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Acetylation and oxidation phenotypes in malignant lymphoma

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Summary. 101 white British adults with Hodgkin's disease or non-Hodgkin's lymphoma were phenotyped for acetylation status using dapsone and for oxidation status with debrisoquine prior to treatment. The frequencies of acetylation and oxidation phenotypes in these patients were compared with reference populations of normal subjects. No significant difference in phenotype frequency was found in the lymphoma patients. This suggests that neither of these metabolic polymorphisms for exogenous compounds is strongly associated with these malignancies. Owing to the small size of the study, however, an effect of these phenotypes could not be excluded.

Introduction

The aetiology of lymphomas is largely unknown, although there is now some convincing evidence for the role of type C retroviruses and the Epstein-Barr virus in some varieties of the disease [3]. In addition, however, there is at least some evidence that the process may be initiated by hydantoins [20]. Excess mortality from lymphoma has been reported for chemists from three countries [19, 23, 28], for rubber processing workers [22], for workers in contact with vinyl chloride [7], phenoxyacetic acid or chlorophenol [13] and for veterinary surgeons [2]. Evidence for dietary associations is slender, but epidemiological surveys find a relationship between mortality from non-Hodgkin's lymphoma and per capita bovine protein consumption [9]. The possible importance of exogenous chemical inducers of the malignant process may mean that the capacity of an individual to metabolise environmental compounds may in part determine the liability of that individual to develop a malignant disease. In some cases this metabolic capacity is inherited. Thus it has been suggested that there is an increased proportion of "rapid acetylators" in patients with breast [4] or colorectal [17] cancer. By contrast, there is an excess of "slow acetylators" among patients who develop bladder cancer subsequent to industrial exposure to arylamines [5, 10]. Another inherited polymorphism, of drug oxidation, has also been linked with increased susceptibility to cancer, with an increased representation of "extensive oxidisers" in patients with carcinoma of the bronchus [14] and larynx [27].

In this paper we have studied the prevalence of two well-known drug-metabolising polymorphisms in patients with lymphoma. *N*-acetyltransferase (NAT) is a cytosolic non-inducible enzyme utilising acetyl CoA. Approximately 55% of the Caucasian population are slow acetylators (SA), being homozygous for the recessive allele. Rapid acetylators (RA) comprise the rest of the population and are either homozygous or heterozygous for the dominant allele [8, 30]. Neither age nor sex influences the prevalence of the acetylator in a population [25, 26].

Mahgoub et al. [21] described the polymorphism of C-oxidation of debrisoquine. The enzyme responsible is debrisoquine 4-hydroxylase, an isoenzyme of the cytochrome P-450 system. In a Caucasian population, 8.9% were poor metabolisers (PM) consequent upon a deficient or absent debrisoquine 4-hydroxylase enzyme. PM are homozygous for a recessive allele, while extensive metabolisers (EM) are either homozygous or heterozygous for the dominant allele [10]. Recently it has been shown that genetic constitution accounts for 79% of interindividual variation in debrisoquine oxidation. Sex, age, alcohol intake and smoking exert no effect on debrisoquine oxidation [29]. In addition, polymorphic *N*-acetylation and D-oxidation exhibit marked interethnic variation in the proportion of the various phenotypes [8].

Methods

Subjects

Lymphoma group. The lymphoma group consisted of 101 white British adults (68 males and 33 females) attending the lymphoma clinic at Guy's Hospital, London with untreated disease between January 1983 and October 1985. A positive histological diagnosis of malignant lymphoma was available on all patients, mostly by lymph node biopsy. Thirty-four of the patients had Hodgkin's disease (HD) and 67 had non-Hodgkin's lymphoma (NHL). Approval to conduct this study was granted by the Committee on Ethical Practice of Guy's Hospital and Medical School. Patients known to be allergic to sulphonamides or deficient in glucose-6-phosphate dehydrogenase were excluded from acetylation phenotyping. It was considered unethical to alter any concurrent medication, and all patients were on their usual diets. Social alcohol intake was permitted, since it has been shown not to interfere with acetylation phenotyping by dapsone [15]. Patients with impaired renal or hepatic function were not excluded from this study.

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Control group. (a) Acetylation status: This group consisted of white 337 British adults – 146 healthy volunteers aged below 30 years (98 males, 48 females) and 191 patients aged over 65 years (46 males, 145 females). The healthy volunteers were medical students and staff at the United Medical and Dental Schools, Guy's Hospital, while the group aged over 65 years comprised patients attending geriatric clinics at Orpington General Hospital, Kent. (b) Debrisoquine oxidation status: The 87 healthy volunteers (53 males, 34 females) in this group were medical students at the United Medical and Dental Schools, Guy's Hospital. They ranged in age from 18 to 28 years (mean 20.2 ± 1.9 years).

Phenotyping

Informed consent was obtained from each subject. Phenotype testing in the lymphoma group was part of the initial series of investigations aimed at diagnosis and/or staging of disease prior to treatment.

Acetylation test. Dapsone (DDS) was employed as a marker for *N*-acetylation. Following an oral dose (100 mg), 5 ml venous blood was withdrawn into a heparinised tube 2–4 h after dosing. Plasma was immediately separated by centrifugation and stored at –20° C pending analysis. Plasma monoacetyldapsone /dapsone (MADDS /DDS) ratios were used to characterise acetylation status. Ratios <0.30 indicate SA and those $\geqslant 0.30$ indicate RA [8, 12, 30].

Debrisoquine oxidation test. After emptying of the bladder, 10 mg debrisoquine (Declinax, Roche Products Ltd.) was administered orally and urine collected from 0 to 8 h inclusive [21]. A 20-ml aliquot was stored at –20° C. Debrisoquine oxidation status is expressed in terms of the metabolic ratio (MR) which is the ratio of the amount excreted in urine as debrisoquine to the amount excreted as 4-hydroxydebrisoquine during the 8-h period. Ratios $\geqslant 12.6$ represent the PM phenotype, while ratios < 12.6 indicate the EM phenotype [8].

Analysis

MADDS and DDS were simultaneously detected in plasma using a non-extractive HPLC method [24].

Debrisoquine and 4-hydroxydebrisoquine were concurrently measured in urine employing gas chromatography with nitrogen-selective detection after derivatisation with acetylacetone following extraction with diethyl ether [18].

Statistical analysis

The significance of variations in acetylation and debrisoquine oxidation phenotypes between the different groups was ascertained by means of the uncorrected χ^2 test employing 2×2 contingency tables [1]. The 5% level was taken as the limit of statistical significance. The approximate relative risk (odds ratio) was calculated according to the formula:

$$\text{odds ratio } (\psi) = \frac{a \times d}{b \times c}$$

and its 95% confidence interval was computed according to the equation

$$\log_e \psi \pm 1.96 \sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$$

after transforming the values back to the original scale [1].

Results

Acetylation

Table 1 gives the distribution of lymphoma patients and controls between SA and RA phenotypes. The proportion of controls who were SA (55.2%) is close to figures reported by others for Caucasian populations [8, 30]. Despite the occurrence of relatively more RA phenotypes in the lymphoma groups (52.4% and 54.8% for NHL and HD respectively), no significant statistical difference exists in their distribution between the groups. The approximate relative risk ratio for the rapid acetylator in NHL was 1.35 (95% confidence interval 0.79–2.31) and that for HD was 1.50 (95% confidence interval 0.72–3.14).

Debrisoquine oxidation

Table 2 demonstrates the distribution of lymphoma patients and controls with poor and extensive MR. The proportion of poor metabolisers of debrisoquine in the control population is similar to that described in other Caucasian populations [8]. No significant association of PM or EM phenotype exists with the presence of lymphoma as

Table 1. Distribution of acetylation phenotypes in HD, NHL and controls

Acetylation phenotypes	Non-Hodgkin's lymphoma ($n = 63$)		Hodgkin's disease ($n = 30$)		Controls ($n = 337$)	
	n	%	*n*	%	*n*	%
Slow acetylators	30	47.6	14	45.2	186	55.2
Rapid acetylators	33	52.4	17	54.8	151	44.8
	$\chi^2 = 0.050$, df = 1 $P > 0.50$ (N.S.)		$\chi^2 = 1.15$, df = 1 $0.50 > P > 0.10$ (N.S.)			
	$\chi^2 = 1.23$, df = 1, $0.50 > P > 0.10$ (N.S.)					

Table 2. Distribution of debrisoquine metabolic ratio in Hodgkin's disease, non-Hodgkin's lymphoma and controls

Metabolic ratio	Hodgkin's disease		Non-Hodgkin's lymphoma		Controls	
	n	%	*n*	%	*n*	%
< 12.6	30	96.7	57	95.0	80	92.0
$\geqslant 12.6$	1	3.3	3	5.0	7	8.0
Total	31	100.0	60	100.0	87	100.0
	$\chi^2 = 0.022$, df = 1 $P > 0.50$ (N.S.)		$\chi^2 = 0.15$, df = 1 $P > 0.50$ (N.S.)			
	$\chi^2 = 0.53$, df = 1, $0.50 > P > 0.10$					

compared to the controls. The relative risk ratio for extensive metabolisers was 1.66 in NHL (95% confidence interval 0.41–6.7) and 2.63 in HD (95% confidence interval 0.3–22.3, reflecting the very small numbers of this series).

Discussion

Several attempts have been made to investigate polymorphic *N*-acetylation and debrisoquine oxidation in relation to cancer incidence. These studies were inspired by the fact that interindividual variations in the rate of drug metabolism may, at least in part, account for the variability in susceptibility to cancer. A strong association has been found between SA phenotype and bladder cancer, particularly the occupational variety [5, 10]. This is in keeping with the proven role of NAT in the detoxification of carcinogenic arylamines incriminated for bladder carcinogenesis [11]. In addition, Bulovskaya et al. [4] demonstrated a preponderance of RA phenotypes in a group of advanced breast cancer patients as compared with healthy controls. Hetzel et al. [14] showed that a group of patients who had bronchial carcinoma and were smokers tended to include significantly lower numbers of PM phenotypes. Nigerian patients with gastrointestinal and hepatic malignancies also included disproportionately large numbers of individuals who were EM phenotypes as compared to normal controls [16]. Our own investigations, however, failed to elicit any association between EM or PM phenotypes and patients with bladder cancer [6].

The results presented in this study suggest a lack of any important association between the acetylation and debrisoquine oxidation phenotypes and malignant lymphomas. However, the size of the present study yields little statistical power, and the observations can do no more than exclude very large effects of the metaboliser phenotypes investigated. The data are thus presented to provide a basis for further investigations of these relatively rare tumours. An alternative interpretation is that the findings suggest the lack of any gross alteration of these metabolic pathways in the presence of lymphoma. Although the lymphoma group and the controls were not matched regarding age and sex, these two factors appear not to influence acetylator status [25] or debrisoquine oxidation capacity [29]. Further subgrouping by histological type of HD or NHL would yield numbers too small to allow valid conclusions.

At the present time, therefore, knowledge of the acetylation phenotype does not appear to contribute to our knowledge of the natural history of the lymphomas. Nevertheless, as further inherited polymorphisms of drug-metabolising enzymes are discovered this question may require reinvestigation.

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