A Variant Alkaline Phosphatase in Renal Cell Carcinoma

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We report a case of renal cell carcinoma in which up to 32% of the abnormally increased alkaline phosphatase activity in serum was contributed by a variant alkaline phosphatase originating in the primary tumor and its secondary deposits. The variant enzyme was probably an altered form of normal renal alkaline phosphatase. The rest of the alkaline phosphatase activity in the serum was of hepatic origin, but no abnormality of the liver was discovered at autopsy.

Additional Keyphrases: isoenzymes • variant enzymes • a possible tumor 'marker'

Increased alkaline phosphatase activity in the serum of a patient with cancer usually indicates the presence of tumor in liver or bone, or both. The spread of tumor to such sites can be further investigated by separation and evaluation of alkaline phosphatases of bony and hepatic origin by various techniques of isoenzyme analysis. These techniques have also shown, in a small percentage of patients with various cancers, the presence of a circulating alkaline phosphatase that is virtually identical to normal placental alkaline phosphatase—the Regan isoenzyme (1). Minor variants of placental alkaline phosphatase also occur in some cancer patients—the Regan variant and Nagao isoenzyme (2, 3). Placental-type alkaline phosphatase has also been demonstrated in the tumor tissue in many cases.

Other alkaline phosphatase variants, resembling the forms normally found in tissues such as bone rather than the placental isoenzyme, have been reported, which also are apparently produced by tumor cells (e.g., 4-6). Identification of these forms is more difficult because of the relatively slight differences between normal alkaline phosphatases from tissues other than placenta or small intestine.

In patients with renal cell carcinoma, the interpretation of serum alkaline phosphatase activity as a marker of the occurrence and distribution of metastatic disease is complicated by the fact that this activity may be abnormal, together with other liver-function tests, in the absence of pathological changes in the liver (7-9). We now report a patient with a renal cell carcinoma in whom an increased activity of liver-type alkaline phosphatase in serum was observed in the absence of metastatic infiltration of the liver, but in which a variant alkaline phosphatase of non-Regan type was also present, both in the serum and in the renal carcinoma tissue.

Case Report

A 61-year-old white man presented at another hospital than ours in October 1979 with a three-month history of lethargy, anorexia, postprandial epigastric pain, and weight loss. Initial investigations revealed normal values for hematocrit, leukocyte count, serum bilirubin, aspartate aminotransferase (EC 2.6.1.1), and amylase (EC 3.2.1.1). Serum alkaline phosphatase (EC 3.1.3.1) was above normal (20 King–Armstrong units/dL; upper reference limit, 13). Results of an upper gastrointestinal series and chest roentgenogram, ultrasound examination of the pancreas, and endoscopic retrograde cholangio-pancreatography were normal. During the next 10 months his serum alkaline phosphatase increased to 70 King–Armstrong units/dL and hepatomegaly was noted. Hepatic scintiscan and liver biopsy were performed, but the appearances of both were normal.

In September 1980 he was referred to the Hammersmith Hospital. On examination the liver was enlarged 7 cm below the costal margin and a mass was detected in the left upper quadrant of the abdomen. A bruist was audible in the left renal angle. Ultrasound examination, intravenous pyelography, and arteriography showed the presence of a renal cell carcinoma in the left kidney. The right kidney was normal.

Biochemical estimations on the serum showed a normal bilirubin concentration, normal aspartate aminotransferase activity, and 945 U of alkaline phosphatase activity per liter (reference interval, <230). The serum calcium concentration was above normal, 2.77 mmol/L (reference interval, 2.15–2.65), and the inorganic phosphate concentration was normal (1.2 mmol/L; reference interval, 0.8–1.4). Serum creatinine was normal (87 µmol/L; reference interval, 55–125).

Chest roentgenograms at this time revealed multiple metastases in both lung fields, but results of a methylene diphenyl phosphonate 99Tc bone scan were normal. Before nephrectomy, the patient suffered a grand mal convulsion and his serum calcium, estimated immediately afterwards, had increased to 3.7 mmol/L; it declined to normal on treatment with intravenous fluids and dexamethasone and remained normal thereafter. Determination of serum immunoreactive parathyin at the time of the hypercalcemic episode showed it to be below the limits of detection. Multiple cerebral metastases were revealed by computerized axial tomography.

On 16 September 1980, left nephrectomy was performed. Histological examination of the tissue removed at nephrectomy showed a clear-cell adenocarcinoma. Despite an initially good postoperative recovery, further grand mal fits occurred, requiring control by phenytoin (300–400 mg daily). Treatment with medroxyprogesterone acetate and testosterone did not induce regression of the pulmonary metastases, and the patient died on 31 October 1980.

The autopsy confirmed the presence of multiple pulmonary and cerebral metastases, but no metastatic disease was detected on careful examination of the liver or skeletal system.

Materials and Methods

Alkaline phosphatase and γ-glutamyltransferase (EC
2.3.2.2) were extracted from the primary tumor and from histologically normal regions of the kidney removed at operation, and alkaline phosphatase was extracted from the liver and a secondary tumor that was removed from the lung after death. We homogenized 0.75-g (wet weight) samples of each tissue for 30 s with an Ultra-Turrax homogenizer (Janke and Kunkel KG) in 3 mL of Tris HCl buffer (10 mmol/L, pH 7.7), and then mixed for 2 h with 4.5 mL of butan-1-ol. The aqueous phase, which contained the enzymes, was separated from the butan-1-ol phase by centrifugation for 30 min and then dialyzed for 24 h against more of the Tris HCl buffer. Alkaline phosphatase and γ-glutamyltransferase activities in the extracts and in sera were measured at 37 °C (10, 11).

The tissue extracts and serum samples were electrophoresed on vertical polyacrylamide slab gels at pH 9.5 with Tris-borate-MgCl₂ buffer (Tris 0.38 mol/L, adjusted to pH 9.5 with a 20 g/L solution of boric acid, and containing 0.5 mmol of MgCl₂ per liter). The gels were stained for alkaline phosphatase activity by incubation with disodium 1-naphthyl phosphate (4 mmol/L) and diazotized 4'-amino-2',5'-diethoxybenzene (Fast Blue BB salt; 3 mmol/L) dissolved in this buffer. In some experiments samples were incubated with neuraminidase (EC 3.2.1.18) for 24 h at 37 °C before electrophoresis.

We investigated the heat-stability characteristics of alkaline phosphatase in serum and tissue extracts by measuring residual activity after incubation at 56 °C (12). Tissue extracts were mixed with an equal volume of human serum that had been heated at 56 °C for 60 min to inactivate endogenous alkaline phosphatase, to ensure comparable conditions of protein concentration and pH during the heat-inactivation studies. Half-inactivation times at 56 °C were calculated from plots of residual activity (on a logarithmic scale) vs duration of incubation.

The degree of inhibition of alkaline phosphatase activity by L-phenylalanine was determined by measuring the activity with and without L-phenylalanine (5 mmol/L) in the reaction mixture.

**Results**

Electrophoresis (Figure 1) and heat-inactivation analysis of serum before nephrectomy showed the predominant activity to be ascribable to liver-type alkaline phosphatase. However, careful inspection of the original gel revealed a diffuse area of alkaline phosphatase activity with an electrophoretic mobility similar to, but not identical with, that of normal bone alkaline phosphatase, and migrating differently from the alkaline phosphatases of liver, small intestine, or kidney origin. Heat-inactivation analysis showed that this alkaline phosphatase had a half-inactivation time of 184 s at 56 °C as compared with mean values for liver and bone alkaline phosphatases in serum of 456 and 112 s, respectively (Figure 2). The value of 184 s is similar to that for normal renal alkaline phosphatase (not usually detectable in serum), for which we found a half-inactivation time of 170 s in serum.

When first detected, the variant alkaline phosphatase with unusual stability characteristics accounted for 32% of the total activity in serum. After nephrectomy, there was a dramatic decline in the activity of the alkaline phosphatase variant and then a slow increase during the subsequent course of the illness (Figure 3). The liver-type alkaline phosphatase activity in serum also declined steeply after nephrectomy, but this improvement was not maintained and the liver phosphatase activity again increased, more rapidly than that of the variant (Figure 3).

The tumor removed at operation contained a high activity of alkaline phosphatase, indistinguishable by electrophoretic and heat-stability characteristics from the serum variant but clearly differing in electrophoretic mobility from the alkaline phosphatase of adjacent normal renal tissue (Figures 1 and 4). The variant alkaline phosphatase was also detected in a secondary tumor removed from the lung after death. Alkaline phosphatase resembling the liver form of the enzyme was not
detected in primary or secondary tumors. Table 1 summarizes the characteristics of the tumor variant alkaline phosphatase and the enzyme prepared from histologically normal liver and kidney tissue.

During the late stages of the illness, γ-glutamyltranspeptidase activity in serum became supranormal (Figure 3); however, the tumor tissue was relatively poor in this enzyme (0.11 U/g of tissue) as compared with normal kidney (1.2 U/g of tissue) or liver tissue, and therefore it is unlikely to be the source of the increased activity in the serum.

Discussion

In the patient described here, a renal cell carcinoma was associated with the appearance in serum of a variant alkaline phosphatase. It seems clear that the renal tumor tissue was the source of this enzyme, because variant enzyme activity was detected in large amounts in primary and secondary tumors, and removal of the primary tumor led to a dramatic but unsustained decline in the activity in serum. The tumor tissue was highly vascular, which would be expected to facilitate the entry of the variant phosphatase into the circulation.

To our knowledge, this is the first time that a variant alkaline phosphatase has been detected in both serum and in the tumor tissue of a patient with renal cell carcinoma. The Regan isoenzyme was reported in the serum of one of a series of 14 patients with this tumor (13), and the Kasahara enzyme, initially associated with primary hepatocellular carcinoma (14), has also been described in serum of a patient with renal carcinoma (quoted in 15). The Kasahara enzyme appears to be a somewhat modified form of placental phosphatase, because its stability to heat is considerably greater than that of non-placental phosphatases. A Kasahara-variant, apparently differing by one sialic acid residue from the form originally described, has been identified in renal carcinoma tissue but not in the serum of that patient (15).

Ectopic production of an essentially normal isoenzyme such as the placental-type Regan isoenzyme in a patient with a tumor may be a result of de-repression of a normal gene. In contrast, when alkaline phosphatases unlike the normally occurring forms are produced, as in the present case, it is more likely that a somatic cell mutation has occurred in the neoplastic tissue. That both genetic de-repression and post-

Table 1. Properties of Alkaline Phosphatases Extracted from Tissues of the Patient and Present in His Serum

<table>
<thead>
<tr>
<th></th>
<th>Primary tumor</th>
<th>Secondary deposit in lung</th>
<th>Normal kidney</th>
<th>Normal liver</th>
<th>Serum</th>
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<tr>
<td>Specific activity, U/g tissue</td>
<td>2.2</td>
<td>3.5</td>
<td>4.1</td>
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<td></td>
</tr>
<tr>
<td>Half-inactivation time at 56 °C (s)</td>
<td>140</td>
<td>168</td>
<td>170</td>
<td>504</td>
<td>498a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>184b</td>
</tr>
<tr>
<td>Inhibition (%) by 5 mmol/L L-phenylalanine</td>
<td>13.0</td>
<td>14.3</td>
<td>14.5</td>
<td>11.7</td>
<td>13.6</td>
</tr>
<tr>
<td>Effect of incubation with neuraminidase on electrophoretic mobility</td>
<td>Retarded</td>
<td>Retarded</td>
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a Liver component. b Variant form.
translational modification of enzymes can take place in cancer cells is shown by the example of the Kasahara-variant and other modified placental-type isoenzymes. Normal renal alkaline phosphatase shows considerable electrophoretic heterogeneity, apparently owing to variation in carbohydrate side chains, and the difference in electrophoretic mobility between normal kidney alkaline phosphatase and the variant described here may indicate the occurrence of a mutation affecting side-chain structure and expressed in both primary and secondary tumor cells. Such a change would not be expected to affect the heat-stability of the molecule. An alternative hypothesis is that the malignant transformation in this case selectively favors the production of what may be normally a minor form of the enzyme, e.g., a highly-sialated molecular species.

Although the presence of a variant alkaline phosphatase in both tumor and serum in renal cell carcinoma has not been described previously, abnormalities in the liver and in "liver-function tests," particularly serum alkaline phosphatase, are well recognized in this condition. Hepatomegaly, splenomegaly, and increased serum alkaline phosphatase in the absence of hepatic metastases, with a return to normal after removal of a renal carcinoma, have been reported frequently. Most authors have not characterized the serum alkaline phosphatase isoenzyme pattern in these cases, but Axelsson et al. (9) histochemically documented an increase in bile canalicular alkaline phosphatase. The nature of this distant effect on the liver is uncertain, and attempts to extract toxic humoral factors have not been successful. The changes in our patient—including hepatomegaly, an increased value for hepatic alkaline phosphatase, declining after nephrectomy, and the increased γ-glutamyltransferase seen in the late stages of the illness—may all represent a similar phenomenon. However, the administration of the enzyme-inducing agent phenytoin complicates the interpretation of the later changes in the activity of γ-glutamyltransferase.

Our demonstration of a renal carcinoma-derived variant alkaline phosphatase offers another tumor marker that may be of value in diagnosis. As the manifestations of renal carcinoma are often subtle, insidious, and prolonged, detection of a variant alkaline phosphatase may permit early diagnosis and surgical cure by removal of the primary tumor before metastasis has occurred.

References


