Acetylation Phenotype Is Not Associated with Breast Cancer[1]

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ABSTRACT

In the present study, we have measured acetylation phenotype in 45 patients who had undergone surgical resection of a primary adenocarcinoma of the breast and in 48 patients or volunteer subjects with no breast disease. Phenotype was determined by measuring the ratio of N-acetylsulfamethazine to N-acetylsulfamethazine plus sulfamethazine in plasma 6 h after a p.o. dose of sulfamethazine. In the control group, there were 31 slow and 17 rapid acetylators, while in the breast patients, there were 25 slow and 20 rapid acetylators. The proportions of slow/rapid acetylators were not significantly different between the 2 groups (Pearson's χ^2 with Yates' correction = 0.45; P = 0.51). The data suggest that acetylation phenotype is not a useful risk prediction measurement in breast cancer.

INTRODUCTION

Hepatic acetylation phenotype is a genetically inherited trait controlled by 2 major alleles of a single locus. Slow acetylators are homozygous for an autosomal recessive gene, whereas rapid acetylators are homozygous or heterozygous for the dominant gene (1, 2). Acetylation phenotype has been associated with spontaneous disorders such as systemic lupus erythematosus, diabetes, and Gilbert's disease, and also with cancers such as those occurring in the bladder, colon, larynx, and breast (2, 3).

The first study of the association between breast cancer and acetylation phenotype was undertaken in a group of Russian patients (4) with "advanced" disease. They found 68% rapid acetylators in breast patients (n = 41) compared with 37% in controls (n = 38). Preliminary results from a study undertaken in Britain (5) were generally supportive of the earlier Russian study. However, when the full results of the British study were published (6), the percentages of rapid acetylators were similar in controls (44.8%; n = 337), benign breast disease (52.9%; n = 136), and malignant breast disease (45.3%; n = 181). In addition, they reported a significant trend for an increase in the percentage of rapid acetylators with increase in disease severity from Stage I to Stage IV. A study of patients with fibrocystic breast disease (7) found a similar percentage of rapid acetylators in patients (39%; n = 36) compared with controls (40%; n = 9), while another Spanish study (8) also found a similar percentage of rapid acetylators in breast cancer patients (39.5%; n = 49) compared with controls (40%; n = 45). However, the latter study did not report the disease stage for their patients. Thus, the association between acetylation phenotype and breast cancer is controversial and in some studies, confounded by inadequate description of disease stage. The present study has documented acetylation phenotype in breast cancer patients and controls in Australia.

MATERIALS AND METHODS

Phenotype was determined in 2 groups of subjects: (*a*) patients who had undergone surgical resection of a primary carcinoma of the breast; and (*b*) patients or volunteer subjects with no breast disease. The breast cancer patients were drawn at random from patients presenting to the Sir Charles Gairdner and Fremantle Hospitals over an 18-month period. Prospective patients were identified by the physician during weekly ward rounds and outpatient clinics. The study was explained in detail to each patient and only those willing to participate were entered into the trial. Control subjects were drawn from other outpatient clinics and from healthy volunteers using the same recruitment procedure. In the control group, 20 were recuperating patients (1 bowel obstruction, 1 esophageal varices, 1 diverticulitis, 1 hernia, 1 benign ovarian teratoma, 1 varicose veins, 1 infected finger, 2 appendicitis, 2 leg ulcers, 2 laparatomy for investigation of abdominal pain, 3 skin grafts, 4 orthopedic repairs) in the above hospitals and 28 were healthy volunteers. All subjects were of Caucasian racial origin. Subjects with diabetes, advanced vascular disease, and renal or hepatic disease were excluded as were patients who were receiving chemotherapy. All subjects gave informed consent and the study protocol was approved by the Human Rights Committee of the University of Western Australia, and by the Ethics Committees of the Sir Charles Gairdner and Fremantle Hospitals. Smoking history was recorded for all subjects and tumor stage and hormone receptor classification were noted for breast patients.

Subjects were fasted from midnight on the day prior to study, and on the study day were given SMZ.[3] (13.5–16.7 mg/kg at 7 a.m.) orally in tablet from (Sulphadimidine Tablets, 500 mg) together with 200 ml of water. Fluids were not restricted, but breakfast was delayed until 10:30 a.m. and alcohol was not permitted on the study day. A single blood sample was collected 6 h after the SMZ dose, and the plasma was separated by centrifugation and stored at −20°C until analyzed for sulfonamide content. SMZ and its N-acetyl metabolite in plasma were determined by high-performance liquid chromatography as previously described (9) and the molar ratio of N-acetyl-SMZ to N-acetyl SMZ + SMZ was used an the index of acetylation phenotype (9) with a ratio of <0.6, indicating a slow acetylator, and >0.6, indicating a rapid acetylator. In all breast patients, phenotyping was undertaken at least 5 days after removal of the tumor.

Estrogen and progesterone receptor levels were determined in tumor tissue using enzyme immunoassay (Abbott Diagnostics, Australia). Negative results in the assay procedure were <4 fmol/mg protein for estrogen and <10 fmol/mg protein for progesterone.

Data were summarized as mean ± SD or mean and range unless otherwise specified. Acetylator phenotype categorization data were compared using a χ^2 analysis.

RESULTS

A total of 45 breast cancer patients (mean age = 52 years; range = 31–82 years) and 48 controls (mean age = 61 years; range = 38–89 years) were studied. As delineated by age 50 and above, 73% of the breast patients and 56% of controls were postmenopausal. The phenotype data for the 2 groups are summarized in Fig. 1 in the form of Probit plots, which clearly show 2 parallel sections (representing the 2 phenotypes) for both cancer patients and controls. As in our previous study (9), the rapid and slow phenotypes were readily discriminated using a plasma ratio of 0.6 as the cutoff point. In the control group,
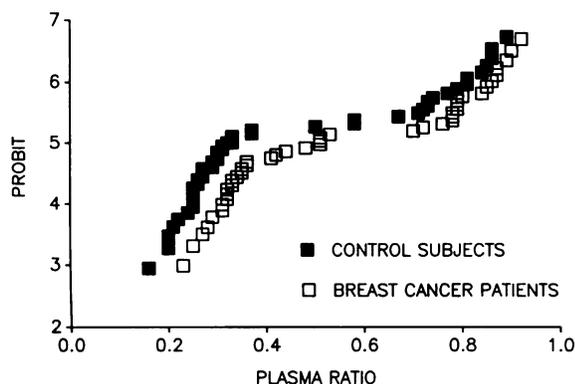
Fig. 1. Probit plot of cumulative number of subjects *versus* plasma ratio (*N*-acetyl-SMZ:*N*-acetyl-SMZ + SMZ) at 6 h after giving 15 mg SMZ/kg p.o. to breast cancer patients (□) and controls (■).

Table 1 *Smoking history for cancer patients and controls*

Group	Acetylation status	No. of	
		Smokers[a]	Nonsmokers
Breast cancer	Slow	2	23
	Rapid	4	16
Control[b]	Slow	8	21
	Rapid	4	13

[a] Includes exsmokers.
[b] No data available for 2 subjects.

Table 2 *Tumor stage and acetylation phenotype in breast cancer patients*

Stage	No. of patients with[a]	
	Slow phenotype	Rapid phenotype
I	16 (61.5)	10 (38.5)
II	5 (41.7)	7 (58.3)
III	0	2
IV	1	0
Unclassified	3	1

[a] Numbers in parentheses, percentage distribution.

there were 31 slow and 17 rapid acetylators, while in the breast patients, there were 25 slow and 20 rapid acetylators. The proportions of slow/rapid acetylators were not significantly different between the 2 groups (Pearson's χ^2 with Yates' correction = 0.45; $P = 0.51$). Since the mean age in our breast patients was 9 years lower than in the controls, we also performed a parallel analysis in an age-matched subset (5 years grouping; $n = 36$ in each group) of our subjects. This analysis also indicated that there was no correlation between acetylator phenotype and susceptibility to breast cancer (Pearson's χ^2 with Yates' correction = 0.9; $P = 0.66$).

Smoking history data are summarized in Table 1. Overall, 26.6% of controls and 13.3% of cancer patients had been or were current smokers. There was no relationship with phenotype ($\chi^2 = 3.45$; $P = 0.33$). Table 2 summarizes disease stage for the breast patients and its relationship to phenotype. Most patients were in Stage I (57.7%) or Stage II (26.7%), and these classifications had similar proportions rapid and slow acetylator phenotypes ($\chi^2 = 5.22$; $P = 0.26$). Hormone receptor data for the breast patients are shown in Table 3. The distribution of the tumor receptor subtypes was similar for rapid and slow acetylators ($\chi^2 = 0.71$; $P = 0.87$).

DISCUSSION

Our study shows that the proportions of slow and rapid acetylators are similar in breast cancer patients and controls.

Table 3 *Hormone receptor classification in breast cancer patients*

Receptor type	No. of patients with	
	Slow phenotype	Rapid phenotype
Estrogen positive	11	11
Estrogen negative	7	4
Progesterone positive	8	6
Progesterone negative	8	8

Thus, our Australian data support earlier findings in Spanish (8) and British (6) breast patients. In agreement with the study of Philip and coworkers (6), we also noted a trend towards more slow acetylators in patients with Stage I disease compared with Stage II disease. The significantly greater proportion of rapid acetylators in the Russian study (4) is unexplained but may reflect the "advanced" nature of the disease and/or the effects of concurrent chemotherapy in their patients.

Although positive tumor receptors for both estrogen and progesterone are now recognized as highly favorable predictors of outcome (10), both our own data and those of Philip and coworkers (6) show clearly that determination of acetylator phenotype does not add a further dimension to such prediction.

However, because of the relatively small sample size, our data must be interpreted with caution. In studies of this type, previous authors (6) have pointed out that very large numbers of subjects are required to yield ideal statistical power in the investigation. If we assume that the control population has 35% of the rapid phenotype [consistent with our present and previous data (9)], it can be calculated (11) for the present study ($n = 45$ and 48), that an 80% increase in the rapid phenotype (*i.e.*, 63% rapid in population) would be detectable at the 5% significance level and with an 80% power of study. Obtaining very large numbers of subjects in one center is not practicable and thus one is usually reliant on several smaller studies being carried out in a number of centers. The combined data can then be used to assess the hypothesis. Multicenter data collection is also highly desirable because acetylation phenotype is dependent on racial origin (1) and because the impact of phenotype in cancer is thought to be dependent on dietary and environmental exposure to amine carcinogens. When the data from the present study are combined with observations from Russia (4), Spain (8), and Britain (6), the world totals become 348 breast cancer patients, of whom 42% were rapid acetylators and 498 controls, of whom 46.6% were rapid acetylators. Thus, there is no significant association between breast cancer and acetylation phenotype ($\chi^2 = 1.51$; $P = 0.21$). Overall, these data strongly suggest that the metabolic activation/deactivation role for *N*-acetyltransferase and related enzyme activities, which has been postulated particularly in cancer of the bladder (12) (slow acetylators definitely more susceptible) and colon and rectum (13, 14) (rapid acetylators may be more susceptible), is unlikely to be relevant in breast cancer.

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