Eur J Cancer, Vol. 26, No. 7, pp. 790-792, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00

Cytochrome P450dbl Phenotypes in Malignant and Benign Breast Disease

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129 female patients with breast cancer and 79 controls undergoing biopsy for benign breast conditions had debrisoquine hydroxylator phenotype established. 129 female hospital patients with known hydroxylator phenotype were used as another control group. The breast cancer cases differed significantly from the benign controls in their debrisoquine phenotype, with 10% being poor metabolisers compared with none of the controls ($P < 0.01$). However, while a comparison of the distributions of metabolic ratio (an inverse measure of debrisoquine metabolism) of breast cancer patients and hospital controls showed a significant difference by rank, there was no significant difference in the proportion of poor metabolisers in these two groups. The cases with benign disease differed from the hospital controls in both metabolic ratio distribution ($P < 0.001$) and frequency of poor metabolisers ($P < 0.05$). Although there was a shift in metabolic ratio distribution, debrisoquine hydroxylator phenotype was not a genetic marker for breast cancer. Why no patients undergoing biopsies for benign conditions were poor metabolisers is unknown.
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INTRODUCTION

NORMAL development, function and involution of the breast are under endocrine control, which is preserved and sometimes amplified in many disease states [1]. Thus endocrine abnormalities may be involved in the aetiology or promotion of breast diseases, particularly malignancy. However, most studies have measured steroid hormone levels in urine and blood, which may poorly reflect the endocrine milieu of mammary epithelium [2].

The interconversion of oestrogens is largely by the cytochrome P450 system [3]. Hydroxylation of oestradiol (E_2) by P450 produces the active 16-hydroxy and 4-hydroxy metabolites, and the weakly active 2-hydroxy E_2 [4, 5]. Transformations occur in the liver but also in oestrogen target tissues, such as the placenta [6] and in breast cancers [7]. Thus cytochrome P450 isoenzymes are likely to play an important role in the autocrine, paracrine and endocrine effect of oestrogens by modulation of local and circulating steroids.

Many purified isozymes of the cytochrome P450s have been studied at the protein and DNA level, to reveal at least ten gene families with between 1 and 20 members in each [8]. For example, aromatase, the P450 that converts androstenedione into oestrone, is the sole member of family P450XIX [9]. It is not known which P450s are responsible for the conversion of oestradiol to the catecholoestrogens in breast tissue itself, but one clue points to the P450IID subfamily, whose major gene product is P450dbl, the debrisoquine 4-hydroxylase [8, 9].

With bufuralol 1'-hydroxylation as a measure of activity, P450dbl has been shown to harness hydroperoxides, such as cumene hydroperoxide, for peroxidation [10]. This non-inducible isoenzyme has a well characterised genetic polymorphism visible as two phenotypes: extensive metabolisers (EM) and poor metabolisers (PM) for debrisoquine 4-hydroxylation [11, 12].

The recessive PM phenotype affects 1 in 10 of the U.K. population, and in such individuals P450dbl is not detectable. It is possible that P450dbl polymorphism is involved in individual risk of smokers developing carcinomas of both bladder and bronchus [13–15]. Fewer PMs were found in the cancer patients, which suggests that activation of precarcinogens by P450dbl increases the risk of malignant transformation. However, in patients with carcinomas of lung and bladder, other investigators did not find a correlation [16, 17].

For these reasons we have examined debrisoquine hydroxylator phenotypes in women with breast cancer undergoing biopsy compared with those in women who had benign breast lesions biopsied in the same period.

PATIENTS AND METHODS

208 patients took part in the study which was blind for ascertainment of debrisoquine 4-hydroxylation phenotype. Informed consent was obtained from each patient who was asked

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to complete a questionnaire on smoking history and concurrent medication. Approval for the study was obtained from the hospital ethical committee.

129 subjects were patients with histologically confirmed breast cancer admitted to Guy's Hospital breast unit between January 1987 and January 1988. 79 women were admitted during the same period to the breast unit with palpable breast lumps which after biopsy proved to be benign. A second control group consisted of 129 female inpatients who had had debrisoquine phenotype established in previous studies. Diagnoses were varied but the major categories were varicose veins (11), hernia (10), essential hypertension (8), diabetes mellitus (6) and cholecystitis (6). None of these hospital controls had malignant conditions. None of the patients were taking quinidine-type drugs or beta-blockers which are known to affect debrisoquine phenotype.

Each patient took 10 mg debrisoquine orally before going to bed. All urine was collected over the next 8 h. The total volume of urine collected was recorded and a sample was sent to the Department of Pharmacology and Toxicology, St Mary's Hospital Medical School. The urine samples were analysed for debrisoquine (D) and 4-hydroxydebrisoquine (4HD) content by automated electron-capture capillary gas chromatography. D and 4HD were derivatised in urine with hexafluoroacetylacetone to yield 3,5-bis(trifluoromethyl) pyrimidine derivatives which were separated and detected by gas chromatography.

The metabolic ratio was calculated as D/4HD and used to define the phenotype. Patients with a ratio between 0.1 and 12.6 were categorised as EMs whereas those with ratios greater than 12.6 were classified as PMs [12]. Experience with this assay in more than 16 000 samples over 13 years in the St Mary's laboratory shows that the metabolic ratio varies little with time and has a reproducibility of over 99.3% [18].

RESULTS

The patients with benign disease were significantly younger than the cancer cases ($P < 0.0001$, t test), but the ages of the cancer patients and hospital controls were not significantly different (Table 1). However, there was no relation between age and debrisoquine phenotype (Fig. 1). In other respects the two groups with breast disease were similar: in particular, both were predominantly white and neither were taking drugs known to effect debrisoquine metabolism. The frequency of smokers in both groups was similar. Smoking, however, has no effect on the debrisoquine metabolic ratio [19].

None of the patients with benign disease had the PM phenotype compared with 13 (10%) of the cancer patients and 8 (6%) of the hospital controls (Fig. 2). The distribution of metabolic

*Table 1. Patients' details**

	Breast cancer	Benign disease
No.	129	79
Age in years	56.1 (12.1)	42.4 (12.5)
Age at first child	25.5 (4.9)	23.5 (4.1)
Age at menarche	13.3 (1.7)	12.8 (1.6)
Parity	103 (80%)	56 (71%)
White	121 (94%)	78 (99%)
Family history of breast cancer		
1st degree	12 (9%)	7 (9%)
2nd degree	6 (5%)	11 (14%)
Oral contraceptive use	32 (25%)	33 (42%)
Smoked (pack per year)		
>1	66 (51%)	40 (51%)
>10	43 (33%)	20 (25%)
No. of PMs	13 (10%)	0
Recovery (%)	22.5 (8.9)	25.4 (9.5)

Mean (S.D.).
*129 hospital controls were aged 58.5 (2.3); 8 (6%) were PMs.

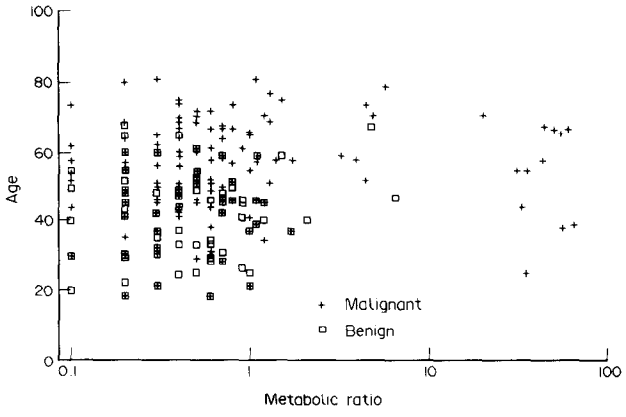

Fig. 1. Metabolic ratio by size.

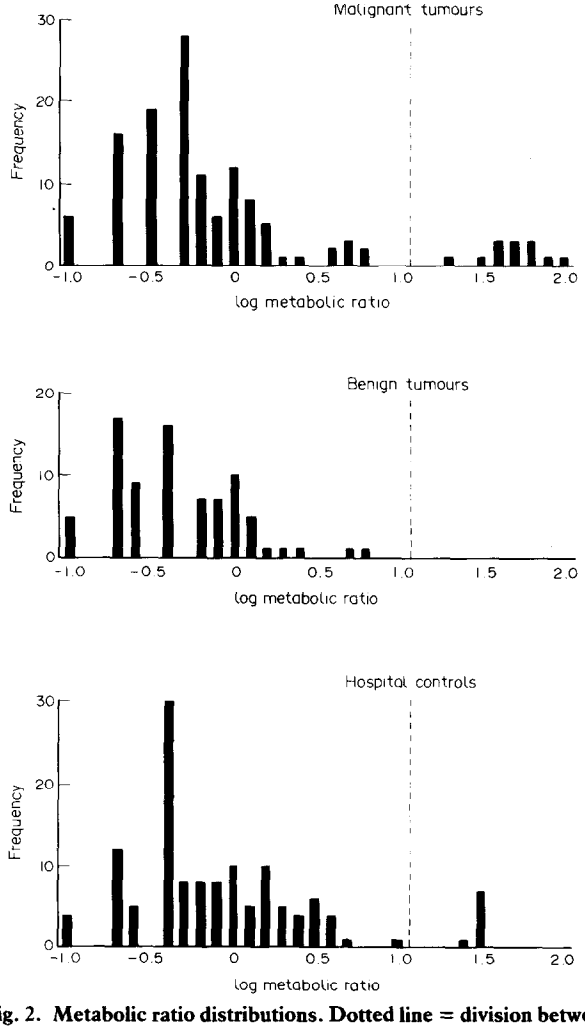

Fig. 2. Metabolic ratio distributions. Dotted line = division between EM and PM (> 12.6) phenotypes.

ratio in the patients with benign disease was significantly different from the cancer cases ($P = 0.04$) and from the hospital controls ($P < 0.001$, z test). The cancer cases were also different from the hospital cases ($P = 0.008$). In the proportion of PMs, cases with benign disease differed significantly from the cancer patients ($P < 0.01$) and from the hospital controls ($P < 0.05$, χ^2) but the cancer cases were similar to the hospital controls. Thus, while there was a slight excess of PMs in the cancer cases, there was an absence of PMs among the patients with benign disease. When subdivided by menopausal status there was no difference in metabolic ratio of premenopausal and postmenopausal cases or controls.

Histological examination of the patients with benign disease revealed: fibroadenoma 17, fibrocystic disease 27, normal 8, inflammation 5, duct ectasia 4, epithelial hyperplasia 4, intraduct papilloma 3, phylloides tumour 2 and other 9. Only 1 patient had atypical epithelial hyperplasia, while another had multiple papillomatosis (both deemed to be premalignant conditions) and neither were PMs (metabolic ratio 0.2 and 0.5, respectively). There was no correlation between the oestrogen or progesterone receptor status and metabolic ratio. In the patients with breast cancer, there was no correlation between stage of disease and metabolic ratio.

DISCUSSION

In this study of debrisoquine hydroxylator phenotypes, there was a significant difference between patients with breast cancer or benign disease: significantly more women with breast cancer had the PM phenotype. This result suggests that the PM phenotype is associated with breast cancer, although the proportion of PMs in the hospital controls fell between the two other groups, being significantly different from both by rank test but significantly different from only the benign group in frequency of PMs. Thus while there was a slight excess of PMs in the cancer group, it was the metabolic ratio of the benign group that was skewed. Why this should be is not known.

The benign controls did not differ from cases in terms of ethnic background, family history of breast cancer or smoking history. The controls were a heterogeneous group with a variety of histological diagnoses. However, the commonest diagnoses were fibroadenoma and fibrocystic disease. Possibly the absence of PMs means that the EMs are more able to activate promoters of benign proliferation within the breast. This significant difference in phenotype of benign and malignant cases is yet more evidence that most patients with benign conditions of the breast do not have a premalignant disease [20].

The findings by Weisz and her co-workers [7] of P450-mediated 2-hydroxylation and 4-hydroxylation of E_2 by breast tumour tissue may provide insights into the development of various breast diseases in which there is a suspected oestrogenic component. The principal oestrogen secreted by the ovary in premenopausal women is circulating unconjugated E_2 which is taken up by the breast and can undergo further metabolism by ductal epithelial cells. Whilst the classical P450 monooxygenation requiring molecular oxygen and NADPH has not been found, a P450 peroxidation by cumene hydroperoxide and other hydroperoxides *in vitro* affects both the 2-hydroxylation and 4-hydroxylation of E_2. Because the 4-hydroxycatecholoestrogen is a potent oestrogen, this P450-mediated peroxidation may significantly modify the hormonal environment of the breast by introducing a paracrine, or even autocrine, component.

The question remains as to whether the *in vivo* phenotypes of EM and PM, which arise from hepatic P450dbl, are reflected in breast tissue. Should this be the case, *in vivo* phenotyping with debrisoquine would yield unique data on the aetiology of oestrogen-related breast diseases. The finding of only EM women in the benign disease cohort, with a shift to higher debrisoquine 4-hydroxylation, may mean that increased production of 4-hydroxy-E_2 by P450dbl-mediated peroxidation among EMs puts them at higher risk of benign breast lesions. For many years it has been suspected that some benign breast conditions resulted from an abnormal tissue response to apparently normal levels of circulating hormone. This finding provides possible indirect evidence of such a tissue abnormality. The link between genetically determined P450dbl activity, peroxidation and oestrogen metabolism in the human breast needs investigation. We are examining the distribution of P450dbl activity in breast tissue from biopsy specimens.

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