Alkaline Phosphatase as Tumor Marker

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ABSTRACT

Applications and limitations of alkaline phosphatase as a tumor marker are discussed. This review focuses on three characteristic isoenzymes that have been found in the serum of cancer patients: (1) the Regan isoenzyme in bronchogenic carcinoma; (2) the Nagao isoenzyme in pleuritis carcinomatosa; and (3) the hepatoma alkaline phosphatase. Tumor variants of alkaline phosphatase are classified based on their heat stability properties resembling or differing from the placental isoenzyme. They can also be differentiated in their behavior to specific inhibitors. The presence of electrophoretic variants and enzyme-immunoglobulin complexes in other cancers are also described.

Introduction

Since the identification and characterization of a unique isoenzyme of alkaline phosphatase, indistinguishable from the placental isoenzyme, in the serum of a patient with bronchogenic carcinoma (the Regan isoenzyme\(^5\)), numerous reports have appeared on the existence of characteristic isoenzymes of alkaline phosphatase in the sera of patients with various malignancies.\(^6,13,20\) Unique isoenzymes have been broadly characterized in relation to the placental isoenzyme.

In addition to the Regan isoenzyme, the Nagao isoenzyme\(^13,14\) and hepatoma alkaline phosphatase isoenzyme have been well characterized.\(^7,8,17,19\)

Enzymes may exist in the circulation complexed to immunoglobulins, and this has led to the identification of alkaline phosphatase-immunoglobulin complexes in malignancies such as prostatic carcinoma.\(^1\)

Methods For Studying Tumor Alkaline Phosphatase

Heat Treatment\(^4,13\)

Tumor alkaline phosphatase can be distinguished from the placental isoenzyme in its sensitivity to heat, the latter being heat-stable at 65°C for 10 minutes.

Electrophoresis\(^4,8,11,13,17\)

Several types of zone media have been utilized including polyacrylamide, starch and agar gels. Subsequent to electrophoresis alkaline phosphatase activity is visualized by staining the gel with a substrate disodium α-naphthyl phosphate, a dye such as Fast blue BB salt in Tris or...
carbonate buffer containing a magnesium salt.

**Immuno-electrophoresis**

This procedure followed by staining for alkaline phosphatase is used for identification of immunoglobulin-enzyme complexes.

**Gel Permeation Chromatography**

Chromatography of sera on an appropriate gel permeation column (such as Sephadex-G-200) is useful for the separation of high molecular weight alkaline phosphatases complexed to an immunoglobulin or abnormal lipid, Lipoprotein-X.

**Specific Inhibitors**

Measurement of alkaline phosphatase activity, following incubation with organ specific inhibitors of alkaline phosphatase, L-phenylalanine, and L-homocitrulline, is used to distinguish placental and non-placental types of tumor alkaline phosphatase. Incubation with L-leucine is used to identify the Nagao isoenzyme.6,13,14 Other inhibitors that can be employed include ethylenediamine tetraacetic acid (EDTA) and urea.8

**Regan Isoenzyme**

This isoenzyme first reported in a patient named Peter Regan with bronchogenic carcinoma is virtually indistinguishable from the placental enzyme in its electrophoretic mobility and sensitivity to heat.5 Like the placental enzyme, it is also sensitive to L-phenylalanine. The degree of inhibition is dependent on the concentration of L-phenylalanine. At 1 mM L-phenylalanine concentration, both the placental and the Regan isoenzyme are inhibited 23 percent whereas at 5 mM the inhibition may be as high as 70 to 80 percent.4,13 Inhibition by L-phenylalanine is of the noncompetitive type. Serum containing Regan isoenzyme can be precipitated by antibody to placental alkaline phosphatase. Following incubation at 65°C for 10 minutes, both the placental and Regan isoenzymes retain virtually all the original activity. The Regan isoenzyme is more sensitive to urea than the placental isoenzyme suggesting differences in three-dimensional structure.4

**Nagao Isoenzyme**

This isoenzyme, first reported in the serum of a patient named Nagao who had pleuritis carcinomatosa, resembles the Regan isoenzyme in that it is stable after incubation at 65°C for 10 minutes, has a similar electrophoretic mobility, and is precipitated by antibody to the placental enzyme.13,14 However, it is more sensitive to L-phenylalanine at a concentration of 1 mM, in that 59 percent of the enzyme activity is inhibited as compared to 23 percent for the Regan isoenzyme.13 Its sensitivity to L-leucine and EDTA is pronounced as compared to the Regan isoenzyme which is only minimally inhibited. In table I, the behavior of Nagao and Regan isoenzymes to L-leucine and EDTA is compared.13 The Nagao isoenzyme has an increased affinity for the substrate phenylphosphate as compared to the Regan isoenzyme. The Km for this substrate using the Nagao isoenzyme was

<table>
<thead>
<tr>
<th>Inhibitor Differences Between Nagao and Regan Isoenzymes</th>
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<tbody>
<tr>
<td>Inhibitor</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>L-leucine</td>
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<tr>
<td>EDTA*</td>
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</tbody>
</table>

*Ethylenediamine tetraacetic acid
0.26 mM as compared to a Km of 2.2 mM obtained with the Regan isoenzyme.\textsuperscript{13}

The Nagao isoenzyme has been reported to be a variant of placental alkaline phosphatase, a hybrid, perhaps, of liver and placental alkaline phosphatase, the D-variant.\textsuperscript{9} However, the identity between the Nagao isoenzyme and the D-variant has been questioned.\textsuperscript{13}

**Hepatoma Alkaline Phosphatase**

Sera of patients with hepatoma have circulating alkaline phosphatase which is heat sensitive. Whereas the Regan and Nagao isoenzymes are virtually stable after incubation at 65°C for 10 minutes, as much as 79 percent of the hepatoma alkaline phosphatase activity is inhibited.\textsuperscript{8} Inhibition studies using 1 mM L-phenylalanine and 0.5 mM L-leucine suggest that it is more akin to the Regan as compared to the Nagao isoenzyme. Similarly, it is not particularly sensitive to L-homoarginine, an inhibitor of liver alkaline phosphatase, thus resembling Regan isoenzyme. However, like the Nagao isoenzyme the hepatoma alkaline phosphatase is highly sensitive to EDTA. In the presence of urea it is more stable than liver alkaline phosphatase but less so than placental alkaline phosphatase.\textsuperscript{17} In Table II is summarized the sensitivity of hepatoma alkaline phosphatase to various inhibitors.\textsuperscript{8}

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>Percent Inhibition</th>
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</thead>
<tbody>
<tr>
<td>L-phenylalanine</td>
<td>1.0</td>
<td>41</td>
</tr>
<tr>
<td>L-leucine</td>
<td>0.5</td>
<td>13</td>
</tr>
<tr>
<td>EDTA*</td>
<td>0.5</td>
<td>83</td>
</tr>
<tr>
<td>L-homoarginine</td>
<td>10.0</td>
<td>28</td>
</tr>
<tr>
<td>Urea</td>
<td>4.0</td>
<td>66</td>
</tr>
</tbody>
</table>

*Ethylenediamine tetraacetic acid

**Other Malignancies**

The presence of alkaline phosphatase with altered electrophoretic mobility and properties in various malignancies such as carcinoma of ovary,\textsuperscript{16} bile duct,\textsuperscript{10} stomach,\textsuperscript{15} pancreas,\textsuperscript{18} colon and rectum\textsuperscript{2} have been reported. However, definitive studies are needed before these isoenzymes can be regarded as specific tumor markers.

Recently an alkaline phosphatase variant was described in the serum of a patient with renal cell carcinoma.\textsuperscript{20} The variant appeared to be an altered form of normal renal alkaline phosphatase. It is more resistant to heat than liver alkaline phosphatase, the half-inactivation time at 56°C being 184 seconds for the variant as compared to 112 and 456 seconds for bone and liver alkaline phosphatases, respectively. The variant also migrates differently from liver, intestinal, and kidney alkaline phosphatase on polyacrylamide gel electrophoresis. The migration is closer to that of the bone isoenzyme, although not identical to it.

The presence of serum alkaline phosphatase complexed to IgG has been reported in a patient with carcinoma of prostate.\textsuperscript{1} The complex had a reduced mobility on cellulose acetate electrophoresis distinct from liver, bone, placental, and intestinal bands. Evidence for an IgG-alkaline phosphatase complex was demonstrated by the presence of enzyme activity in the immunoprecipitin arc and by dissociation of the complex with trypsin. The inhibitor characteristics and heat sensitivity of the complex were intermediate between that of liver and bone alkaline phosphatase.

**Summary**

The usefulness of alkaline phosphatase as a tumor marker will require more definitive studies on the variants that have been described. Limitations in present
methodologies include accurate resolution and identification of isoenzyme bands on electrophoresis, since these are complicated with closely migrating bands. Identification of a variant based on its sensitivity to L-phenylalanine is limited since inhibition also occurs with


References
