Placental Alkaline Phosphatase in Tumour Tissue and Serum

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Summary: Using immunochemical techniques, alkaline phosphatase isoenzymes were determined in tissue samples of breast carcinomas and carcinomas of the gastrointestinal tract. In breast carcinomas only 19% of the patients expressed significant placental alkaline phosphatase activity, compared with 78% in gastrointestinal tumours. The intestinal isoenzyme was found in 50% of the breast carcinomas and in nearly all of the other examined tissues. The two isoenzymes usually represent 1% of the total alkaline phosphatase activity, but in a few cases they may constitute between 10 and 90%. In the serum of the patients under examination, elevated total alkaline phosphatase activity was found in only 7%, and elevated placental alkaline phosphatase in 6% of the cases. No cases of elevated serum intestinal alkaline phosphatase were found. We therefore consider that serum placental alkaline phosphatase is a poor tumour marker for a general screening.

Placentare alkalische Phosphatase in Tumorgewebe und Serum

Zusammenfassung: In Gewebeproben von Mamma-Carcinomen als auch Carcinomen des Gastrointestinaltraktes wurden mit empfindlichen immunologischen Methoden die Isoenzyme der alkalischen Phosphatase bestimmt. Bei Brustkrebs zeigten nur 19% der Patienten signifikante Aktivität der placentaren alkalischen Phosphatase, verglichen mit 78% bei gastrointestinalen Tumoren. Das intestinale Isozym wurde in 50% bei Brustcarcinom und in nahezu allen anderen malignen Geweben gefunden.

Meist betrug die katalytische Aktivität der beiden Isoenzyme unter 1% der gesamten katalytischen Aktivität der alkalischen Phosphatase, in wenigen Fällen zwischen 10 und 90%. Im Serum der untersuchten Patienten wurde nur in 7% der Fälle überhaupt erhöhte katalytische Aktivität der gesamten alkalischen Phosphatase beobachtet, nur in 6% der Fälle placentare alkalische Phosphatase und in keinem der Fälle erhöhte intestinale alkalische Phosphatase gefunden. Wir glauben daher, daß die placentare alkalische Phosphatase für ein generelles Screening ein unbedeutender Tumormarker ist.

Introduction

Since its discovery as the Regan isoenzyme, serum placental alkaline phosphatase in a non-pregnant person has been considered to be a product of oncodevelopmental gene expression, and regarded as tumour marker (1). Owing to the use of different assay methods and the immunological cross-reactivity of the placental and intestinal alkaline phosphatase, controversy has arised regarding the sensitivity of the placental alkaline phosphatase as a tumour marker.

With highly sensitive tests, low levels of placental alkaline phosphatase have been found in the sera of some healthy persons (2, 3), and slightly increased levels in smokers (4, 5).
Also, placent al alkaline phosphatase has been found as a minor component in the lung (6), testis (7), ovary (8) and cervix (9, 10, 6). Thus, in the normal adult, placent al alkaline phosphatase might be produced by a slight expression of developmental genes, which is increased in cancer cells.

We developed a sensitive and easily performed immunoassay for the quantitative determination of placent al alkaline phosphatase and also for intestinal alkaline phosphatase in serum and tissue extracts. In a screening test of 174 sera from treated cancer patients, we found elevated placent al alkaline phosphatase values in only 10% of the samples (11). This unsatisfactory result led us to the quantitative determination of placent al alkaline phosphatase in tumour tissue and its possible appearance in the sera of the patients prior to therapy and surgery.

Materials and Methods

Biological materials

Tissue samples were obtained at operation. Sera gained prior to surgery and the tissue were stored no longer than 24 h at 4 °C or at −20°C until used. The tissue (samples between 0.3 and 8.0 g) was homogenized in 10 mmol/1 Tris/HCl pH 7.5, containing 2 mmol/l MgCl₂, 0.025 mmol/l ZnCl₂ and 0.025 mmol/l p-nitrophenyl phosphate as substrate and 5 mmol/l L-leucyl-glycyl-glycine to inhibit placent al alkaline phosphatase. At a concentration of 5 mmol/l this inhibitor inactivates 90% of the placent al isoenzyme, but only 15% of the intestinal alkaline phosphatase. As only a part of the placent al isoenzyme is bound to the anti-intestinal al kaline phosphatase coated tubes, a good discrimination is possible. After 60 min the reaction was stopped by addition of 100 μl 5 mmol/l NaOH, containing 10 mmol/l EDTA, and the absorbance was measured at 405 nm against a buffer blank. The enzymatic activity was referred to standard samples with known activity of the isoenzyme in 10 μl serum or incubation buffer.

Placent al alkaline solid-phase direct immunoassay

One ml of solution, containing 8 mg anti-placent al alkaline phosphatase IgG in 3 mmol/l phosphate buffer, pH 6.3, was added to an immuno-bead suspension (200 mg in 20 ml buffer, Bio-Rad Laboratories, Richmond/CA) and incubated for 1 h at room temperature. To this 40 mg 1-ethyl(dimethyl-amin o propyl)-car bodiimide hydrochloride (EDAC) was added, mixed vigorously and stored at 4 °C for 3 h. The mixture was equally divided amongst four 25 ml centrifuge tubes and each aliquot was sus pended in phosphate buffered saline to make 25 ml. The tubes were centrifuged at 1000 g for 10 min. The supernatant was decanted, the pellet washed three times by suspension in phosphate buffered saline and centrifugation, followed by 2 washes in 1.4 mmol/l NaCl/phosphate buffered saline and 2 washes in phosphate buffered saline. The mixture was resuspended in phosphate buffered saline and allowed to stand on ice for 3 h in order to render the antibody. After centrifugation, the pellet was resus pended in 20 ml 10 mmol/l Tris/HCl buffer, containing 2 mmol/l MgCl₂, 0.025 mmol/l ZnCl₂ and 0.02% Na₃PO₄. Protein determination in the supernatant revealed that, on average, 35% of the IgG fraction is coupled to the immuno-beads.

The bead suspension (50 μl) (possessing covalently bound anti-placent al alkaline phosphatase) was added to the serum or tissue extract. After 1 h incubation at 37 °C and washing and centrifugation steps (phosphate buffered saline, containing 3% polyethylene glycol 6000, 10 min 10000 g) the pellet was resuspended in 1 ml 10 mmol/l Tris/HCl buffer, pH 7.5, containing 2 mmol/l MgCl₂, 0.025 mmol/l ZnCl₂ and 3% polyethylene glycol 6000 and heated for 90 min at 60 °C to inactivate the partially bound intestinal isoenzyme. Placent al alkaline phosphatase remains unaffected by this heating step, retaining 100% of its catalytic activity, while the intestinal isoenzyme is completely inactivated. With shorter heating periods, up to 10% of the intestinal alkaline phosphatase might be still active. After centrifugation for 10 min at 1000 g, the pellet was resuspended in 900 μl 1 mol/l diethanol amine/HCl pH 9.8 containing 0.5 mmol/l MgCl₂ and 10 mmol/l p-nitrophenyl phosphate. The catalytic activity was determined as described for intestinal alkaline phosphatase.

Intestine alkaline solid-phase direct immuno assay

Polystyrene tubes (50 × 7 mm, Greiner, Nürtingen) were coated with anti-intestinal alkaline phosphatase IgG by incubating them with 1 ml of antibody solution (10 mg/ml in 10 mmol/l phosphate buffer, pH 7.0) for 2 days at room temperature. Non-absorbed antibodies were removed by suction and the tubes were washed three times with phosphate buffered saline (9 g NaCl, 0.27 g KH₂PO₄, 1.43 g Na₂HPO₄·H₂O ad 100 ml H₂O, pH 7.4). Tissue extracts (900 μl) or serum (100 μl) were pipetted into the tubes together with 800 μl of 10 mmol/l Tris/HCl buffer (pH 7.5, containing 2 mmol/l MgCl₂ and 0.025 mmol/l ZnCl₂ and 3% polyethylene glycol 6000) and the mixture was incubated for 1 h at 37 °C followed by 24 h in the cold. After removing the solution by aspiration and washing three times with 1 ml phosphate buffered sa-
say coefficient of variation (determined in 100 µ human serum, 800 µ buffer with purified enzyme added) was 5.7% (mean 2.44 U/l, n = 10).

This solid-phase assay, using antibody-coated tubes, could also be applied to the determination of placental alkaline phosphatase in tissue extracts. On the other hand, the low concentration of serum placental alkaline phosphatase might lead to poor analytical recoveries, using 1 ml serum for the determination.

The immuno-beads assay for placental alkaline phosphatase determination (catalytic concentration range = 0.2–3 U/l) showed an analytical recovery of 60–80% for enzyme standards in buffer or in 1 ml serum. The intra-assay coefficient of variation (determined in 1 ml human serum with purified enzyme added) was 3.3% (mean 0.3 U/l, n= 10) and 5.0% (mean 2.2 U/l, n= 10).

**Tab. 1. Alkaline phosphatase isoenzymes in cancerous and benign tissue extracts.**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Total alkaline phosphatase mU/g¹ (mean value)</th>
<th>Placental alkaline phosphatase mU/g¹ (mean value)</th>
<th>Intestinal alkaline phosphatase mU/g¹ (mean value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast fibroma</td>
<td>1</td>
<td>200</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>36</td>
<td>200–5200 (1007)</td>
<td>0–36</td>
<td>0–11</td>
</tr>
<tr>
<td>Breast carcinoma bone metastasis</td>
<td>9</td>
<td>1500–6500 (20522)</td>
<td>0–2045</td>
<td>0–174</td>
</tr>
<tr>
<td>Stomach benign</td>
<td>4</td>
<td>1100–4200 (2000)</td>
<td>0</td>
<td>1–882</td>
</tr>
<tr>
<td>Stomach carcinoma</td>
<td>19</td>
<td>400–26300 (3813)</td>
<td>0–2000</td>
<td>0–1050</td>
</tr>
<tr>
<td>Sigma diverticulitis</td>
<td>3</td>
<td>500–11000 (733)</td>
<td>0–4</td>
<td>6–18</td>
</tr>
<tr>
<td>Sigma carcinoma</td>
<td>11</td>
<td>600–2000 (1265)</td>
<td>0–38</td>
<td>3–53</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>12</td>
<td>500–11000 (1310)</td>
<td>0–76</td>
<td>3–889</td>
</tr>
<tr>
<td>Rectum carcinoma</td>
<td>14</td>
<td>140–6100 (2.5)</td>
<td>0–18</td>
<td>0–94</td>
</tr>
<tr>
<td>Bronchial carcinoma</td>
<td>1</td>
<td>95000</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bronchial carcinoma bone metastasis</td>
<td>1</td>
<td>3700</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gall bladder carcinoma</td>
<td>2</td>
<td>1000–5200</td>
<td>0–4</td>
<td>0.2–21</td>
</tr>
<tr>
<td>Duodenal carcinoma</td>
<td>1</td>
<td>300</td>
<td>69</td>
<td>21</td>
</tr>
<tr>
<td>Kidney carcinoma</td>
<td>1</td>
<td>1600</td>
<td>2</td>
<td>35</td>
</tr>
<tr>
<td>Ovarial carcinoma</td>
<td>1</td>
<td>3700</td>
<td>104</td>
<td>22</td>
</tr>
</tbody>
</table>

¹) mU alkaline phosphatase per gram tissue.
²) No. positive cases.
³) No. negative cases.
This explains the poor positive results in sera: Placental alkaline phosphatase was detected in only 7% of the tested sera. In cases of carcinomatoid tissue with a very high placental alkaline phosphatase content (1000 mU/g tissue, e.g. in one bone metastasis of a breast carcinoma and one stomach carcinoma) placental alkaline phosphatase was also found in the sera.

No placental alkaline phosphatase was detected in benign stomach or breast tissue, but it was present in 2 out of 3 tested cases of sigma diverticulitis. Intestinal alkaline phosphatase was detected (a few mU/g tissue) in 50% of the breast carcinoma tissue and most bone metastases; as expected, it was also found in the intestinal tissue. The very variable intestinal alkaline phosphatase content of the stomach tissue, benign and cancerous, must be explained by the inclusion of duodenum tissue in the surgery samples.

Very high total alkaline phosphatase activity content (15–65 U/g tissue) was found in 3 bone metastases of breast carcinomas and in 2 stomach carcinomas. With the exception of one bone metastasis, the latter tissue samples had only a little intestinal alkaline phosphatase and no placental alkaline phosphatase catalytic activity.

**Discussion**

Our experiments do not confirm the literature findings, that in cancer patients total alkaline phosphatase catalytic activity is raised and placental alkaline phosphatase is found quite frequently (12). In our patient collective (cancer of the breast and digestive tract), prior to any therapy, serum placental alkaline phosphatase was found in only 7% of 170 tested samples, and raised total alkaline phosphatase catalytic activity occurred with similar low frequency. Owing to the sensitivity of the immunoassays used, the placental isoenzyme was detected in various normal tissues and in a few normal sera, where it represented a low percentage of total alkaline phosphatase activity. We measured placental alkaline phosphatase in over 50% of the examined tumour tissues, but in most cases the enzyme appeared in such a low catalytic concentration that its release into serum by cell turnover would give undetectable serum levels; with even more sensitive tests it might be shown to be in the range found in normal serum. In accordance with literature data (13, 14) we found, in the few samples tested (see also l.c. (11)), a good correlation between seminoma patients and serum placental alkaline phosphatase. Most of the gastrointestinal tumours showed the placental isozyme in the tissue, but not in the sera. Especially in these cases the high intestinal alkaline phosphatase content of the intestinal mucosa compared with the low intestinal alkaline phosphatase in fasted individuals, which shows a dependence on blood grouping and secretory status (15), must be kept in mind. These factors have never been considered in relation to serum placental alkaline phosphatase, but high intestinal alkaline phosphatase catalytic activity has been measured in faeces (16). Thus, placental alkaline phosphatase expressed in intestinal tumours might also reflected in the faecal excretion of the enzyme.

No placental alkaline phosphatase was found in benign stomach tissue, but it was found in 60% of the examined stomach carcinomas and in 75% of intestinal carcinomas, and in sigma diverticulitis.

Demonstration of a low placental alkaline phosphatase catalytic activity is not necessarily related to cancerous tissue. Measured in sera, placental alkaline phosphatase must be regarded as an unsatisfactory tumour marker.

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**Tab. 2. Alkaline phosphatase isoenzymes in sera of cancer patients.**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Total alkaline phosphatase U/l (range)</th>
<th>No. of patients &gt;190 U/l</th>
<th>Intestinal alkaline phosphatase U/l (range)</th>
<th>No. of patients &gt;8 U/l</th>
<th>Placental alkaline phosphatase U/l (range)</th>
<th>No. of patients &gt;0.4 U/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma</td>
<td>62</td>
<td>(28–246)</td>
<td>2</td>
<td>(0–14.5)</td>
<td>2</td>
<td>(0–0.7)</td>
<td>3</td>
</tr>
<tr>
<td>Stomach carcinoma</td>
<td>29</td>
<td>(30–356)</td>
<td>2</td>
<td>(0–2)</td>
<td>0</td>
<td>(0–1.0)</td>
<td>2</td>
</tr>
<tr>
<td>Rectum carcinoma</td>
<td>21</td>
<td>(30–293)</td>
<td>1</td>
<td>(0–2)</td>
<td>0</td>
<td>(0–0.3)</td>
<td>0</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>20</td>
<td>(20–1337)</td>
<td>1</td>
<td>(0–2)</td>
<td>0</td>
<td>(0–1.6)</td>
<td>2</td>
</tr>
<tr>
<td>Sigma carcinoma</td>
<td>20</td>
<td>(30–250)</td>
<td>1</td>
<td>(0–2)</td>
<td>0</td>
<td>(0–3)</td>
<td>0</td>
</tr>
<tr>
<td>Seminoma</td>
<td>4</td>
<td>(163–241)</td>
<td>2</td>
<td>(0–2)</td>
<td>0</td>
<td>(0–9.6)</td>
<td>3</td>
</tr>
<tr>
<td>Bronchial carcinoma</td>
<td>2</td>
<td>(89–93)</td>
<td>0</td>
<td>(0–2)</td>
<td>0</td>
<td>(0,2–0.8)</td>
<td>1</td>
</tr>
<tr>
<td>Different carcinoma</td>
<td>12</td>
<td>(42–651)</td>
<td>3</td>
<td>(0–2)</td>
<td>0</td>
<td>(0–0.5)</td>
<td>1</td>
</tr>
</tbody>
</table>

1) Reference value 40–190 U/l (Merck, Klinisches Labor, see l.c. (17)).
2) Reference value 0–8 U/l (see Mössner (11)).
3) Reference value 0–0.4 U/l (see Mössner (11)).
References


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