ACTA BIOCHIMICA POLONICA
Vol. 39 1992 No. 3

*Short Communication*

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# ENZYME ACTIVITIES IN HUMAN BREAST TUMOURS

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Received 26 August 1991, Revised 15 July, 1992

**The activities of six enzymes associated with carbohydrate metabolism were measured both in carcinomas and in normal breast tissues. The following differences were observed. 1. The carcinoma showed higher enzyme activities than the normal mammary tissue. 2. The ratios of glutamate dehydrogenase, hydroxybutyrate dehydrogenase, glutathione reductase and catalase to lactate dehydrogenase were lower in carcinomas than in normal tissues. Similarly, the ratios of glutamate dehydrogenase, hydroxybutyrate dehydrogenase, glutathione reductase and catalase to glucose-6-phosphate dehydrogenase were also significantly lower in carcinomas. 3. There were no significant differences in enzyme activities between I and II stage of the disease and the metastatic tissues, however, there were significant differences between I and III stage. The significance of these findings is discussed in terms of the alterations in the balance between the metabolic pathways.**

Most investigations on human breast cancer are directed towards the search for biochemical parameters which will be helpful in determining the clinical stage of the disease. It has been observed that malignant tumours

usually exhibit a high rate of glycolytic activity. The studies on Morris and Novikoff hepatoma have shown that their growth rates in animals are related to the activity of enzymes associated with carbohydrate metabolism [1]. In some human cancers alterations in the content of glycolytic enzymes are successfully used as markers of diagnostic or prognostic value. Also, elevated glycolytic enzyme activities have been demonstrated in human breast cancer [2].

It can be expected that premalignant changes could be detected by biochemical methods before histological changes become evident. Thus, a search for tumour markers to identify breast cancer at an early stage has been undertaken. Previous studies showed that, when cancer was present the activities of a number of enzymes were altered and some correlations were observed between the enzyme activities, estrogen receptors and response to chemotherapy [3].

The present study concerns the activities of six enzymes in human breast tissues in comparison with their activities in normal tissues from the same patients. The aim was to determine whether any biochemical abnormality can be detected in that breast tissue which is at a high risk of developing cancer before morphologic changes are found.

## MATERIALS AND METHODS

Cancers were classified and staged by WHO histological typing of breast tumours [4] which is based on the degree of tumour differentiation, pleomorphism of nuclei, number of hyperchromatic nuclei and mitotic figures. Stage I tumours are of lower malignancy and are better differentiated than stage II and III tumours. The following cases have been involved in the study: 5 patients with fibroadenoma, 15 with primary tumours and 5 from metastatic tumour was obtained. There were 3 patients with I stage, 6 patients with II stage and 6 with III stage of breast carcinoma.

Breast tumours and their adjacent normal tissues were collected from patients after mastectomy. Small pieces of tissues were fixed in 10% formalin for histological examination to define normal and malignant tissues. After fixation, the tissues were dehydrated, embedded, sliced and stained with hematoxylin and eosin.

The remaining part of the tissues was used for determination of enzyme activities. In each case a 10% homogenate was prepared in phosphate

buffered saline (0.01 M, 0.9% NaCl, pH 7.2). The homogenate was centrifuged at $10000 \times g$ at 4°C and the supernatant was collected. Protein content was determined by Bradford's method [5]. The activity of lactate dehydrogenase (LDH) was determined by the colorimetric method [6]. The change in absorbance at 340 nm due to oxidation of NADH was used as a measure of glutamate dehydrogenase (GLDH) activity [7]. Hydroxybutyrate dehydrogenase (HBDH) [8], glucose-6-phosphate dehydro-genase (G6PDH) [9], glutathione reductase (GR) [10] and catalase [11] were determined by spectrophotometric methods.

Enzyme activities were expressed as μmoles cofactor converted per minute per unit of tissue weight. The enzyme activities were also calculated on the basis of units per 0.5 g wet tissue and per 1 mg tissue protein (not shown). The results were additionally analysed as enzyme/LDH and enzyme/G6PDH ratios.

Mean values were tested by Student's *t*-test.

## RESULTS AND DISCUSSION

There was a significant increase in LDH, HBDH, G6PDH and catalase, and decrease in GLDH and GR activities in carcinomas in comparison to normal tissues (Table 1). When the values obtained for fibroadenomas were compared with I stage cancers, significantly higher values for the LDH and

Table 1
*Enzyme activity in breast tissues*

| | No of cases | LDH[1] | GLDH | HBDH | G6PDH | GR | Catalase |
|---|---|---|---|---|---|---|---|
| | | | | U/0.5 g wet tissue | | | |
| Normal tissue | 25 | 97.4 | 25.5 | 205.4 | 8.9 | 39.1 | 2.4 |
| Fibroadenomas | 5 | 125.8* | 20.5 | 208.5 | 6.8 | 29.8* | 3.0 |
| Cancers: | | | | | | | |
| primary | 15 | 223.8** | 16.5* | 226.5* | 12.1* | 28.4* | 3.4* |
| metastatic | 5 | 230.5** | 17.1* | 293.2** | 25.6** | 35.2 | 3.8* |

*$P < 0.02$
**$P < 0.001$ with respect to normal tissue
[1]For abbreviations see Materials and Methods

G6PDH were seen in the latter tissue. The differences between the malignant tissues were then evaluated (Table 1 and 2). No significant differences were found between I and II stage, but there were significant differences between I and III stage (Table 2). The enzyme to LDH ratios and enzyme

Table 2

*Enzyme activities in breast carcinoma tissues*

| Stage | No of cases | LDH[1] | GLDH | HBDH | G6PDH | GR | Catalase |
|---|---|---|---|---|---|---|---|
| | | | | U/0.5 g wet tissue | | | |
| I | 3 | 220.8 | 16.4 | 219.5 | 12.2 | 20.2 | 2.4 |
| II | 6 | 221.2 | 15.9 | 222.2 | 12.3 | 22.6 | 2.5 |
| III | 6 | 204.7* | 17.8* | 286.5** | 18.4** | 32.6** | 3.9** |

*$P < 0.02$

**$P < 0.001$, P with respect to stage I

[1]For abbreviations see Materials and Methods

to G6PDH ratios were analysed, because LDH as well as G6PDH are very important and useful biochemical markers for metastatic cancers. The enzyme to LDH ratios were lower in all cases of tumour tissues. The enzyme to G6PDH ratios were also lower in the carcinomas, except the LDH/G6PDH ratio (Table 3).

Table 3

*The ratios of various enzymes to LDH and G6PDH*

| | Carcinoma tissues | | Normal tissues | |
|---|---|---|---|---|
| | enzyme/LDH | enzyme/G6PDH | enzyme/LDH | enzyme/G6PDH |
| LDH[1] | 1.00 | 18.48** | 1.00 | 10.93 |
| GLDH | 0.07** | 1.37** | 0.26 | 2.86 |
| HBDH | 1.01** | 18.70* | 2.11 | 23.05 |
| G6PDH | 0.05* | 1.00 | 0.09 | 1.00 |
| GR | 0.13** | 2.35** | 0.40 | 4.39 |
| Catalase | 0.01* | 0.19* | 0.03 | 0.27 |

*$P < 0.02$

**$P < 0.001$ with respect to normal tissues

[1]For abbreviations see Materials and Methods

It may be concluded that a correlation exists between tumour progression and the activity of glycolytic enzymes. Hennipman & Oirschot [12] observed higher activities of LDH, aldolase, hexokinase and pyruvate kinase in metastatic cancers. It seems that the data quoted are somewhat convergent, suggesting higher activities being associated with biologically more aggressive disease [13]. Poorly differentiated carcinoma showed significantly higher activities of LDH than well differentiated tumours [1]. Since all neoplastic tissues are characterized by a higher production of lactate than their respective original tissue due to the increased activity of LDH, various attempts have been made to investigate LDH and other enzymes of glucose metabolism. Also Balinsky & Platz [3] observed significantly increased activities of LDH in malignant tumours. High activity of LDH in the primary tumours is an useful predictor of the good response to cytotoxic chemotherapeutic drugs. The increased activity of the glycolytic enzymes is probably due to the increased energy requirements of malignant tumour tissues. High activity of LDH was found in the fibroadenomas, primary and metastatic cancers also in this study. Forteleoni & Argiolas [14] reported that G6PDH deficiency did not seem to be a protective factor against cancer development. Glucose-6-phosphate dehydrogenase plays an important role in the carcinogenic process and will be a good marker for the early diagnosis of tumours. Activity of G6PDH was significantly increased in tumour as compared with normal and benign tissues [15]. The same results were obtained in this study. Glutathione reductase has been reported to be present in cancer cells resistant to chemotherapeutic agents, and to cause cellular drug resistant to chemotherapeutic agents, and to cause cellular drug resistance [16]. In Table 1 a significant decrease of GR activity in fibroadenomas and primary cancers is seen, while this enzyme activity is only slightly lower in metastatic cancers than in normal tissue. Hydrogen peroxide is toxic at high concentrations to both eukaryotic and prokaryotic cells [17]. The mechanism whereby cells inhibit and/or repair $H_2O_2$-mediated damage may be of significance in studies on the resistance to anticancer xenobiotics. Catalase can play a critical role in detoxification of $H_2O_2$. Akman *et al.* [17] and Buckley & Tanswell [18] demonstrated increased intracellular catalase activity in MCF7 breast cancer line. This enhanced activity was due to stabilization of the catalase mRNA produced in this line [17]. However, in this study no significant differences in catalase activity were found between the normal tissues and fibroadenomas and between stage I and stage II (Tables 1 and

2). Siginificant differences were observed between normal and carcinoma tissues and between stage I and III tumours.

Analysis of the data showed some differences in enzyme activities between the normal and neoplastic tissues of the breast. It remains to be seen whether they correlate with either the recurrence rates or response to treatment in individuals. The elevated activities of glycolytic enzymes are indicative of cancer, although normal activities do not rule out its presence. Continued studies are needed to determine whether changes in the enzyme activities precede histological changes in patients ultimately developing breast cancer, so that enzyme assays could be possibly used to identify the precancerous state.

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