Lack of a relationship between the polymorphism of debrisoquine oxidation and lung cancer

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- 1 Determination of debrisoquine oxidation phenotype was carried out in 119 healthy subjects, 135 patients with chronic bronchitis and 153 patients with lung cancer, all of Caucasian origin.
- 2 A non-Gaussian distribution of the log D/HD ratio was observed in the three groups.
- 3 Assuming an antimode of 1.12, the proportion of PMs was found to be 6.7% in healthy subjects, 8.9% in chronic bronchitics and 6.5% in patients with lung cancer. These differences were not significant.
- 4 The presence of a lung tumour itself had no influence on phenotype in a group of 14 patients who were phenotyped before and after surgery.
- 5 We conclude that a link between debrisoquine phenotype and lung cancer is unlikely.

Keywords debrisoquine phenotype genetic polymorphism bronchitis lung cancer

Introduction

Polymorphism of drug oxidation in man was first shown to occur by Maghoub *et al.* (1977). The urinary ratio of debrisoquine (D) to its main 4-hydroxy metabolite (HD) in an 8 h urine collection following a single oral dose of 10 mg debrisoquine hemisulphate was shown to be distributed bimodally in a British Caucasian population, 8.9% of whom were poor metabolizers (PMs) (Price-Evans *et al.*, 1980). Polymorphism of debrisoquine oxidation reflects enzyme deficiency in cytochrome P450IID6 (Gonzalez *et al.*, 1988, Nebert & Gonzalez, 1987). This isoenzyme is not inducible (Eichelbaum *et al.*, 1986) but the administration of certain drugs can decrease its activity (Inaba *et al.*, 1986; Speirs *et al.*, 1986). Ayesh *et al.* (1984) suggested a relationship between the EM phenotype of debrisoquine and bronchocarcinoma. They showed that, in a population of patients with a lung cancer, the EM phenotypes were over represented. However, Drakoulis *et al.* (1986) and Speirs *et al.* (1990) were unable to confirm this finding. Because of these conflicting results, we have attempted to reassess the possible association between debrisoquine phenotype and lung cancer.

Methods

The protocol was approved by the local Ethics Committee (Centre hospitalo-universitaire de Besançon).

Healthy subjects

One hundred and nineteen French Caucasian subjects (78 men and 41 women) gave their informed consent to take part in the study. Their ages ranged from 22 to 61 years (mean $\pm$ s.d., 30.3 ± 7.6). They had no history of hepatic or renal disease. Their alcohol and cigarette consumption was moderate and they were not taking any medication at the time of the study.

Chronic bronchitis and lung cancer groups

All patients were treated in the Centres Hospitalo-Universitaire of Besançon and Creteil, France. The chronic bronchitis group (135 patients, 124 men and 11 women, mean age: 64.7 ± 10.8 , range from 41–93 years) were smokers without any clinical, X-ray or (where performed), endoscopic evidence of lung cancer. The lung cancer group (153 patients, 143 men, 10 women,

mean age 62.41 ± 9.3 , range 39–86 years) had histologically proven bronchogenic carcinoma. The cell types comprised squamous cell ($n = 108$), small cell ($n = 38$), undifferentiated carcinoma ($n = 13$) and adenocarcinoma ($n = 25$); four patients had no definite histology. All patients had a smoking history of more than 20 pack years calculated according to the formula:

number pack/years =

$$\frac{\text{number of cigarettes/day} \times \text{number of years}}{20 \text{ (number of cigarettes per pack)}}$$

All patients had normal or near normal renal and hepatic function as judged by routine clinical and biological data (SGPT, alkaline phosphatase and plasma creatinine levels below twice normal ranges). Medication taken at the time of study included antibiotics, bronchodilators, cardiovascular drugs (not quinidine), antipsychotic, and analgesic drugs, but no antimitotic drugs. Some of these drugs are known to be specific inhibitors of P450IID6. At the time of the phenotyping:

- In the cancer group, six patients were given antidepressant drugs (amitriptyline, clomipramine, imipramine), four had neuroleptic drugs (acepromazine, levopromazine, propericiazine), one received codeine.
- In the chronic bronchitis group, three patients were given antidepressant drugs (amitriptyline, clomipramine), four had neuroleptic drugs (levopromazine, propericiazine), four had captopril and one received codeine.

Study design

Each subject was fasted from 20.00 h the day before the study. They were given 10 mg debrisoquine hemisulphate at 08.00 h (Declinax® Hoffmann-La Roche, Basle), and were allowed to have breakfast 2 h after. Urine was collected, under nurse supervision, over 8 h into plastic containers. Total urine volumes were noted and aliquots (3×20 ml) were stored at -20°C until analysis. The logarithm of the metabolic ratio of debrisoquine (D)/4-OH-debrisoquine (HD) was used to define the oxidation phenotype (Maghoub *et al.*, 1977).

Debrisoquine and 4-OH-debrisoquine were assayed in urine by the method of Lennard *et al.* (1977).

Statistics

The normality of the distribution of the log D/HD ratio in each group was examined using the Kolmogorov-Smirnoff test. The Kruskal-Wallis test allowed simultaneous comparison of the distributions of the D/HD ratio in the three groups. Using the median test we investigated any dissimilarities in the log D/HD distribution between control and lung cancer patients and control and chronic bronchitic patients (Siegel, 1956).

We were able to obtain usable data for 153 patients with lung cancer and 135 patients with chronic bronchitis. To compare our results with those of Ayesch *et al.* (1984), we calculated the '*a posteriori*' statistical power of our analysis according to Feinstein (1975). Thus the β risk was 20% and the statistical power ($1 - \beta$) of our analysis was 80%.

Results

The mean ages of the lung cancer and chronic bronchitis patients were similar, although greater than that of the healthy subjects ($P < 0.05$, Table 1). There was no difference in smoking history between the patients with lung cancer and those with chronic bronchitis (Table 1). A bimodal distribution of the log D/HD ratio was apparent in the healthy population (Figure 1) but not in the other two groups (Figures 2 and 3). However, neither the lung cancer nor the chronic bronchitis groups showed a Gaussian distribution (Table 1). Using 1.12 as the anti-mode for the three distributions, the percentage of PMs found was 6.7% in the healthy volunteers, 8.9% in chronic bronchitis patients and 6.5% in lung cancer patients.

The percentage of PM phenotypes was not significantly different amongst the three populations as a whole (Table 1) or between the healthy volunteer groups and the lung cancer or chronic bronchitis groups. Log D/HD

Table 1 Characteristics and statistical data of the three populations studied

	Healthy subjects*†‡ (n = 119)	Chronic bronchitis patients*‡ (n = 135)	Cancer patients*† (n = 153)
Age (years) $\pm$ s.d.	30 ± 8	65 ± 11^a	63 ± 9^a
Cigarette smoking (pack-year)	N.D.	49 ± 29	48 ± 25^b
log D/HD	-0.05 ± 0.63	-0.02 ± 0.64^c	-0.02 ± 0.67^c
PM Phenotype	n = 8	n = 12	n = 10
Mean	6.7%	8.9%	6.5%
Confidence interval	2.2–11.2%	4.1–13.7%	2.6–10.4%
Kolmogorov-Smirnoff test	z = 2.395	z = 1.837	z = 1.910
Normality of the distribution	P < 0.001	P = 0.002	P = 0.001

* Global comparison Kruskal-Wallis Anova-one way, $\chi^2 = 1.705$ NS.

† Median test, $\chi^2 = 0.01$ NS.

‡ $\chi^2 = 3.61$ NS.

^a $P < 0.05$ between chronic bronchitis, lung cancer patients and healthy subjects.

^b Not significant between chronic bronchitis and lung cancer patients.

^c Not significant between chronic bronchitis, lung cancer patients and healthy subjects.

N.D. Not determined.

Figure 1 Frequency distribution of the log debrisoquine hydroxylation ratio (MR) in healthy subjects ($n = 119$).

Figure 3 Frequency distribution of the log debrisoquine hydroxylation ratio (MR) in lung cancer patients ($n = 153$). Patients treated with a drug having a possible inhibitory effect on P450IID6 are indicated in black.

Figure 2 Frequency distribution of the log debrisoquine hydroxylation ratio (MR) in patients with chronic bronchitis ($n = 135$). Patients treated with a drug having a possible inhibitory effect on P450IID6 are indicated in black.

values of the patients with lung cancer or chronic bronchitis who were receiving, at the time of the phenotyping test, a drug with a possible inhibitory effect on P450IID6 were evenly distributed as shown in Figures 2 and 3.

The log D/HD ratio was also measured before and between 1 to 2 months after surgery in 14 cancer patients (13 men and 1 woman), and no statistically significant difference was observed (Table 2).

Discussion

We have not confirmed the results of Ayesh *et al.* (1984), who found an over-representation of EMs of debrisoquine in a population of lung cancer patients compared with a group of patients with chronic bronchitis. The power of our statistical analysis ($1-\beta = 80\%$) does not preclude a possible type II statistical error. However, our data are in agreement with those of Drakoulis *et al.* (1986) and Speirs *et al.* (1990). Two studies (Jacqz *et al.*,

Table 2 Comparison of log D/HD values before and after surgery in a group of 14 patients with lung cancer

Subjects	Sex	Age (years)	Tumour histology	log metabolic ratio	
				Before surgery	After surgery
1	M	64	squamous	-0.19	0.28
2	M	71	squamous	-0.28	-0.24
3	M	75	squamous	0.96	0.53
4	M	69	squamous	0.23	0.75
5	F	55	squamous	-0.14	0.04
6	M	61	adenocarcinoma	0.73	0.60
7	M	63	squamous	0.50	0.37
8	M	65	squamous	-0.36	0.01
9	M	53	squamous	-0.59	-0.43
10	M	75	squamous	-0.29	0.36
11	M	55	squamous	-0.97	-0.61
12	M	59	undifferentiated	-0.12	-0.16
13	M	64	adenocarcinoma	-0.62	-0.72
14	M	44	small cell	-0.32	-0.22
Mean $\pm$ s.d.		64.3 $\pm$ 8.7		-0.10 $\pm$ 0.54	0.04 $\pm$ 0.46
				NS	

1988; Larrey *et al.*, 1987) found no more than 3.5% of PMs in a French Caucasian population. In contrast, Bechtel *et al.* (1986) and Siest *et al.* (personal communication) found a percentage similar to that reported for the British population. Moreover, Alvan *et al.* (personal communication) performed a meta-analysis of published and unpublished data on the distribution of the log D/HD ratio in various Caucasian populations which showed no significant difference in PM percentage among the various studies. Therefore, we cannot ascribe the difference between our results and those of Ayesh *et al.* (1984) to a difference in the frequency of PM subjects in French and British Caucasians. The lung cancer and chronic bronchitis patients were older than the healthy subjects and were taking medication at the time of study. However, there was no evidence of a systematic shift of log D/HD ratios to higher values in those patients taking drugs suspected to be inhibitors of cytochrome P450IID6 (Figures 2 and 3). Thus, the difference from the results of Ayesh *et al.* (1984) cannot be explained by such a bias.

A clear bimodality was not apparent in the log D/HD distributions for either chronic bronchitis patients (Figure 2) or those with lung cancer (Figure 3). However, as established by the Kolmogorov-Smirnoff test, none of the three distributions were normal and no difference was established between them (Table 1). Thus, it is unlikely that the choice of the same antimode value (1.12) for the assignment of phenotype is responsible for the difference from the findings of Ayesh *et al.* (1984).

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In a subgroup of 14 patients with lung cancer who underwent surgery it was possible to define the phenotype before and after operation (Table 2) to assess the possible influence of the tumour *per se* on phenotype. All of these patients were classified as EMs before surgery and remained so afterwards. This finding supports that of Speirs *et al.* (1990), who showed in a group of 17 patients who were cured of lung cancer at the time of phenotyping, that the MR distribution did not differ from that of other lung cancer patients or healthy volunteers. Thus, the tumour itself does not seem to influence the phenotype.

We cannot explain the difference between our results and those of Ayesh *et al.* (1984). The lower limit of the 95% confidence limits for the prevalence of the PM phenotype in our lung cancer population overlaps the upper limit for the Ayesh population and the power of the present study was only 80%. Nevertheless, we conclude from our data that a clinically significant link between debrisoquine phenotype and lung cancer is unlikely. It is possible that the EM phenotype is one among many other risk factors for lung cancer.

We would like to thank Dr J. Magnette for his helpful advice on statistical analysis.

This work was supported by Grant n° 86C 0856 from the French Ministry of Research and Technology within the frame of the COST B1 project.

(Received 25 June 1990,
accepted 14 November 1990)