Altered Polyamine Profiles in Prostatic Hyperplasia and in Kidney Tumors

Udo Dunzendorfer and Diane Haddock Russell

ABSTRACT

Polyamine concentrations were evaluated in normal human prostatic tissue as well as hyperplastic prostate. Normal tissues had high concentrations of putrescine and spermidine with intermediate spermine concentrations, whereas there was a dramatic increase in the spermine concentration in patients with hypertrophy of the prostate. Although not highly significant, spermidine concentrations were elevated slightly in benign hyperplasia, whereas the putrescine content was decreased compared to normal tissue. Polyamine concentrations were measured also in human kidney tumors and corresponding healthy kidney tissue. The concentration of spermidine in renal carcinomas was significantly elevated when compared to histologically normal areas of the same kidney. The spermine concentration of the tumor was generally lower but not highly significant (p < 0.01). These data suggest that polyamines are accumulated above normal levels in pathological conditions such as benign hyperplasia of the prostate and renal carcinoma. In both cases, spermidine turnover rate may be influenced by carcinogenesis.

INTRODUCTION

Elevated urinary excretion of polyamines has been well documented in patients with metastatic cancer (2, 4, 6-8, 13, 15, 17, 20, 23, 27, 28). The level of putrescine in the urine reflects the growth fraction of a tumor, whereas the spermidine concentration is related to the tumor cell loss factor (6, 15, 17, 18, 21, 22). Alterations in tumor kinetics related to success or failure of therapy, therefore, can be assessed readily by polyamine determinations (6, 17). Abnormal polyamine excretion also has been reported in patients with psoriasis and cystic fibrosis, other pathologies with increased cell turnover (3, 11, 12, 16, 24). Intracellular polyamine concentrations are highest in tissues with a high percentage of the cells in cell cycle, i.e., tissues undergoing rapid growth (1, 14). Polyamines are markedly elevated in tumors during development (19).

To elucidate further the alterations in intracellular polyamine concentrations in normal and neoplastic tissues, we analyzed putrescine, spermidine, and spermine concentrations in normal and hyperplastic tissues of the human prostate. Polyamines of prostatic origin are normally found in high concentrations in semen (9). Polyamine concentrations were measured also in kidney tumors and in corresponding tumor-free areas of the same kidney. The major alteration in the hyperplastic prostate was a significant increase in spermine concentration (56.8 nmol/mg of protein in hyperplastic prostate versus 24.4 nmol/mg of protein in normal prostate, p < 0.005). In kidney tumors, there was a significant increase in the spermidine/spermine ratio. This ratio is known to be highest in tissues with high rates of RNA and protein synthesis. Abnormal polyamine levels in extracellular fluids in pathological states appear to reflect altered specific polyamine accumulation patterns as well as alterations in growth fraction and cell loss factor.

MATERIALS AND METHODS

Materials

Normal prostate tissue was obtained from kidney donors (n = 2) and from young males (n = 3) after accidental death. Tissue was obtained by open surgery without the use of a coagulant or irrigating fluids. The specmen was examined histologically, and only those of mixed hyperplasia (glandular and stromal) were used in the study. Tissues were stored at -30° within 1 hr. Prostate tissues exhibiting benign hyperplasia (n = 23) and renal tumor samples (n = 13), as well as corresponding tumor-free areas as established by histology, were obtained and frozen directly after operation at -30°. The control tissue for renal tumors was composed of cortical areas free of tumor inasmuch as adenocarcinoma of the kidney forms in proximal tubules, mainly in cortex.

Tissue Preparation

Tissues were homogenized in 4 volumes of cold 10% trichloroacetic acid and centrifuged at 50,000 x g for 20 min. The supernatant was diluted 1/20 so that polyamine concentrations were close to 220 pmol/20 µl. Standards of 200 pmol/20 µl of each amine were run routinely, and trace amounts of 14C-polyamines were added to assess recovery rates. Recovery rates were consistently 100%.

Cation Exchange Analysis Procedure for Polyamines

Analyzer. A Durrum D-500 amino acid analyzer (Durrum Instrument Corp., Sunnyvale, Calif.) equipped with a fluorescence detector assembly with a 2-mm-path-length flow cell was used for polyamine analyses. All sample functions, including injection and peak area analysis, were accomplished by a PDP8/M computer (Digital Equipment Co., Maynard, Mass.). As many as 80 sample cartridges can be
was prepared by adding 25 g of KOH to 800 ml of deionized water. The pH was adjusted to 5.6 with concentrated hydrochloric acid. For Buffer 2R, stock buffer was diluted to 1.9 M KCl and 0.07 M potassium citrate. For Buffer 2L, stock buffer was diluted 1/2 and contained 1.2 M KCl and 0.035 M potassium citrate per liter. For Buffer 1L, stock buffer was diluted 1/5 and contained 0.5 M KCl and 0.02 M potassium citrate per liter. Buffers were filtered through a Millipore filter (47-mm diameter, 0.45-μm pore size) before use.

Buffers. The stock buffer solution (Buffer 3R) consisted of 2.4 M potassium chloride (KCl), 0.09 M potassium citrate, and 5 ml of thioglycolic per liter of double-deionized distilled water. The pH was adjusted to 5.6 with concentrated hydrochloric acid. For Buffer 2R, stock buffer was diluted to 1.9 M KCl and 0.07 M potassium citrate. For Buffer 2L, stock buffer was diluted 1/2 and contained 1.2 M KCl and 0.035 M potassium citrate per liter. For Buffer 1L, stock buffer was diluted 1/5 and contained 0.5 M KCl and 0.02 M potassium citrate per liter. Buffers were filtered through a Millipore filter (47-mm diameter, 0.45-μm pore size) before use.

Fluorescent Reagent. o-Phthalaldehyde, obtained from Aldrich Chemical Co. (San Leandro, Calif.), was prepared in the following manner. One liter of borate buffer, pH 10.4, was prepared by adding 25 g of KOH to 800 ml of deionized water. Boric acid was added to the KOH solution until the pH was 10.4 ± 0.1 (S.D.). Deionized water was added to adjust the volume to 1 liter. The following chemicals were then added to the borate buffer: 4.5 ml of 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.), 3 ml of BRU (30% solution) (Pierce Chemical Co., Rockford, Ill.), and 5.8 g of KSCN (Sigma Chemical Co.). The o-phthalaldehyde crystals were dissolved in 20 ml of glass-distilled methyl alcohol, and this mixture was added to the buffer solution and stirred gently. The solution was purged with nitrogen for 10 min in the amino acid analyzer reagent reservoir.

Elution Program. Elution and separation of the polyamines was accomplished in 62.5 min utilizing a 4-buffer system as follows. Buffer 1L was directed to the column for 30 min to elute the amino acids that do not bind to the column at the pH and ionic strength of this buffer; Buffer 2L was then used for 10 min to elute putrescine; Buffer 2R was directed to the column for 11 min to elute cadaverine and spermidine; finally, Buffer 3R was used for 11.5 min to elute spermine. Buffer flow rate was 18.5 ml/hr and the o-phthalaldehyde flow rate was 9.5 ml/hr. Reagent flow was started 10 min prior to switching to Buffer 2L. Column temperature was held isothermally at 60-62°C and column pressure generated during analysis was approximately 1600 psi. Full-scale deflection on the recorder was set at 0.1 absorbance unit. o-Phthalaldehyde reacts with primary amines to form intensely blue-fluorescing condensation products with an activation maximum at 340 nm and an emission maximum at 455 nm. Following each analysis the column was regenerated with a solution of 1 M KOH and then equilibrated for 10 min with Buffer 1L. As little as 20 pmol of each polyamine could be accurately detected.

RESULTS

Polyamine Concentrations in Normal and Hyperplastic Prostate. Normal prostates have high concentrations of putrescine and spermine with intermediate spermidine concentrations (Table 1). In patients with hypertrophy of the prostate, there was a significant increase in the spermine concentration (Table 1). Although not highly significant, spermidine concentrations were slightly elevated whereas putrescine content was decreased compared to patients with normal prostates.

Polyamine Concentrations in Kidney Tumors and in Corresponding Healthy Kidney Tissue. The concentration of spermidine in renal carcinomas is significantly elevated (p < 0.005) when compared to histologically normal areas of the same kidney (Table 2). Spermine concentration of the tumor was generally lower but not highly significant (p < 0.01). The levels of spermidine and spermine in normal kidney tissue and in corresponding tumor samples of the same organ are significantly correlated (r = 0.68; p < 0.05). This indicates that the metabolism of spermidine and spermine in renal carcinomas is not basically altered.

Table 3 summarizes the statistical analysis of alterations in polyamine content in normal and pathological kidney tissue and normal and pathological prostate.

DISCUSSION

These data suggest that polyamines are accumulated above normal levels in pathological conditions such as
### Table 2

**Polyamines in tissue of patients with renal carcinomas**

Control determination of polyamines was carried out in corresponding tissue samples free from tumor as proven by histology.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Histology</th>
<th>TNM</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
<th>Spermidine/Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>56</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.99</td>
<td>3.2</td>
<td>27.0</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>54</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.50</td>
<td>4.2</td>
<td>9.2</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>63</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.08</td>
<td>2.3</td>
<td>6.9</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>64</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.11</td>
<td>4.6</td>
<td>3.5</td>
<td>1.3</td>
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<tr>
<td>5</td>
<td>M</td>
<td>58</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.06</td>
<td>2.4</td>
<td>1.9</td>
<td>1.3</td>
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<tr>
<td>6</td>
<td>M</td>
<td>72</td>
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<td>T,N,M</td>
<td>0.18</td>
<td>6.1</td>
<td>4.5</td>
<td>1.4</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
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<td>Normal</td>
<td>T,N,M</td>
<td>0.35</td>
<td>4.2</td>
<td>3.7</td>
<td>1.1</td>
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<tr>
<td>8</td>
<td>F</td>
<td>50</td>
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<td>T,N,M</td>
<td>0.13</td>
<td>3.7</td>
<td>3.4</td>
<td>1.1</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>52</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.15</td>
<td>5.4</td>
<td>3.1</td>
<td>1.7</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>65</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.05</td>
<td>1.7</td>
<td>4.6</td>
<td>0.4</td>
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<tr>
<td>11</td>
<td>F</td>
<td>23</td>
<td>Pathological</td>
<td>T,N,M</td>
<td>1.60</td>
<td>4.1</td>
<td>3.8</td>
<td>1.1</td>
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<tr>
<td>12</td>
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<td>80</td>
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<td>0.9</td>
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<td>13</td>
<td>F</td>
<td>62</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.14</td>
<td>1.3</td>
<td>1.4</td>
<td>0.9</td>
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<tr>
<td>14</td>
<td>F</td>
<td>66</td>
<td>Pathological</td>
<td>T,N,M</td>
<td>0.08</td>
<td>1.1</td>
<td>3.4</td>
<td>0.3</td>
</tr>
<tr>
<td>15</td>
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<td>60</td>
<td>Normal</td>
<td>T,N,M</td>
<td>2.90</td>
<td>2.1</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>54</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.15</td>
<td>3.3</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>60</td>
<td>Pathological</td>
<td>T,N,M</td>
<td>0.07</td>
<td>2.5</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>60</td>
<td>Normal</td>
<td>T,N,M</td>
<td>0.26</td>
<td>5.7</td>
<td>7.2</td>
<td>0.8</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>56</td>
<td>Normal</td>
<td>T,N,M</td>
<td>Trace</td>
<td>Trace</td>
<td>3.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*TNM, tumor-nodes-metastasis classification.

### Table 3

**Tissue level of polyamines in kidney and prostate**

The results were analyzed by paired and unpaired Student's t test.

<table>
<thead>
<tr>
<th></th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D.</td>
<td>p</td>
<td>Mean ± S.D.</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0.11 ± 0.29</td>
<td>0.2</td>
<td>2.18 ± 1.58</td>
</tr>
<tr>
<td>Pathological (RC)</td>
<td>0.29 ± 0.44</td>
<td></td>
<td>3.91 ± 1.52</td>
</tr>
<tr>
<td><strong>Prostate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6.53 ± 4.70</td>
<td>0.2</td>
<td>3.31 ± 1.57</td>
</tr>
<tr>
<td>Pathological (BHP)</td>
<td>3.42 ± 2.55</td>
<td></td>
<td>4.89 ± 2.10</td>
</tr>
</tbody>
</table>

The high concentration of spermine in hyperplastic prostate is of interest and may be related to the dependence of \( S - \)adenosyl-L-methionine decarboxylase, the rate-limiting enzyme in both spermidine and spermine synthesis, on androgens (26). Androgens and luteinizing hormone are significantly altered with increasing age (5, 10). The imbal-
ance of hormones in elderly men has been postulated as a cause of proliferation of paraurethral glands.

In summary, specific polyamine accumulation patterns are altered in hyperplastic and neoplastic states. Altered regulation of the biosynthesis and metabolism of spermidine and spermine may be involved. Since putrescine or spermidine is required for the synthesis of spermidine and spermine, respectively, S-adenosyl-L-methionine decarboxylase activity may have altered substrate requirements under these pathological conditions. It is already known that cell loss factors are higher in certain tumors (25) and, as previously discussed, excretion of polyamines is elevated in patients with neoplasias.

REFERENCES

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