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Phosphohexose Isomerase in Hypernephroma

Significance as Serum Tumor Marker, Comparison to Other Glycolytic Enzymes and Isozyme Patterns in Normal and Tumor Tissue

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Summary. In hypernephroma an overall diagnostic sensitivity of 72% and a specificity of 87% was found for the serum tumor marker phosphohexose isomerase (PHI). Both in early stage disease and in well differentiated tumors a sensitivity of about 60% was reached. In contrast the sensitivity of three other glycolytic enzymes tested was found to be less than 20%. Since the cancer induced elevation of PHI activity in the tumor was found to be comparable to those of the other test enzymes, elevated PHI serum activities cannot be attributed to overproportional PHI synthesis and unspecific cell-lysis. In 6 of 10 cases studied differences in the PHI isozyme pattern between the tumor and the normal tissue were found suggesting the occurence of cancer associated structural alterations of PHI.

Key words: Phosphohexose Isomerase – Hypernephroma – Serum and Tissue Activities – Isozyme Pattern

Introduction

The usefulness of the glycolytic enzyme phosphohexose isomerase (PHI) in monitoring cancer patients is well documented [7, 15, 20, 21, 24, 29, 30–32]. Recently we were able to demonstrate that serum PHI can also be helpful in the diagnosis of gastrointestinal cancer [2]. One purpose of this study was to evaluate the diagnostic validity of PHI in 90 patients with histopathologically confirmed hypernephroma prior to primary treatment. Most authors agree that PHI is the most suitable tumor marker from all glycolytic enzymes [7, 15, 20, 21, 31, 32]. On the other hand little is known about the origin of the raised serum activities of PHI in cancer bearing patients. Therefore, this work also evaluated the significance of serum PHI as a tumor marker for hypernephroma in comparison with other glycolytic enzymes. To address this problem PHI activity and the activities of three other glycolytic test enzymes were assayed in histologically proven cancerous and normal renal specimens and in serum probes of 24 hypernephroma patients. Since tumor associated structural alterations of various enzymes have been reported [13, 14, 19] the occurence of cancer associated alterations of PHI were also studied. For this purpose the isozyme patterns of PHI in host and cancerous tissue specimens from the same patient were compared by means of the isoelectric focusing technique.

Patients, Materials and Methods

90 patients with confirmed hypernephroma were studied. Serum samples were taken prior to primary treatment and the histopathological staging [28] and grading [34] was performed in all cases. The sensitivity of PHI is given as the percentage of tumor patients with elevated PHI serum activities. To determine the specificity, PHI activity was also assayed in the serum of 24 patients with various benign urological diseases and disorders. The specificity is given as the percentage of tumor free patients with normal PHI serum activities. For comparison in 24 tumor patients the serum activities of the glycolytic enzymes hexokinase (HK), aldolase (ALD) and pyruvate kinase (PK) were also determined.

Additionally in these cases the activities of the four glycolytic enzymes in cancerous and in normal host tissues were assayed. Host (non cancerous) and tumor tissue probes from the kidney were obtained immediately after surgical removal and the histopathological examination of each specimen. Only solid, surgically and histologically homogenous tumor tissue, and closest noninvolved, histologically normal tissue as control was used. Blood and coagula were removed very thoroughly. All further steps were carried out at 4 °C. Normal and carcinoma specimens were prepared identically. An aliquot of 400 mg of each specimen was minced and homogenized in a 10 fold volume of hypotonic *K*rebs-*S*aline-*B*uffer (KSB:NaCl 136 mM, KCl 4.7 mM, $CaCl_2 \times 2H_2O$ 1.29 mM, $K_2HPO_4 \times 3H_2O$ 1.18 mM, $NaH_2PO_4 \times 2H_2O$ 5 mM, $MgSO_4 \times 7H_2O$ 0.77 mM; KSB: H_2O/1:4; pH 7.5). Then the homogenate was sonicated by 6 pulses of 10 s each at 50 Watt with cooling intervals of 1 min to make sure the temparature of the preparation remained below 10 °C. Subsequently the extract was rehomogenized and centrifuged at 12,000 g for 10 min. In the serum probes and in the supernatants the activities of the glycolytic enzymes were determined spectrophotometrically at 25 °C according to the methods described elsewhere: Phosphohexose isomerase (PHI) [3], hexokinase (HK) [4], aldolase (ALD) [5], pyruvate kinase (PK) [6]. In the serum probes the enzyme

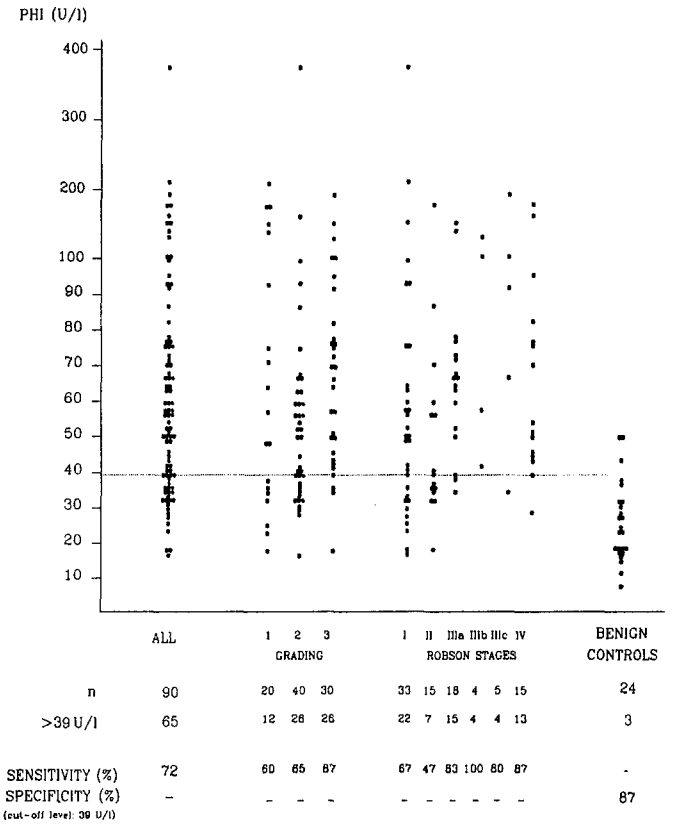

Fig. 1. Serum activities of phosphohexose isomerase (PHI) in patients with hypernephroma and benign urological diseases prior to primary treatment. The sensitivity is expressed as the percentage of tumor patients with elevated serum activities (cut-off: 39 U/l). The specificity is expressed as the percentage of patients with correct normal serum activities in the control group with benign urological diseases

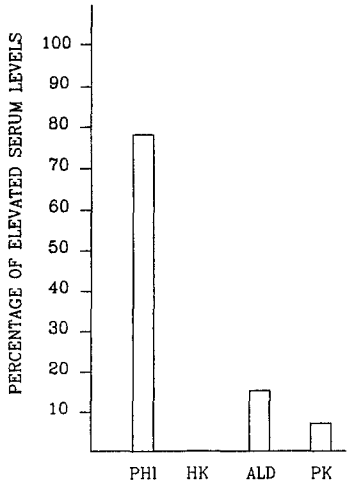

Fig. 2. Diagnostic sensitivity of phosphohexose isomerase (PHI) in comparison with hexokinase (HK), aldolase (ALD) and pyruvate kinase (PK) in 24 patients with hypernephroma. The sensitivity is given as the percentage of patients with elevated serum activities prior to primary treatment (cut-off levels are given in "Patients, Materials and Methods")

activity is expressed in Units per litre (U/l), in tissue specimens the enzyme activity is expressed in Units per gramme soluble protein (U/g). The protein content of the tissue probes was measured with the biuret method. PHI isozyme patterns in the tumor and corresponding host tissue specimens were analysed by the isoelectric focusing technique. In 10 cases tissue specimens were prepared as described and the supernatants were dialysed against 1% glycine buffer, pH 7.5 overnight at 4 °C. LKB Multiphor 2117, LKB Power Supply Unit 2103, and Servalyt Precotes pH 3–10 were used for the isoelectric focusing experiments. A Julabo Paratherm FT20p electronic thermostat served to cool the plate to 4 °C during the run. The dialysed probes were diluted with LKB ampholine pH 3–10, 2%, achieving a final PHI activity of 1,000–3,000 μU in a sample volume of 20 μl. A constant power of 4.0 Watt was preset and the precote was prefocused until the voltage reached 500 Volt. Then the probes were applied to a voltage limit of 2,000 Volt. When this voltage was reached the run was carried out for a further 30 min, so that the whole procedure took 3–4 h. Subsequently the precote was specifically stained for PHI, according to the method described by Carter and Parr [11], using phenazine methosulphate and the tetrazolium salt MTT. For each run, the protein standard Test Mix 9, supplied by Serva, was used to determine the isoelectric points (pI) of the PHI bands. The standard was visualized by Coomassie staining.

Normal Serum Activities

Serum PHI activity was originally determined in a collective study of 42 healthy men and women within an age range from 20 to 55 years. From the mean and the standard deviation obtained, a cut-off level of 39 U/l was established, with false positive results in this group being less than three percent. This upper limit of 39 U/l was confirmed in a two years period of clinical application [1, 2] and agrees with the results of Schwartz [33]. Since there are no reports on a serum cut-off level for HK a value of 2 U/l was elaborated. For ALD an upper limit of normal serum activity of 3.1 U/l according to Feissli et al. [17] was used. The serum cut-off level of 26 U/l for PK is proposed by Otto et al. [26].

Results

In Fig. 1 the sensitivity and specificity of the serum tumor marker PHI is illustrated. In 90 patients with histologically proven hypernephroma an overall diagnostic sensitivity of 72% was reached. Even in early stage disease and in highly differentiated hypernephroma serum PHI showed a sensitivity of about 60%. There was a continuous increase in elevated serum levels from 60% in the well differentiated tumors (Grading 1) up to 87% in the poorly differentiated tumors (Grading 3). An increasing trend in the sensitivity from 47%–67% in the early stages (Robson I, II) to about 80%–90% in the advanced stages (Robson III, IV) with metastases was found. In a control group of 24 patients with benign urological diseases in 87% of the cases normal serum activities of PHI were found.

Figure 2 illustrates the exceptional position of PHI as a serum marker for hypernephroma in comparison with other glycolytic enzymes. In 24 cases studied elevated PHI serum levels were found in 79% of the patients. In contrast the sensitivity of all other glycolytic test enzymes ranged from

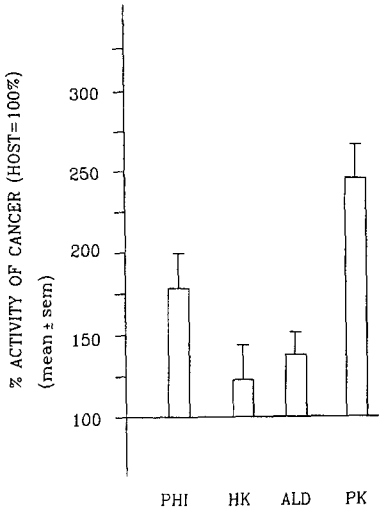

Fig. 3. Cancer associated increase in the enzyme activities of phosphohexose isomerase (PHI), hexokinase (HK), aldolase (ALD) and pyruvate kinase (PK) in cancerous tissue of 24 patients with hypernephroma. Enzyme activities in the cancerous tissue are expressed as a percentage of those in the corresponding host tissue

Table 1. Isozyme patterns of phosphohexose isomerase in normal and tumor tissues from patients with hypernephroma

Patient	Normal			Tumor		
	Band A	Band B	Band C	Band A	Band B	Band C
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	+	+
5	+	+	+	+	+	–
6	+	+	+	–	+	+
7	+	+	+	–	–	+
8	+	+	+	–	–	+
9	+	+	–	+	–	–
10	+	+	–	–	+	–

Band A: pI = 9.1; Band B: pI = 8.9; Band C: pI = 8.6

+ = presence of Band; – = absence of Band

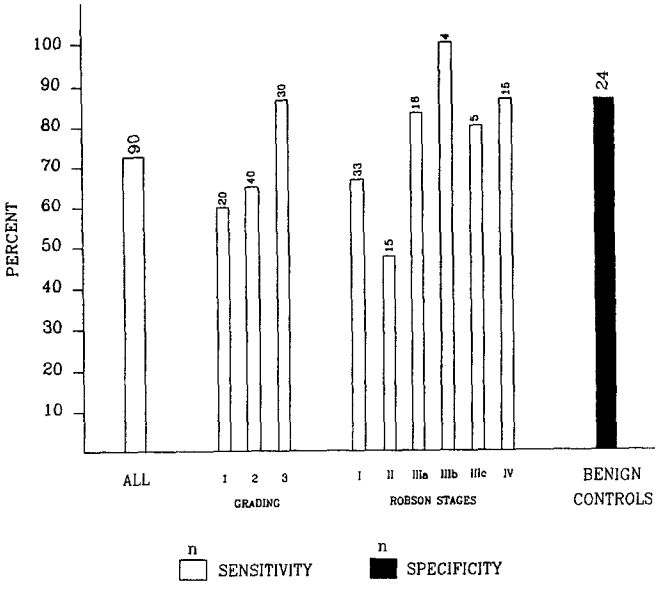

Fig. 4. Diagnostic validity of phosphohexose isomerase in hypernephroma. The sensitivity is expressed as the percentage to tumor patients with elevated serum activities (cut-off: 39 U/l) prior to primary treatment. The specificity is expressed as the percentage of patients with correct normal serum activities in a control group with benign urological diseases and disorders

0% for hexokinase to 17% for aldolase. To address this phenomenon, enzyme activities were additionally measured in normal and tumor probes derived from surgical specimens of these patients.

In Fig. 3 the cancer associated rise in the tissue activities of the four glycolytic enzymes is shown. The levels of the enzymes in the cancerous tissues were expressed as a percentage of those in the corresponding host. In contrast to the results obtained for the serum enzyme activities, tissue levels revealed that all glycolytic enzyme activities are increased in the tumor tissue. The highest rise was measured for PK (2.5fold) followed by PHI (1.8fold), ALD (1.4fold) and HK (1.2fold). To discern whether tumor associated variants of PHI do occur in hypernephroma extracts of cancerous and host tissues obtained from 10 patients were subjected to isoelectric focusing (Table 1). Three variants of PHI could be demonstrated in the tissues studied: a major basic band A with an isoelectric point of 9.1 and two, more acidic bands B and C, with a pI of 8.9 and 8.6 respectively. In 60% of the cases a different isozyme pattern in the cancerous and in the corresponding host tissue was found. In the majority of the normal specimens a triplet pattern could be detected, whereas in 6 of 10 tumor probes one or two bands of the triplet were lost.

Discussion

The objective of the current study was to evaluate the diagnostic validity of serum PHI in patients with hypernephroma. The results are summarized in Fig. 4. In the 90 cases studied PHI reached an overall diagnostic sensitivity of 72%. Even in early stages without metastases and in well differentiated tumors a sensitivity of about 60% was reached. In a control group of 24 patients with benign urological diseases only in 3 cases slightly elevated PHI serum levels were detected yielding a specificity of 87%. The exceptional position of PHI as the most sensitive tumor marker of all glycolytic enzymes was confirmed also for hypernephroma (Fig. 2). In contrary to the serum activities the rise of PHI activity in tumorous tissue remained within the magnitude of that of the other glycolytic enzymes (Fig. 3). It therefore appears that overproportional production of PHI in the tu-

mor cell and leakage into circulation by simple cell-lysis can be excluded as a cause for the exclusive rise of PHI in the tumor sera. Another approach to address this phenomenon was to look for cancer associated alterations or modifications of PHI in the malignant cell. The occurence of such alterations has been shown for other enzymes [13, 14, 19] and might account for enhanced leakage of PHI from the tumor. In 6 of 10 cases studied different PHI isozyme patterns were found when comparing host and cancerous tissue of the same patient (Table 1). Most of the normal specimens showed a triplet pattern. Since PHI is known to be a dimeric enzyme this pattern indicates that the patient is heterozygous for the PHI locus [12, 16, 18, 25]. In 4 of the cancerous tissues only a single band could be detected, indicating homozygosity for the PHI locus. The loss of heterozygosity during the process of malign transformation has been described for a series of malignancies [22, 23, 27]. From the data obtained it appears that loss of constitutional heterozygosity might also occur in hypernephroma. It must be pointed out, however, that the observed differences in the isozyme patterns can also be attributed to a different stability of the PHI variants in normal and tumorous tissue. Such PHI isozymes with different stability against inactivating conditions are known from human genetics [8]. Such inactivation of PHI variants might be promoted by increased levels of proteolytic enzymes which have been reported in malign transformed cells [9, 10]. Further studies aiming at the isolation, purification and characterization of PHI from hypernephroma specimens are now in progress.

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