Possible Link Between Zinc Intake and Colon Cancer

Moon K. Song, Madalene C. Y. Heng, Rolando Rolandelli, Marvin E. Ament, Ming K. Heng*

Both zinc and calcium are essential nutrients. Low intake of zinc and of calcium may increase the incidence of colorectal cancers (1-3). Both zinc and calcium regulate a large number of intracellular enzyme activities, including the activities of adenylate cyclase (4) and of phospholipases (5), and act as second messengers (6,7). These activities are involved in the cascade of reactions in the regulation of gene transcription and cell growth. Thus, adequate intake of zinc and of calcium is important for health and the prevention of disease. Because calmodulin (CaM) is activated by calcium (8) but is inhibited by zinc (9) and because CaM and cyclic adenosine monophosphate (cAMP) phosphodiesterase are activated in sequence (10), it is possible that CaM and cAMP levels in colon tumor cells change in relation to the dietary intake of zinc and of calcium, and these changes may be either protective against disease or carcinogenic.

We undertook the present study (a) to determine the relationships between the metabolism of zinc, calcium, cAMP, and CaM in colon tumor cells and (b) to establish a relationship between these metabolic changes and those of zinc nutriture-altered normal colon cells.

Surgically removed human colon carcinomas and adjacent normal colon tissues from the same patients were obtained from the Departments of Surgery of the Sepulveda DVA Medical Center and of the Kaiser Permanente Medical Group, Los Angeles. They were immediately transported on dry ice to the Departments of Pathology of both medical centers and stored at -70 °C in our laboratory until used for the biochemical analysis.

Colon tumor cells from rats were prepared by a modified method described by Reddy and Maeura (11). The colon tumor cells were induced by subcutaneous injection of azoxymethane (15 mg/kg body weight) into male F344 rats once weekly for 2 weeks, and the tumor cells were harvested 30 weeks after carcinogen injection.

The procedures for the preparation of the human and rat cells were in accord with the guidelines of the Human and Animal Study Committees of the Sepulveda DVA Medical Center.

Frozen tumor and normal cells from human subjects and from rats were homogenized three times for 2-second intervals with a tapered Teflon plunger with a glass tube, transferred into a 5-mL-capacity polypropylene test tube, and centrifuged in an IEC Model PR-6 Centrifuge (International Equipment Co., Needham Heights, Mass.) at 3000g for 15 minutes. The supernatant was analyzed for zinc, calcium, cAMP, and CaM. The concentrations of zinc and calcium were determined with an Atomic Absorption Spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.), and the concentrations of cAMP and CaM were determined by use of radioimmunoassay kits (DuPont Co., Wilmington, Del.).

Table 1 shows that, although the zinc concentrations in the rat tumor cells were significantly higher than those in the adjacent normal colon cells (P<.01), the zinc concentrations in the human tumor cells tended to be lower than those in the adjacent normal cells. It is possible that zinc concentrations in human colon tumor cells were lower than in normal cells because patients were put on zinc-depleted, clear fluids for a few days prior to surgery. Under these conditions, metabolism of zinc would be greater in tumor cells than in normal cells because zinc is required for tumor propagation. This hypothesis was supported by the facts that (a) the ratios between the concentrations of zinc and calcium in both human subjects and rats were significantly higher in colon carcinomas than in adjacent normal cells (P<.001 and P<.01, respectively) and (b) the ratios between zinc and calcium concentrations in normal colon cells isolated from rats fed a zinc-excess diet were also significantly higher than those in normal colon cells from rats fed either a zinc-adequate or a zinc-deficient diet (P<.01).

Furthermore, Table 2 shows that the ratios of cAMP to CaM concentrations in colon tumor cells of both human subjects and rats were significantly higher than those in adjacent normal colon cells (P<.001). Thus, the metabolism of zinc and of calcium was closely related to that of CaM and of cAMP in both the human subjects and the rats. These ratios were also similar to those found in rats fed a zinc-excess diet compared with those fed a zinc-adequate or a zinc-deficient diet. Therefore, we believe that it is highly likely that human colon tumor cells have excess concentrations of zinc.

Although there are several biochemical similarities between colon cells from rats fed a zinc-excess diet and colon tumor cells from both human subjects and rats (Table 1 and 2), the impact of these biochemical similarities on the pathophysiology of colon cancer is largely unknown. Since cAMP, CaM, and prostaglandin E2 regulate each other's activities (12-14) and since zinc and calcium influence prostaglandin metabolism (15,16), dietary effects of calcium and zinc on colon cancer induction may be mediated via mechanisms regulating prostaglandin synthesis. Prostaglandins induce colorectal cancer (16), and partial inhibition of prostaglandin synthesis by aspirin or indomethacin is associated with a reduced risk of human and animal colon cancers (17-20). Since pros-
taglandin E₂ stimulates zinc uptake and zinc-retaining capacities of rat enterocytes (27) and since concentrations of prostaglandin E₂ in human colon tumor cells (16) and zinc in rat colon tumor cells (Table 1) are almost twice as high as those in normal colon cells, the role of prostaglandins in the pathophysiology of colon cancer appears to be via their influence on cellular zinc metabolism. Zinc stimulates gene transcription and cell proliferation (22), and an increased cellular zinc concentration may contribute to tumor cell propagation. Therefore, maintaining the optimal cellular zinc concentration may be critical for preventing colon cancer or tumor growth.

### References

12. Brewar GI: Calmodulin, zinc and cal-

### Table 1. Concentrations of zinc and calcium in cytosols of colon tumor cells and adjacent normal colon cells isolated from F344 rats and human subjects and in rats fed various amounts of zinc in the diet

<table>
<thead>
<tr>
<th>Source</th>
<th>Experimental condition*</th>
<th>No. of subjects</th>
<th>Mean ± SE</th>
<th>Ratio (zinc/calcium) X 10³</th>
<th>Ratio (zinc/calcium/2) X 10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>Normal cells adjacent to tumor cells</td>
<td>7</td>
<td>167.7 ± 10.4</td>
<td>3130.1 ± 210.0</td>
<td>56.3 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>Tumor cells</td>
<td>7</td>
<td>294.4 ± 26.7</td>
<td>1765.6 ± 126.7</td>
<td>171.7 ± 19.4</td>
</tr>
<tr>
<td></td>
<td>Male rats fed a zinc-deficient diet</td>
<td>6</td>
<td>77.5 ± 9.2</td>
<td>1007.7 ± 171.9</td>
<td>84.9 ± 14.3</td>
</tr>
<tr>
<td></td>
<td>Male rats fed a zinc-adequate diet</td>
<td>6</td>
<td>82.8 ± 5.6</td>
<td>999.3 ± 99.3</td>
<td>88.5 ± 12.4</td>
</tr>
<tr>
<td></td>
<td>Male rats fed a zinc-excess diet</td>
<td>6</td>
<td>176.0 ± 39.1</td>
<td>14660 ± 175.4</td>
<td>117.9 ± 10.2§</td>
</tr>
<tr>
<td>Humans</td>
<td>Normal cells adjacent to tumor cells</td>
<td>7</td>
<td>92.7 ± 7.6</td>
<td>12427 ± 22.4</td>
<td>74.6 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>Tumor cells</td>
<td>7</td>
<td>78.9 ± 10.7</td>
<td>6543 ± 11.1§</td>
<td>121.3 ± 10.3§</td>
</tr>
</tbody>
</table>

* Eighteen 3-month-old male rats were divided into three groups of six rats each and were fed for 2 weeks one of the following diets: a zinc-deficient diet (1 µg zinc/µg of diet), a zinc-adequate diet (40 µg zinc/µg of diet), or a zinc-excess diet (1 mg zinc/µg of diet).
† The values for the ratios between zinc and calcium were average values. Therefore, the mean ratio values are not necessarily the same as the exact ratios between the mean value of zinc and calcium.
‡ P<.001 when comparing the values for normal colon cells with those for tumor cells by paired Student's t statistic.
§ P<.05 compared with the values in rats fed a zinc-deficient or zinc-excess diet.
||/ P<.05 compared with the values for rats fed a zinc-adequate diet.
€/€ P<.05 compared with the values for rats fed a zinc-deficient or zinc-adequate diet.

### Table 2. Concentrations of cAMP and CaM in cytosols of colon tumor cells and adjacent normal colon cells isolated from F344 rats and human subjects and in rats fed various amounts of zinc in the diet

<table>
<thead>
<tr>
<th>Source</th>
<th>Experimental condition*</th>
<th>No. of subjects</th>
<th>Mean ± SE</th>
<th>Ratio (cAMP/Calcium) X 10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>Normal cells adjacent to tumor cells</td>
<td>6</td>
<td>9.1 ± 0.9</td>
<td>435.0 ± 43.5</td>
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<td></td>
<td>Tumor cells</td>
<td>6</td>
<td>11.3 ± 1.6</td>
<td>340.0 ± 68</td>
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<td>Male rats fed a zinc-deficient diet</td>
<td>6</td>
<td>7.0 ± 1.3</td>
<td>506.7 ± 94.6</td>
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<td>Male rats fed a zinc-adequate diet</td>
<td>6</td>
<td>9.6 ± 2.2</td>
<td>411.5 ± 44.1</td>
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<td>Male rats fed a zinc-excess diet</td>
<td>6</td>
<td>18.4 ± 3.18</td>
<td>358.0 ± 57.7</td>
</tr>
<tr>
<td>Humans</td>
<td>Normal cells adjacent to tumor cells</td>
<td>7</td>
<td>10.7 ± 1.3</td>
<td>560.3 ± 165.3</td>
</tr>
<tr>
<td></td>
<td>Tumor cells</td>
<td>7</td>
<td>15.2 ± 2.0</td>
<td>520.0 ± 37.7</td>
</tr>
</tbody>
</table>

* See Table 1 footnote (*) for explanation of experimental conditions.
† The values for the ratios between cAMP and CaM were average values. Therefore, the mean ratio values are not necessarily the same as the exact ratios between the mean value of cAMP and CaM.
‡ P<.001 when comparing the values for normal colon cells with those for tumor cells by paired Student's t statistic.
§ P<.05 compared with the values for rats fed either a zinc-deficient or a zinc-adequate diet.
||/ P<.05 compared with the values for rats fed a zinc-deficient diet.
€/€ P<.05 compared with the values for rats fed a zinc-deficient or zinc-excess diet.


Notes

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