Putrescine metabolism in human brain tumors

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Summary

The metabolism of the polyamines, putrescine, spermidine and spermine, was studied in human brain and brain tumors. Samples of brain and tumors were incubated with ³H-putrescine and the amounts of labeled polyamines were measured. The amount of putrescine conversion was found to be greater in tumors than in normal brain samples. Furthermore, the metabolism of putrescine in brain tumors was related to tumor type and appeared to correlate with the degree of malignancy. The significance of these findings with regard to positron emission tomographic scanning and therapy of patients with malignant gliomas is discussed.

Introduction

Polyamines are present in the central nervous system (CNS) (1-4) and have been found to be increased in the cerebrospinal fluid (CSF) of patients with a variety of CNS malignancies (5-7). In gliomas, CSF and intratumoral concentrations of putrescine correlate with the degree of malignancy suggesting elevated polyamine metabolism (2, 8, 9). As a clinical marker of CNS neoplasia, however, CSF polyamines have been most useful for medulloblastoma where they appear to be a possible indicator of disease progression (10-12). This may be a reflection of the proximity of this tumor to the CSF pathways (13, 14).

We recently reported selective uptake and metabolism of radiolabeled putrescine in an experimental implanted brain tumor in the rat (15). From these studies we suggested that a ¹¹C labeled putrescine might prove useful as a marker for human brain tumors using positron emission tomography (PET). PET now permits localization of brain tumors and distinguishes the metabolic differences that exist between the lesion and the surrounding brain (16-18). Current approaches with PET for brain tumors have utilized substances that are both readily available and modeled for tomographic reconstruction such as glucose, oxygen, amino acids and their analogs. These markers lack ideal resolution since they are metabolized by both normal brain and tumors. A compound taken up and metabolized solely by the tumor would be a powerful addition to this approach since it would enhance the specificity of the technique and facilitate the assessment of the response of human brain tumors to radiation and chemotherapy.

To study this further we examined human brain tumors incubated in vitro with ³H putrescine to determine if the metabolism of putrescine correlated with the histologic appearance of the tumors as a preliminary study prior to carrying out PET scans in patients with brain tumors.

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Materials and methods

Samples of brain tumors were obtained at the time of surgery planned for the treatment of patients with a variety of intracranial tumors. Following removal, tissue samples of tumors and adjacent normal brain tissue removed as part of the surgical exposure were immediately packed in ice and transferred to the laboratory. The remaining portions of the tumors were sent for routine histopathologic examination. Tissue slices were prepared for incubation within 30 minutes of removal. Two or 3 slices were prepared from each specimen which weighed between 75 and 200 mg wet weight. Slices were incubated at 37 °C in 5 ml of a balanced solution with the following composition in mMol/L: NaCl 120; KCl 5.0; CaCl2 0.75; MgSO4 1.2; NaH2PO4 1.0; NaHCO3 1.0; glucose 10; and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) 25 adjusted to pH 7.4 (19). The solution was gassed with O2:CO2 (95:5%) and the pH readjusted to 7.4. The tissue slices were preincubated for 10 minutes.

Following preincubation 3H-putrescine (New England Nuclear, Boston MA, 26.6 Ci/mMol) was then added for a final specific activity of 120 nCi/ml which was equivalent to 4.5 nM of putrescine. The incubation was continued for 30 minutes at which time the slices were removed, blotted and frozen by immersion in liquid nitrogen.

The frozen tissue slices were weighed and placed in 10 volumes of ice cold 0.2 N HClO4 prior to sonication at 0°C. The sonicate was kept on ice for one hour and then centrifuged at 800×G for 15 minutes. An aliquot of the supernatant was removed and the water soluble amines were dansylated according to the method of Igarashi et al (20). The dansylated amines were extracted into toluene and resolved by silica gel G thin layer chromatography using chloroform: isopropanol (25:1) (21). Putrescine, spermidine and spermine were then scraped and counted for radioactivity by liquid scintillation counting. The HClO4 pellets were digested in 2 ml of 1 N NaOH at 60°C for 18 hours. Protein was determined according to Lowry et al using bovine serum albumin as the standard (22).

Results

We have determined the amount of 3H putrescine converted to spermidine and spermine in samples of human brain tumors as an indication of the overall activity of polyamine metabolism in these tumors. Twenty-two tumors were examined. Samples of tumors studied included 6 astrocytomas, 5 meningiomas, 4 neurofibromas, and 7 miscellaneous intracranial tumors (2, ependymomas, 1 craniopharyngioma, 1 angioblastic meningioma and 3 metastatic tumors). Polyamine conversion in single samples of cerebral and cerebellar cortex obtained at the time of surgery in these patients was also determined (Table 1).

The results obtained from the 6 histologically confirmed astrocytomas are summarized in Table 2. Although there was some variation in the percent of labeled putrescine converted to spermidine and spermine among these tumors, all specimens showed a definite active conversion. The mean percent conversion in this group was 8.9% of the labeled putrescine. Table 3 displays the data for 5 intracranial meningiomas. The mean percent conversion of putrescine to spermidine and spermine was 3.8%. Although this suggests a difference in polyamine metabolism between these types of tumors, the results are not significantly different (p=0.2, Student's t-test).

Table 4 includes similar data obtained from 4 acoustic neuromas that appeared to be typical nerve sheath tumors on histological examination. There was virtually no conversion of putrescine

<table>
<thead>
<tr>
<th>Source</th>
<th>% protein</th>
<th>nCi/gm wet weight</th>
<th>% conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Putrescine</td>
<td>Spermidine</td>
<td>Spermine</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>8.73</td>
<td>120</td>
<td>1.6</td>
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<tr>
<td>Cerebellar cortex</td>
<td>8.64</td>
<td>185</td>
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