Putrescine as a Biochemical Marker of Malignant Brain Tumors

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ABSTRACT

Putrescine, spermidine, and spermine levels were determined in normal brain and central nervous system-related tumor tissues obtained at operation from 50 patients. The biochemical data were correlated with morphological histopathological descriptions of the same tissues. There was little variation in putrescine levels in normal cerebral cortical tissue. Subcortical white matter had lower putrescine but higher spermidine content than those of the overlying cortex. Putrescine levels were elevated in all astrocytomas assayed, and the magnitude of this elevation was proportional to the malignancy of the tumor as determined by histopathological criteria. In contradistinction, putrescine content of 'benign' tumors was generally equal to or lower than that of the normal cerebral cortex. Spermidine and spermine levels varied widely in the tumors assayed and did not correlate with criteria of malignancy. It is concluded that putrescine may be a good biochemical marker of malignancy in central nervous system-related tumors.

INTRODUCTION

Polyamine biosynthesis and accumulation are closely associated with cellular growth and proliferation (2, 22) be it physiological, such as the response of the hepatic remnant to partial hepatectomy (17) and the prostate to the administration of androgens (12), or neoplastic (1). Recently, there has been increasing interest in the polyamines as biochemical markers that can be clinically useful in the diagnosis and follow-up of cancer patients (3). This interest was generated mostly by the finding of high levels of putrescine and spermidine in the urine and sera of patients harboring malignant tumors (4, 13, 14). Also, high levels of putrescine and spermidine were found in the cerebrospinal fluid of patients with malignant brain tumors (11). The high levels of the diamine, putrescine, and the triamine, spermidine, in the serum, urine, or cerebrospinal fluid of tumor-bearing patients presumably reflect extracellular leakage of these intracellular amines resulting from spontaneous cell necrosis (4, 11, 14).

The need for basic information regarding the levels of putrescine, spermidine, and spermine in human tumors and the correlation of these levels with known criteria of malignancy is essential for adequate understanding of results describing the concentration of these amines in body fluids. We report here on the levels of putrescine, spermidine, and spermine in tissue samples obtained from 50 patients undergoing neurosurgical procedures for the excision of central nervous system-related tumors. The samples represent a variety of tumor types and in some instances normal brain tissue. A preliminary summary of our findings has been reported previously (7).

MATERIALS AND METHODS

Tissues were obtained during neurosurgical procedures for the excision of intracranial and intraspinal mass lesions under general anesthesia. After excision, tissue samples were immediately divided into 2 portions. The smaller portion was quickly frozen and stored at $-60\degree$ until assayed for its putrescine and polyamine content. The larger portion was processed for conventional histopathological evaluation. In many instances, several samples of tissues were obtained from the same patient, and each sample was coded and processed separately.

For biochemical assays, the frozen tissue samples were homogenized in 9 volumes of cold distilled water in motor-driven glass homogenizers. Immediately after homogenization, a small volume of the homogenate was taken for the putrescine assay. To the remainder, an appropriate volume of 6 M perchloric acid was added to a final acid concentration of 0.4 M. The portion taken for the putrescine assay was heated in a water bath at 95\degree for 20 min and centrifuged for 10 min at 20,000 x g, and 10- to 40-\mu l aliquots of the supernatant (representing 1 to 4 mg of tissue) were assayed for putrescine by the enzymatic-isotopic technique (5). The acidified homogenates were allowed to stand in an ice bath for 30 min and were centrifuged for 10 min at 20,000 x g, and 0.5- to 2-ml aliquots of the supernatant were assayed for spermidine and spermine as described previously by Kremzner et al. (10). The amounts of putrescine, spermidine, and spermine in aliquots of the tissue extracts assayed were within the linear range of the respective assay procedure. Putrescine was assayed in triplicates with less than 7% interassay variation. Spermidine and spermine were assayed in duplicates with an interassay variation less than 5%. The recovery of known amounts of authentic putrescine, spermidine, and spermine added to tissue homogenates was studied with each batch of determinations, and the recovery rates were used in calculating the tissue amine content.

Samples for histopathological evaluation were fixed in formalin (10 g/100 ml) and processed in the usual manner for paraffin sectioning. Sections from all biopsy specimens were stained routinely with hematoxylin and eosin. Those from glial tumors were also stained with phosphotungstic acid-hematoxylin to confirm the presence of glial fibers. Reticulum stain and Masson trichrome stains were used whenever needed to identify mesodermal tumors and to demonstrate areas of collagenous fibrous tissue, respectively. Histopathological descriptions and diagnoses were made on each sample of tissue by C. H. Sutton without knowledge of the biochemical results. The grading of primary glial brain tumor was carried out according to the criteria of Kernohan and Sayre (8). In many instances,
although more than one sample of tissue was obtained from
the same patient, each sample was evaluated separately, and
the biochemical data derived from each sample were tabulated
under the appropriate histopathological diagnosis made on that
piece of tissue. As an example, several samples of tissue were
obtained from a patient with a large glioma. One sample,
suspected at operation to contain tumor, was found to repre-
sent normal brain tissue; another sample of tissue was diag-
osed as Grade III astrocytoma; while a third sample was
histopathologically described as Grade II astrocytoma. The
biochemical data obtained from the 3 samples were tabulated
under 3 different headings, normal cerebral cortex, Grade III
astrocytoma, and Grade II astrocytoma, respectively. When
different tissue samples taken from one patient showed similar
histopathological appearance, the biochemical data were av-
eraged and their means were used for data analysis. There
was little variation in biochemical results obtained from histo-
pathologically similar samples obtained from the same patient.
Tissue samples that showed moderate to marked histopatho-
logical heterogeneity were excluded. Similarly excluded were
samples of tissue showing significant edema or reactive
changes. Samples from patients who had had radiation ther-
apy, chemotherapy, and/or heat therapy to their brain tumors
were also excluded.

RESULTS

Levels of putrescine, spermidine, and spermine in normal
human brain tissue and in various tumor types of the nervous
system are detailed in Table 1. There was little variability in the
putrescine content of normal cerebral cortical tissue despite
age differences and variations in the site of the cortical biopsy.
Putrescine levels in samples from 9 patients ranged from 42.3
to 55.3 nmol/g (mean ± S.E., 47.3 ± 1.3 nmol/g) (Table 1;
Chart 1). Spermidine and spermine values obtained from the
same cerebral cortical samples were more variable (Table 1;
Charts 2 and 3). Normal subcortical white matter was found to
have lower (p < 0.01) putrescine levels and higher (p < 0.005)
spermidine levels (Table 1). The levels of putrescine, spermi-
dine, and spermine in cerebellar cortical samples were not
significantly different from those of the cerebral cortex (Table 1).

All astrocytomas had elevated levels of putrescine (Table 1;
Chart 1). Even the least malignant of these tumors had putres-
cine levels higher than the range of values obtained from
normal brain tissue. Putrescine levels in the supratentorial
astrocytoma groups increased progressively in direct relation-
ship to the histopathological criteria of malignancy of these
tumors (Table 1; Chart 1). In astrocytoma Grade II, III, and IV
groups, where the number of observations allowed adequate
statistical analysis, putrescine levels were higher than normal
cerebral cortical levels at p < 0.001. Also, putrescine levels in
Grade IV astrocytomas were significantly higher than those of
Grade II or Grade III tumors (p < 0.01), while putrescine levels
in Grade II and Grade III astrocytomas did not differ signifi-
cantly. Spermidine and spermine levels in the various astrocy-
tomas varied over a large range, which overlapped with values
obtained from normal brain tissue (Table 1; Charts 2 and 3).
Only in Grade II astrocytomas were spermine levels significa-
tively higher than those of normal cerebral cortex (p < 0.01).
Putrescine was minimally to moderately elevated in cerebellar
astrocytomas and in the single sample of the spinal cord
astrocytoma (Table 1).

A variety of slowly growing and relatively benign intracranial
or intraspinal tumors were also studied for their putrescine and
polyamine contents. Samples representing cerebellar hemangi-
obloma, meningioma, chordoma, neurofibroma, schwann-
oma, and chemodectoma all had low putrescine levels with
the exception of one vestibular nerve schwannoma. The sper-
midine and spermine contents of all these tumors were within

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Putrescine nmol/g</th>
<th>Spermidine nmol/g</th>
<th>Spermine nmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal brain tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral cortex (gray and white matter)</td>
<td>47.3 ± 1.3</td>
<td>336 ± 42</td>
<td>228 ± 25</td>
</tr>
<tr>
<td>Subcortical white matter</td>
<td>34.8 ± 5.2</td>
<td>750 ± 107</td>
<td>234.149</td>
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<tr>
<td>Cerebellar cortex</td>
<td>42.4 ± 8.3</td>
<td>345 ± 25</td>
<td>412, 288</td>
</tr>
<tr>
<td>Astrocytoma (supratentorial)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>77.6, 64.2</td>
<td>286, 375</td>
<td>193 (1)</td>
</tr>
<tr>
<td>Grade II</td>
<td>94.0 ± 10.4</td>
<td>439 ± 51</td>
<td>499 ± 110</td>
</tr>
<tr>
<td>Grade III</td>
<td>132.7 ± 11.3</td>
<td>447 ± 47</td>
<td>253 ± 36</td>
</tr>
<tr>
<td>Grade IV</td>
<td>239.6 ± 27.6</td>
<td>545 ± 95</td>
<td>310 ± 36</td>
</tr>
<tr>
<td>Astrocytoma of cerebellum (Grades I—III)</td>
<td>77.1 ± 10.7</td>
<td>240 (1)</td>
<td>227 (1)</td>
</tr>
<tr>
<td>Astrocytoma of spinal cord (Grade II)</td>
<td>62.3 (1)</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>Cerebellar hemangioblastoma</td>
<td>18.0 (1)</td>
<td>310 (1)</td>
<td>281 (1)</td>
</tr>
<tr>
<td>Meningioma</td>
<td>35.4 ± 6.2</td>
<td>320 ± 60</td>
<td>251 ± 69</td>
</tr>
<tr>
<td>Chordoma</td>
<td>13.2, 3.0</td>
<td>Not detectable</td>
<td>150 (1)</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>231.7, 49.4</td>
<td>417 (1)</td>
<td>446 (1)</td>
</tr>
<tr>
<td>Neurofibroma</td>
<td>Not detectable</td>
<td>419 (1)</td>
<td>283 (1)</td>
</tr>
<tr>
<td>Chemodectoma</td>
<td>4.6 (1)</td>
<td>369 (1)</td>
<td>Not done</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>a Mean ± S.E. (When the number of observations is 3 or more, the values given are the mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b Numbers in parentheses, number of observations.</td>
</tr>
<tr>
<td></td>
<td>c Different from normal cerebral cortex at p &lt; 0.01.</td>
</tr>
<tr>
<td></td>
<td>d Different from normal cerebral cortex at p &lt; 0.005.</td>
</tr>
<tr>
<td></td>
<td>e Different from normal cerebral cortex at p &lt; 0.001.</td>
</tr>
<tr>
<td></td>
<td>f Different from astrocytoma Grades II and III at p &lt; 0.01.</td>
</tr>
</tbody>
</table>

DECEMBER 1979
Putrescine and polyamine levels varied widely (Table 2). The type of the primary tumor and necrosis within the tumor, which was frequently encountered in our samples, seem to be important factors in determining the amine content of the tumor. Necrosis was associated with a marked reduction in tissue amines, especially putrescine. This is best illustrated in results from Patient 18, where samples from the same tumor nodule showed remarkable differences in histological appearance (Fig. 2) and amine levels (Table 2).

**DISCUSSION**

Spermidine and spermine values for normal brain obtained in this study are consistent with results from previous studies of human brain obtained at autopsy (9, 20) and during operation (9). This study also confirms the observation that spermidine is substantially higher (2- to 3-fold) in white than in gray matter, which has been previously reported in brains of other mammalian species (6, 15, 18, 20, 21). The specific role, if any, that spermidine might have regarding the structure or function of white matter remains unknown.

Putrescine levels in mammalian brain are much lower than those of spermidine and spermine (6, 9, 15), and the rapid turnover rate for putrescine renders it less stable in autopsy material than are those of spermidine and spermine (19). Kremzner (9) had previously reported on the putrescine content of various regions of human brain obtained at autopsy as well as on biopsy material from the frontal cortex in 3 instances. His results showed wide variation (ranging from 10 to 40 nmol/g) and are generally lower than those obtained in this study. It is possible that the low putrescine levels obtained by Kremzner (9) from autopsy material were due to ongoing catabolism of putrescine under autolytic conditions. As far as we know, there are no other reports on the level of putrescine in the normal human brain available with modern methodological techniques.

Using the same assay procedure (5), the levels of putrescine in the normal human cerebral cortex are about 2- to 3-fold higher than those in the cerebral cortex of the adult rat (5, 6), while putrescine levels in the cat cerebral cortex are intermediate between those of the human and of the rat (6).

The major thrust of the data presented in this manuscript is the level of tissue putrescine in various tumors of the nervous system. Evidence was presented to support the hypothesis that tissue putrescine levels were related to the malignancy of the tumor as determined by histopathological criteria. There was no overlap between results obtained from astrocytomas and normal brain tissue (Table 1; Chart 1). Normal cerebral cortical values were distinctly different ($p < 0.001$) from values for supratentorial astrocytomas (Grade II, III, and IV groups). Astrocytomas of the cerebellum and the spinal cord, which are more benign in their clinical course than supratentorial astrocytomas, had putrescine levels that were intermediate between those of normal cortical tissue and those of the supratentorial astrocytoma groups (Table 1).

On the other hand, benign or slowly growing intracranial tumors such as meningioma, chordoma, neurofibroma, chemodectoma, and cerebellar hemangioblastoma had relatively low levels of putrescine that did not exceed the range for normal cerebral cortex. The only exception to this general statement is the high putrescine value for the only acoustic tumor.
finding. schwannoma in this study. We have no explanation for this finding.

The tissue levels of spermidine and spermine varied considerably among the normal cerebral cortical group as well as among the various tumor types, and the ranges of values for the different groups showed considerable overlapping (Table 1; Charts 2 and 3). There was some tendency for spermidine values to increase with increasing histopathological malignancy (Table 1; Chart 2), but this did not attain statistical significance ($p > 0.05$). No correlation between tissue spermine levels and malignancy was evident (Table 1; Chart 3).

Metastatic tumors to the brain varied widely in their content of putrescine, spermidine, and spermine. There are several possible reasons for this variation: (a) variation in the origin and cell type of the primary tumor. Unfortunately, we did not have a large number of metastatic tumors of any one type to allow for statistical analysis of any particular subgroup; (b) variation in the viability of tumor cells. There were several instances where histopathological correlation with the biochemical data revealed that putrescine and spermidine attain low concentrations in necrotic tissue. This correlates well with the clinical finding of increased putrescine and spermidine in the urine (4, 13, 14), sera (4), and cerebrospinal fluid (11) of tumor-bearing patients after successful chemotherapy or radiotherapy of the tumor; (c) variation in the cellularity of the tumor. On the whole, hypercellular tumors had higher levels of the amines, especially putrescine, than hypocellular tumors.

It is to be expected that tissue putrescine levels will be elevated in rapidly growing tissues such as neoplasms. There is extensive evidence showing that polyamine synthesis is intimately associated with nucleic acid and protein synthesis (2, 22). Since the enzymatic decarboxylation of ornithine by ornithine decarboxylase is the rate-limiting step in polyamine synthesis and since putrescine is the immediate product of this reaction, putrescine tissue levels are expected to faithfully portray polyamine biosynthesis. Furthermore, because of the short half-life of putrescine, in contrast to the slow turnover rates of spermidine and spermine (16, 19), steady-state tissue concentrations of putrescine are more likely to provide a dynamic estimate of on-going polyamine biosynthesis than do similar estimates for spermidine or spermine. Indeed, in experimental hepatomas of varying growth rates, the steady-state tissue concentrations of putrescine correlated better with the growth rate of the tumor than did the tissue levels of either spermidine or spermine (23).

The superior correlation between the malignancy of the tumor and its tissue level of putrescine compared to the tissue levels of spermidine and spermine complement the results of Marton et al. (11), who showed that elevated cerebrospinal fluid levels of putrescine, rather than that of spermidine, correlated best with the presence of malignant brain tumors.

ACKNOWLEDGMENTS

We thank S. Wehle, M. Ganapathi, and G. Castiglione for excellent technical assistance. We also thank Drs. H. L. Rosomoff, L. K. Page, and B. A. Green for making tumor material available from their patients.

REFERENCES


Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor Description</th>
<th>Putrescine (nmol/g)</th>
<th>Spermidine (nmol/g)</th>
<th>Spermine (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Adenocarcinoma of breast</td>
<td>123.5</td>
<td>425</td>
<td>435</td>
</tr>
<tr>
<td>49</td>
<td>Adenocarcinoma of breast</td>
<td>71.5</td>
<td>235</td>
<td>110</td>
</tr>
<tr>
<td>18</td>
<td>Adenocarcinoma, undetermined origin</td>
<td>31.1</td>
<td>169</td>
<td>320</td>
</tr>
<tr>
<td>27</td>
<td>Adenocarcinoma of colon (marked necrosis)</td>
<td>100.3</td>
<td>338</td>
<td>554</td>
</tr>
<tr>
<td>41</td>
<td>Adenocarcinoma of lung (moderate necrosis)</td>
<td>9.1</td>
<td>78</td>
<td>490</td>
</tr>
<tr>
<td>7</td>
<td>Angiosarcoma (hypocellular)</td>
<td>26.1</td>
<td>195</td>
<td>31</td>
</tr>
<tr>
<td>24</td>
<td>Large cell carcinoma of lung (marked necrosis)</td>
<td>11.9</td>
<td>133</td>
<td>Not done</td>
</tr>
<tr>
<td>28</td>
<td>Large cell carcinoma of lung (marked necrosis)</td>
<td>33.4</td>
<td>147</td>
<td>Not done</td>
</tr>
<tr>
<td>30</td>
<td>Small cell carcinoma of lung (hypercellular)</td>
<td>15.1</td>
<td>91</td>
<td>604</td>
</tr>
<tr>
<td>40</td>
<td>Hypernephroma</td>
<td>262.8</td>
<td>348</td>
<td>269</td>
</tr>
<tr>
<td>47</td>
<td>Adenocarcinoma of breast</td>
<td>115.1</td>
<td>333</td>
<td>331</td>
</tr>
</tbody>
</table>
Fig. 1. A, recurrent meningioma showing evidence of marked hypercellularity and lack of psammoma body formation. Putrescine, spermidine, and spermine levels were 51.0, 440, and 69 nmol/g, respectively. B, hypocellular meningioma with abundant psammoma bodies. Putrescine, spermidine, and spermine levels were 18.3, 89, and 100 nmol/g, respectively. H & E, x 137.

Fig. 2. Adenocarcinoma of undetermined origin metastasizing to the brain of Patient 18 (see Table 2 for amine levels). A, center of the tumor nodule which shows moderate necrosis and degeneration of tumor cells. B, growing margin of the same tumor nodule where tumor cells appear healthier and are invading brain. H & E, x 137.
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