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The activity of phosphohexose isomerase in primary breast carcinomas and response to chemotherapy in patients with metastatic disease

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Summary. The activity of phosphohexose isomerase [PHI] was measured in 48 primary carcinomas from patients with breast cancer, and its usefulness as a predictor of response to cytotoxic drugs at the metastatic stage was evaluated. There was a statistically significant difference in the activity of PHI between responders and non-responders to these treatments. These preliminary findings are currently being evaluated in an extended series.

Introduction

Just as the ability to predict the likelihood of recurrence in a patient with early disease should assist clinicians in the selection of patients for adjuvant therapies, prior knowledge of the possibility of inducing regression of metastatic lesions by additive and/or ablative treatments should help to avoid unnecessary treatments. In most centres patients with disseminated carcinoma of the breast are initially treated by endocrine treatments before receiving cytotoxic drugs later on, and there has been a greater emphasis on research into parameters which will predict the likelihood of response to endocrine rather than cytotoxic treatments [1, 2, 9, 13]. Currently the presence of oestrogen binding protein in the carcinoma is taken as an indicator of likely response to endocrine treatment [13], but there are no established parameters which will help to predict the response to cytotoxic drug therapies. The attempts to use oestradiol binding proteins in the carcinomas as a test for selection of chemotherapy have resulted in confusion, in that the initial findings of Lippman et al. [12] and Jonat and Maass [10] were not confirmed by others [6, 11, 15, 17]. Hilf et al. [8] have developed a regression model based on the activities of certain glycolytic enzymes and have shown that the model correctly predicts the likelihood of the outcome of cytotoxic drug therapies in the majority of cases. For the past 10 years we have been estimating the activities of six enzymes of carbohydrate metabolism in carcinomas from patients with either stage I or stage II disease and have reported our findings on the usefulness of these measurements in predicting the likelihood of recurrence in patients with primary disease [3–5]. During this period a reasonable number of these patients have developed metastatic disease and have undergone both endocrine and cytotoxic drug therapies. In this paper we report our preliminary finding of statistically significant differences in the activity of phosphohexose isomerase (PHI) in primary carcinomas between responders and non-responders to cytotoxic drug therapies.

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Materials and methods

Clinical. Six hundred patients with stage I or stage II disease have been followed for up to 10 years after mastectomy for breast cancer. During this period 147 of them developed metastatic disease, 48 of whom were evaluable for response to cytotoxic drug treatment. Throughout this period we have followed a protocol in which the majority of these patients were treated initially by various endocrine treatments and subsequently with cytotoxic chemotherapy on further progression of the disease. Thus, 35 of these patients were treated with both types of therapies, whereas 13 received cytotoxic drugs alone. Of the 35 patients who underwent each of the treatments 2 responded to both, 2 to endocrine therapies only, and 8 to cytotoxic therapies only, while 24 were unresponsive to either treatment. Of the 13 patients who received cytotoxic drug therapies, 6 responded. Thus there were 16 responders and 32 non-responders to cytotoxic treatments. The endocrine treatments consisted of ovarian ablation, tamoxifen with or without prednisolone, and occasionally androgens or progestogens. Cytotoxic treatments included combinations of doxorubicin, cyclophosphamide, methotrexate, 5-fluorouracil, vincristine and mitomycin C, or single use of doxorubicin or mitoxantone. Before systemic treatment was begun all patients had a full physical examination, during which palpable lesions were measured and visible lesions photographed. All patients had had a chest radiograph, isotopic bone scan with radiographs of areas of increased uptake, full blood count and biochemical screen; other tests such as liver scans were done when indicated. Baseline lesions were selected for serial assessment. Patients were followed at regular intervals, 3- or 4-weekly depending upon treatment regimen, when reassessment of baseline lesions was made and repeat photographs taken: Relevant radiographs were repeated 3 monthly. Objective responses were categorised by UICC criteria [7].

Biochemical. Details of the collection, storage, and measurements of the activities of phosphofructokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate-dehydrogenase, lactate dehydrogenase, α-glycerolphosphate

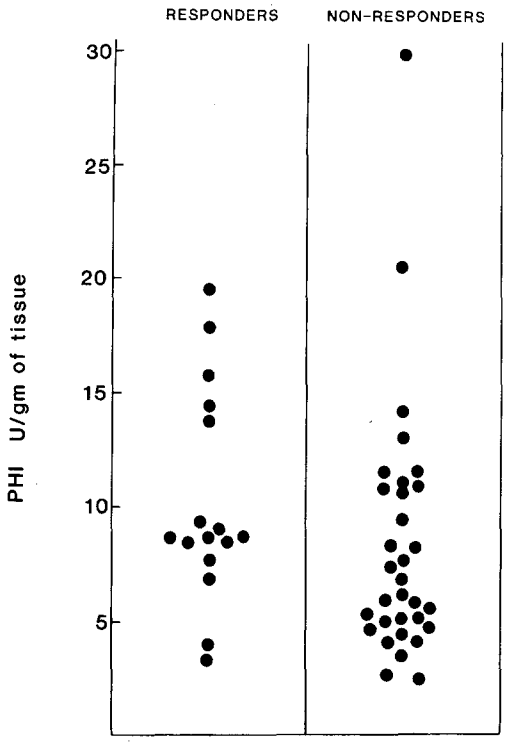

Fig. 1. The activity of phosphohexose isomerase (PHI) in primary breast carcinomas and response to cytotoxic drug treatments in 48 patients with metastatic disease. The activity of PHI was significantly higher in carcinomas from patients who responded to these treatments than in others who failed to respond. The results were analysed by the Mann-Whitney test for non-parametric comparison of two groups. Mann-Whitney's Z-test for tied values: -1.998; $P=0.042$ (two-tailed)

dehydrogenase and phosphohexose isomerase in carcinomas have been presented in detail in previous communications [3–5]. Briefly, a small piece of tissue weighing about 400 mg was obtained from each of these patients during mastectomy performed as a first treatment for the disease. The tissue was wrapped in aluminium foil, frozen immediately in liquid nitrogen and transferred to the laboratory where it remained frozen in liquid nitrogen until further processing. The enzyme activities were estimated within a month of collection. The tissues were semi-thawed, cleared of surrounding fat, cut into small pieces and weighed. They were then homogenized in a homogenization medium described by Shonk and Boxer [16]. The homogenates were centrifuged at 800 *g* for 20 min in a refrigerated centrifuge (4 °C) and the supernatants were decanted off to be used as the source of enzymes. The activities of the enzymes were measured biochemically by observing the optical density changes at 340 nm as a result of the oxidation or reduction of pyridine nucleotides [16]. The results are expressed as units per gram of tissue where a unit is defined as that amount of enzyme which will catalyze the transformation of one micromole of substrate per minute. The results were analysed by the Mann-Whitney test for non-parametric comparison of two groups.

Results and discussion

Although we have analysed our data on all the enzymes, with the exception of PHI, the activities of enzymes in the carcinomas failed to show any statistically significant differences between responders and non-responders to cytotoxic drug therapies. The data on PHI activities are presented in Fig. 1. Higher activities of the enzyme were found in the carcinomas from patients who responded to chemotherapy at the metastatic stage (median = 9.2 units/g) than in carcinomas from non-responders to such treatment (median = 6.3 units/g). Attempts to analyse the data in terms of predicting response to endocrine therapies were not successful.

The usefulness of the measurements of the activity of PHI in prognosis in human breast cancer has been known for sometime. Muir and Fawcett [14] reported that the activity of the enzyme in carcinomas showed a gradual rise between well and poorly differentiated carcinomas, and these findings have been confirmed by us [3]. However, when our results were subjected to life-table analyses the activity of the enzyme failed to predict the likelihood of recurrence in patients with primary disease. As far as prediction of response to treatments for metastatic disease is concerned, with the exception of the report by Hilf et al. [8] mentioned earlier, there are no data to indicate that the measurements of the activities of certain glycolytic enzymes can help to predict the outcome. Since we have not estimated the activities of pyruvate kinase and isocitrate dehydrogenase in the carcinomas from our patients we are unable to confirm the usefulness of the regression model devised by these workers. However, since PHI forms one component of the four-enzyme model, our preliminary findings that significantly higher activities of PHI were found in patients who responded to cytotoxic drugs suggest that it might be worthwhile to estimate the activities of the four enzymes they mention in their paper. In conclusion, although the data presented in this paper showed a statistically significant difference between the two groups, the results need to be treated with extreme caution at this stage. We are currently attempting to confirm these findings in an extended series.

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