoma
Multiforme and Anaplastic Astrocytoma

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ABSTRACT

Eighteen cerebrospinal fluid polyamine determinations in 12 patients with glioblastoma multiforme and 76 determinations in 37 patients with anaplastic astrocytoma were evaluated. Cerebrospinal fluid polyamine levels showed no significant relationship to the degree of malignancy or to enhanced tumor volume or volume of tumor central low density as determined by contrast-enhanced computerized tomography. A significant correlation was found between polyamine levels and the proximity of the tumor to the cerebral ventricles. Polyamine levels were correlated with clinical status as determined by neurological examination, radionuclide scan, and computerized tomography. Compared with those of stable patients, cerebrospinal fluid polyamine levels were significantly elevated in patients with recurrent tumors; however, elevation of polyamine levels did not appear to precede tumor recurrence. A large fraction of the results were false-positive or false-negative results. In contrast to our findings in patients with medulloblastoma, it appears that cerebrospinal fluid polyamine level determinations may be of little use for monitoring tumor progression in patients with glioblastoma multiforme and anaplastic astrocytoma.

INTRODUCTION

The polyamines PU, SP, and spermine are low-molecular-weight bases that are ubiquitous in body tissues. Numerous studies have linked polyamines to nucleic acid synthesis (1, 3, 4, 8) and demonstrated accelerated polyamine production in rapidly dividing tissues (1, 3, 4, 8, 13). Tumor tissue usually has higher polyamine concentrations than does normal, nonneoplastic tissue.

Increased polyamine levels are found in the urine, serum, and CSF of cancer patients. Elevated levels may reflect increased polyamine production by rapidly dividing cells or the release of polyamines from dead tumor cells (6). These elevated levels have been correlated with the degree of cancer and the extent of disease, and decreased levels have been reported following successful treatment (2, 5, 9–11, 13, 14, 16).

Possibly because production of polyamines is not specific for tumor cells, elevated polyamine levels have not proven to be useful for screening patients for cancer. Polyamine levels may be valuable to follow patients with certain types of cancer, to predict tumor recurrence, and to monitor response to treatment (5, 11, 14).

Marton et al. (10) showed an absolute correlation between CSF polyamine levels and clinical status determined by neurological examination, radionuclide and CT scans, myelography, and CSF cytology in 15 of 16 patients with medulloblastoma. Medulloblastomas are most frequently located in the midline of the cerebellum; more often than not, they expand into the fourth ventricle. Because medulloblastomas tend to spread through the CSF pathways, recurrent tumors are usually located within or adjacent to the ventricular system or subarachnoid space. In patients harboring medulloblastoma, elevated CSF polyamine levels are an accurate indicator of tumor progression and, at times, the earliest indication of recurrence.

In this paper, we report the relationship of CSF polyamine levels to the degree of cancer, clinical stability or progression, tumor size, amount of necrosis, and tumor location in patients with glioblastoma multiforme and anaplastic astrocytoma.

MATERIALS AND METHODS

Sample Procurement and Preparation. Samples of CSF were obtained either by lumbar puncture or by puncture of a s.c. Ommaya reservoir connected to the ventricular system in patients with a diagnosis of glioblastoma multiforme or anaplastic astrocytoma. Patients were being evaluated for recurrence or were undergoing therapy. All specimens were obtained just before therapy. Samples of CSF from patients with nonneoplastic neurological disease were obtained via lumbar puncture; no ventricular fluid reference samples have been obtained thus far.

Samples were acidified, centrifuged at 900 × g for 10 min at 2°C, and then frozen at −20°C until they were prepared for analysis. One- to 2-ml aliquots of CSF were lyophilized, and the residues were reconstituted in 0.5 ml of 6 N HCl and hydrolyzed at 110°C for 14 to 16 hr. The hydrolysates were lyophilized, reconstituted in 150 µl of 4% 5-sulfosalicylic acid, and centrifuged at 8000 × g for 10 min. The supernatants were used for chromatographic analysis.

Chromatography. Ion-exchange chromatographic separation of the polyamines with fluorometric detection was carried out on a Durrum D-500 amino acid analyzer (Dionex Instrument Corp., Sunnyvale, Calif.) as previously described (10).

RESULTS

The reference group of 29 patients with neurological disease other than central nervous system tumors and of 5 healthy adult volunteers has been described (10).

Polyamine levels may be elevated in the CSF of patients who have neurological diseases other than tumors. Patients who had these acute processes, such as central nervous system
infection, brain infarction, or subarachnoid hemorrhage, were excluded from the present patient population. CSF polyamine determinations were performed in 18 CSF samples from 12 patients with glioblastoma multiforme and 76 CSF samples from 37 patients with anaplastic astrocytoma. Concomitant clinical status was determined by neurological examination and radionuclide and CT scans. Disease progression was defined as a definite decline in 2 of these 3 tests.

Patients reviewed in this paper were stratified into 2 groups according to the criteria of Rubinstein (12) and Russell and Rubinstein (15) as harboring either glioblastoma multiforme (Grade IV astrocytoma) or anaplastic astrocytoma (Grade III astrocytoma). In this classification system, anaplastic astrocytomas are tumors of astroglial origin that show some malignant features but that are not sufficiently malignant to warrant a designation of glioblastoma multiforme. All patients reported in this paper harbored malignant tumors.

CSF PU and SP levels in all patients with both glioblastoma multiforme and anaplastic astrocytoma, irrespective of the stability or activity of disease, were significantly higher than those of the reference group (Table 1). However, the values for patients with glioblastoma multiforme did not differ significantly from those for patients with anaplastic astrocytoma. There was no significant difference in CSF polyamine levels between patients who remained stable for at least 4 months after CSF examination and patients whose tumor recurred within 2 or 4 months of the examination (Table 2). Compared with patients who had stable disease, CSF polyamine levels were significantly elevated in patients whose tumor recurred by the time of the examination. However, 5 of 14 patients (36%) with recurrent disease had both PU and SP levels in the same range as did the reference group (mean ± 2 S.D.). Both PU and SP levels were elevated in 12 of 47 (26%) stable patients. For patients who remained clinically stable for 4 months after the polyamine determination but who eventually demonstrated recurrence, CSF polyamine levels bore no relation to the time of recurrence.

For patients whose tumors had a measurable volume of enhancement on the CT scan, there was no significant corre-
tion between elevated CSF PU concentration and the presence of a tumor in the subependymal region (adjacent to ventricle) as found by the CT scan and a less significant association between elevated CSF SP level and subependymal tumor location.

**DISCUSSION**

Studies of the kinetic characteristics of brain tumors (7) suggest that the proportion of proliferating tumor cells is greater for glioblastoma multiforme than for anaplastic astrocytoma. Because polyamine levels are highest in rapidly proliferating tissue, polyamine production might be expected to be higher in glioblastoma. In our patient population, however, the values for CSF polyamines were not significantly different in patients with either glioblastoma multiforme or anaplastic astrocytoma.

As a tumor increases in size, its growth fraction and growth rate tend to decrease. The rate of polyamine production is at the maximum when the mass of proliferating tumor cells is maximum. However, our understanding of tumor cell kinetics does not allow prediction of the clinical state at which maximum polyamine values occur. Although CSF polyamine values for patients with recurrent tumors are significantly greater than CSF polyamine values of stable patients, they do not significantly differ from the values determined 2 and 4 months before recurrence. For the reasons discussed above, an increase in tumor volume is not necessarily associated with an increase in the number of proliferating cells and an increase in polyamine production. Our data do not show a significant relation between enhancing tumor volume and CSF polyamine levels.

Heby and Anderson (6) postulated that dead or dying tumor cells release polyamines into the body fluids of tumor patients. Because a large proportion of cells in the central low density region of a tumor is necrotic, a rise in CSF polyamine levels might be expected with increasing volume of tumor central low density seen on CT scans. Our data do not support this hypothesis for tumors with this feature.

The lack of significant correlation between CSF polyamine levels and tumor pathology, clinical stability, tumor recurrence, volume of tumor, and necrosis may reflect a failure to take into account factors affecting the transport of polyamines into the CSF. Mechanisms of polyamine transport through normal and diseased brain and of the clearance of polyamines from the CSF are not well understood. Our data suggest these factors may be important.

CSF polyamine levels are significantly increased in patients whose tumors show enhancement with contrast material on CT scan (regardless of volume) compared to patients whose tumors show no detectable enhancement. Because tumor enhancement reflects the presence of "leaky" tumor capillaries, elevated polyamine levels may be the result of increased capillary permeability or other factors affecting polyamine transport from tumor into CSF. Moreover, patients with tumors in close proximity to the ventricular system have significant elevations of CSF polyamines, which are probably transport related. This finding may explain the excellent correlation between polyamine levels and tumor recurrence in patients with medulloblastoma (10) because most if not all recurrent medulloblastomas are adjacent to the ventricular system or subarachnoid space.

The location of supratentorial tumors had no correlation with polyamine levels. Our sample included one patient with a cerebellar anaplastic astrocytoma and no patients with brainstem tumors; therefore, the relation between the size and malignancy of infratentorial tumors and polyamine levels could not be evaluated. Because these tumors are often adjacent to the ventricular system or basal cisterns, a correlation between clinical progression and CSF polyamine levels is possible and should be investigated. The sample site (lumbar subarachnoid space or cerebral ventricle) and the presence or absence of malignant cells in the CSF might also influence the polyamine levels. Because our data included only one ventricular fluid sample and one sample containing malignant cells, we could not evaluate these possible effects.

In this patient population, CSF polyamine levels could not be used to predict tumor recurrence. Even at the time of recurrence, 5 of 14 (36%) patients had both PU and SP levels in the same range as did the reference group. Twelve of 47 (26%) stable patients had elevated PU and SP levels. This high incidence of false-positive and -negative results indicates the difficulty of utilizing CSF polyamine levels to predict tumor recurrence in patients with supratentorial glioblastoma multiforme or anaplastic astrocytoma.

Although polyamines may reflect growth activity, or the lack thereof, for a variety of brain tumors, the fact that these compounds cannot reach the CSF may be limiting. Studies of PU capillary permeability in rat brain and diffusion in cat brain indicate that this is true. PU passes capillaries readily, escapes into the general circulation, and also has limited diffusion in brain tissue. Therefore, polyamine levels appear to be a poor diagnostic tool for hemispheric gliomas that are distant from CSF pathways, but they clearly represent a useful test for medulloblastoma and perhaps will be useful for other tumors adjacent to the CSF pathways.

**ACKNOWLEDGMENTS**

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Table 5

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of determinations</th>
<th>PU (pmol/ml)</th>
<th>SP (pmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Range</td>
</tr>
<tr>
<td>Frontal</td>
<td>40</td>
<td>369</td>
<td>278</td>
</tr>
<tr>
<td>Parietal</td>
<td>19</td>
<td>300</td>
<td>78</td>
</tr>
<tr>
<td>Temporal</td>
<td>23</td>
<td>322</td>
<td>268</td>
</tr>
<tr>
<td>Adjacent to ventricle</td>
<td>12</td>
<td>515</td>
<td>450</td>
</tr>
<tr>
<td>Not adjacent to ventricle</td>
<td>82</td>
<td>320</td>
<td>179</td>
</tr>
</tbody>
</table>

a p > 0.2 for these values taken in pairs.

Significantly different: p < 0.001 for PU; p < 0.05 for SP.

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REFERENCES

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