HEME BIOSYNTHESIS IN HUMAN BREAST CANCER—MIMETIC "IN VITRO" STUDIES AND SOME HEME ENZYMIC ACTIVITY LEVELS*

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(Received 27 March 1990)

Abstract—1. Porphyrin biosynthesis from δ-aminolevulinic acid (ALA) was investigated using the technique of tissue explant cultures, in both human breast cancer and its original normal tissue.
2. The activity of ALA-dehydratase, porphobilinogenase and uroporphyrinogen decarboxylase was directly determined in both tumor and normal mammary tissues.
3. Porphyrin synthesize capacity of human breast carcinoma was 20-fold enhanced, as compared with normal tissue, at least between the stages of porphobilinogen and coproporphyrinogen formation.
4. The activity of the three enzymes examined was always lower in normal tissue than in tumoral tissue.
5. Present findings show that porphyrin biosynthesis is increased in breast cancer tissue.

INTRODUCTION

Human breast cancer is one of the most frequent type of malignant tumors found in females, leading to the highest rate of mortality as compared with that due to any other tumors affecting this sex. In practice the treatment of choice is surgery followed by radiotherapy, chemotherapy and hormone therapy. More recently, other treatment reported to be rather effective, has been photodynamic therapy (PDT) (Dougherty et al., 1979), consisting in the administration of the so-called hemotoporphyrin derivatives (HPD) or Photofrin II a more purified product already approved by the FDA for its use in humans. PDT is based on the property of certain porphyrins to efficiently accumulate in neoplastic tissue (Rasmussen-Taxdall et al., 1955; Lipson et al., 1961; Gregorie et al., 1968; Dougherty et al., 1978; Gomer and Dougherty, 1979), and the photodynamic properties of these compounds, which would then allow selective killing of the tumorous cells with little damage to the surrounding normal cells.

A good deal of research on this interesting area has been mostly concerned with the tissular distribution of porphyrins after injection (Gomer and Dougherty, 1979; Bugelski et al., 1981) and the subcellular localization of accumulated porphyrins (Kessel, 1981; Sandberg and Romslo, 1981; Jori et al., 1986). However, the reasons for this porphyrins discrimination between normal and tumor cells are not yet known.

As a first approach to the problem, we consider of value to investigate comparatively the functionality of the heme pathway in both neoplastic and the corresponding normal tissue, from human.

From preliminary studies carried out to establish the optimum experimental conditions, it was found that porphyrin synthesis from the precursor δ-aminolevulinic acid (ALA) is significantly enhanced in explant cultures of human breast carcinoma (Navone et al., 1988).

We will present here results on our investigation on the biosynthesis of porphyrins by human breast cancer tissues in comparison with the original normal tissue, using the novel technique of tissue explant cultures and on the other hand we will report on the direct measurement of the levels of some of the heme enzymes in the same kind of tissues. From these findings we can ensure that in human breast carcinoma, porphyrin biosynthesis capacity is notoriously increased, at least from the stage of porphobilinogen (PBG) formation up to that of coproporphyrinogen, when compared with its original normal tissue.

MATERIALS AND METHODS

Tissues

Tissues came from the Instituto Nacional de Oncologia "Angel H. Roffo". Human breast carcinoma was used.
account and correct for the probable cellular lysis, incubation, histology was examined in each tissue; it original normal tissue instead the cytoplasm had lost definition probably was observed that the nuclear area was well preserved in the neoplastic tissue when compared with the average porphyrin formation was enhanced mg of protein. It should be noted that in the case of conditions. The enzymic activity measurements.

Enzymes were measured in homogenates prepared by disrupting the tissue in 0.25 M sucrose at 4°C at the ratios 1:5 (w/v) for ALA-dehydratase (ALA-D) and 1:3 (w/v) for porphobilinogenase (PBGase) and uroporphyrinogen decarboxylase (URO-D), using an Ultra-Turrax (Janke & Kunkel GmbH & Co. KG., F.R.G.) homogenizer. The resulting suspension was centrifuged at 10,000 g for 15 min and the supernatant employed for enzymic activity measurements.

Table 1. Porphyrin biosynthesis by both neoplastic and its corresponding normal mammary tissue

| Patient | Age | Diagnosis | Histological malignancy | N (LU/mg tissue x FRU) |
|---------|-----|-----------|-------------------------|-----------------------|
| 1       | 22  | dc        | GIII                    | 0.150                 |
| 2       | 66  | dc        | GII                     | 0.130                 |
| 3       | 63  | dc        | GIII                    | 0.004                 |
| 4       | 40  | dc        | GIII                    | 0.131                 |
| 5       | 26  | dc        | GII                     | 0.001                 |
| 6       | 30  | dc        | GIII                    | 0.120                 |
| 7       | 62  | kc       | GIII                    | 0.001                 |
| SD      |     |           |                         | 0.077                 |

*Expressed as FRU/mg tissue x LU (see Materials and Methods).
†Normal breast tissue.
‡Tumoral tissue.
§Ductal carcinoma.
¶Fibulillar carcinoma

Serial and comparative studies were performed in samples from 7 patients. It was found that in an average porphyrin formation was enhanced ca 20 fold in the neoplastic tissue when compared with the original normal tissue (Table 1). Before and after incubation, histology was examined in each tissue; it was observed that the nuclear area was well preserved instead the cytoplasm had lost definition probably due to cellular membrane damage, it must be noted that, as indicated in Materials and Methods, to account and correct for the probable cellular lysis, LDH activity was measured in the maintenance medium (Vázquez et al., 1986). The number of parenquimatous cells in both neoplastic and normal tissue was also quantified by means of a histological evaluation. Thus, it resulted that the density of cellular population in the former tissue in some cases corresponded to 80% of the sample, while in those samples showing prevalence of fibrous reached to only 20%. In normal mammary tissue values were between 10 and 30%. It should also be noted that no correlation whatsoever was found between the grade of histological malignancy and the magnitude of porphyrin biosynthesis enhancement.

Table 2. ALA-D activity in tumor and normal mammary tissues

| Patient | Age | Diagnosis | Histological malignancy | N (LU/mg tissue x FRU) |
|---------|-----|-----------|-------------------------|-----------------------|
| 1       | 50  | dc        | GIII                    | 1.32                  |
| 2       | 56  | dc        | GII                     | 0.48                  |
| 3       | 63  | dc        | GIII                    | 3.46                  |
| 4       | 38  | dc        | GIII                    | 0.23                  |
| 5       | 41  | dc        | GII                     | 8.27                  |

*Expressed as nmol of PBG/mg protein (see Materials and Methods).
†Normal breast tissue.
‡Tumoral tissue.
§Ductal carcinoma.
¶Fibulillar carcinoma

†Normal breast tissue.
‡Tumoral tissue.
§Ductal carcinoma.
¶Fibulillar carcinoma

The above findings, although undoubtedly indicating increased capacity for heme synthesis by the neoplastic tissue would only be an indirect measure of the phenomenon; direct quantification of enzyme activity would reflect more definitely and possibly individualize the changes occurred if any. So, ALA-D, PBGase and URO-D were determined in both neoplastic and normal tissue.

**ALA-D**

It was measured in samples from 5 patients (Table 2). In every pair activity was higher in tumor than in normal cells, activity of the latter was between 6 and 60% of the corresponding matched malignant cells. However, the variability among different samples was so great that signification tests were negatives. Moreover, ALA-D is usually in excess over the other enzymes of the pathway, therefore we will not risk any definite comment about its relative increased activity in tumor cells, as yet.
Table 3. PBGase activity in tumor and normal mammary tissues

| Patient | Age | Diagnosis | Histological | SA* |
|---------|-----|-----------|--------------|-----|
|         |     |           | malignancy   | T   | N/T |
| 1       | 63  | dc        | GIII         | 3.67| 37.00|0.09|
| 2       | 42  | dc        | GI           | 5.30| 13.33|0.39|
| 3       | 50  | dc        | GI           | 0.10| 6.70 |0.01|
| 4       | 60  | dc        | GI           | 2.50| 35.00|0.07|
| R       |     |           |              | 2.89| 2.89 |
| SD      |     |           |              | 2.19| 15.26|

*Expressed as FRU/mg protein (see Materials and Methods).

Table 4. URO-D activity in tumor and normal mammary tissues

| Patient | Age | Diagnosis | Histological | SA* |
|---------|-----|-----------|--------------|-----|
|         |     |           | malignancy   | T   | N/T |
| 1       | 64  | dc        | GIII         | 0.61| 19.80|0.03|
| 2       | 50  | dc        | GI           | 9.60| 20.83|0.46|
| 3       | 26  | dc        | GI           | 11.80|33.00|0.35|
| 4       | 43  | dc        | GI           | 10.30|38.30|0.27|
| R       |     |           |              | 8.68| 27.58|
| SD      |     |           |              | 5.06| 9.12 |

*Expressed as FRU/mg protein (see Materials and Methods).

DISCUSSION

Previous (Navone et al., 1988) and present results demonstrate that porphyrin pathway is enhanced in tumor, at least from PBG to coproporphyrinogen formation. These findings are in agreement with those found in mice spontaneous mammary tumor (see ‘Appendix’).

It is important to mention that all attempts to measure δ-aminolevulinic acid synthetase (ALA-S) failed. However, we were able to detect ALA-S in the mice spontaneous mammary tumor; although the levels were low, they were of the same order as those measured in the liver of the same animal (see ‘Appendix’). Consequently, in view of the similarity between the enzyme pattern observed in human neoplastic tissue and that coming from the animals, we would attribute the failure in detecting ALA-S in human breast tumor to technical problems. It should be taken in consideration both the know lability of ALA-S and the time elapsed between the obtainment of the samples and their processing.

Concerning to results obtained by others, information is so far scarce and sometimes, contradictory.

Rasetti et al. (1967) found that ALA-D was significantly reduced in a variety of tumors of the digestive tract and in the same paper these authors reported increased PBG biosynthesis in neoplastic tissue com-

ing from lung and uterus. On the other hand, this enzyme was 3–5 times lower in leukocytes from patients with mienlocytic acute leukemia as compared with controls (Takaku and Wada, 1968), while hydroxymethylbilane synthetase (HMB-S), one of the two components of PBGase, was found to be higher in peripheral lymphocytes of patients with lympho-

proliferative diseases such as chronic lymphocytic leukemia and lymphoma (Lahav et al., 1987).

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*Acknowledgements—This work was supported by grants from the National Research Council (CONICET), University of Buenos Aires and Banco de la Nación Argentina. Alcira M. del C. Batlle holds the post of Superior Scientific Research Fellow at the CONICET. Nora Navone and César Polo are Research Fellows at the CONICET. Alberto Frisardi is grateful to Liz Susana Fonseca for the excellent drawings. A. M. del C. Batlle is also thankful to Ministerio de Educación y Ciencia (MEC), Spain and the Association for International Cancer Research (AICR), U.K. for special help.
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APPENDIX

N. M. Navone, C. F. Polo, A. L. Frisardi and A. M. C. Battle

Heme Enzyme Pattern in Mouse Mammary Carcinoma

Abstract

1. We carried out a comparative study on the activity levels of ALA-D, PBGase, HMB-S and URO-D in different mouse tissues including liver, brain, kidney and mammary tumor.

2. No significant differences were observed in enzyme activity levels in specimens of the same tissue coming from either normal mice or tumor bearing mice.

3. However, tumoral ALA-D activity was significantly different as compared with the hepatic, renal and brain enzyme. PBGase and HMB-S activities were of the same order in tumor, liver and kidney.

4. The ratio between HMB-S and PBGase was nearly 2 and approximately the same in all the tissues.

5. Tumoral URO-D activity was rather the same of that of liver and kidney.

6. Taking into account the above results we conclude that only a partially altered pattern could exist in neoplastic tissue as compared with a typical hemopoietic tissue such as liver.

7. In spite of this, it is interesting to note the high activity levels of PBGase, HMB-S and URO-D, unexpected in cells derived from a glandular tissue.

Introduction

We have demonstrated that, compared to normal mammary tissue, human breast carcinoma showed an enhancement in porphyrin biosynthesis in vitro (Navone et al., 1988). Stout and Becker (1986, 1987) have also found some alterations in the heme pathway both in genetically and chemically induced mouse liver tumor as well as in rat liver nodules and carcinomas. Because of these observations we decided to carry out a comparative study on the activity levels of ALA-D, PBGase, HMB-S and URO-D in different mouse tissues, including liver, brain, kidney and mammary tumor.

Materials and methods

Male mice, strain BALB/c (20–25 g), were used. Animals were fed Purina 3 diet and given water ad libitum. Spontaneously mouse mammary carcinoma (M5 type) were used. Mice received a 1 mm³ inocula of tumor. injected under the skin overlying the flanks up to the axilla. The animals were sacrificed one month after the implantation. The mice previously heparinized were killed under ether anesthesia by cardiac puncture and bled.

Homogenates of the tissues were prepared as described by Batlle et al. (1967) and the supernatant of 10,000 g centrifugation for 20 min was used as enzyme source.

ALA-D activity was measured by the method of Batlle et al. (1967); PBGase and HMB-S by the method of Batlle et al. (1978); URO-D was determined using the method of Polo et al. (1990). Enzyme units were defined as the amount of enzyme that catalyzes the formation of 1 nmol of product under the standard incubation conditions. Specific activity was expressed as U/mg protein. Proteins were measured by the method of Lowry et al. (1951).

Results and discussion

No significant differences were observed in enzyme activity levels in specimens of the same tissue coming from either normal mice or tumor bearing mice (Fig. A1). However, tumoral ALA-D activity was significantly different as compared with the hepatic, renal and brain enzymes (P < 0.01, 0.01 and 0.05 respectively) [Fig. A1(a)]. On the other hand, PBGase activity levels were of the same order in tumor, liver and kidney [Fig. A1(b)]. This was unexpected because it is often found that enzyme activity levels in cells derived from a glandular tissue differ from that measured in cells such as the hepatocytes, where heme plays an important and active role as prosthetic group of a number of proteins involved in detoxifying systems. Moreover, the ratio between PBGase and ALA-D activities was also different to that found in the other tissues examined [Fig. A1(a, b)].
Heme biosynthesis in human breast cancer

In analogy with PBGase, tumoral HMB-S also showed values similar to those of liver and kidney [Fig. A1(c)]. As to the ratio between HMB-S and PBGase it was nearly 2 and approximately the same in all the studied tissues.

Tumoral URO-D activity as already discussed for PBGase and HMB-S was rather the same of that of liver and kidney [Fig. A1(d)].

To the best of our knowledge studies on these enzymes in tumors are scarce and were carried out only in human specimens. Significant diminished ALA-D activity was found in a variety of tumors from human gut, when compared with normal tissues (Rasetti et al., 1967). However these same authors observed increased porphobilinogen biosynthesis in tumor tissues from lung and uterus as compared with the corresponding normal specimens (Rasetti et al., 1967).

In leukocytes from patients suffering either acute or chronic myelocytic leukemia 3-5-fold decreased ALA-D activity was detected in contrast with normal and mature lymphocytes and granulocytes (Takaku and Wada, 1968).

HMB-S activity from peripheral lymphocytes was higher in patients with malignant lymphoproliferative diseases such as chronic lymphocytic leukemia and lymphoma than in normal individuals (Lahav et al., 1987).

Taking into account the above results coming from our and other groups, we conclude that a partially altered enzyme pattern could exist in neoplastic tissue as compared with a high heme producing tissue such as liver. Changes observed for ALA-D activity were quite different depending on the original tissue. It is interesting to note that HMB-S activity seems to be enhanced in all neoplastic tissues examined so far. Furthermore, the results reported here are also in agreement with studies carried out with human breast carcinoma.