substances that can modulate the MDR phenotype have become available [19, 20].

Long follow-up might also increase our knowledge of the prognostic importance of P-glycoprotein positivity in breast cancer, as already demonstrated by Chan *et al.* [21] for soft tissue sarcoma of childhood.

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Polymorphic Oxidation of Debrisoquine in Lung Cancer Patients

Julio Benítez, José M. Ladero, Carlos Jara, Juan A. Carrillo, Jesús Cobaleda, Adrián Llerena, Emilio Vargas and Juan J. Muñoz

Oxidative polymorphism of debrisoquine (DBQ) was assessed in 84 patients (81 male) with histologically proven bronchogenic carcinoma and in 143 healthy male smokers. 80 (95%) patients and 133 (93%) controls, with a metabolic ratio (MR) below 12.6, were classified as extensive metabolisers of DBQ (no significant difference between patients and controls). Only 1 of the 73 patients with epidermoid or microcytic carcinomas was classified as a poor metaboliser (PM) ($P = 0.031$ compared with controls). 63 patients (75%) and 110 controls (77%) showed a very fast oxidative rate, with MR values under 1 (not significant). The EM phenotype of DBQ might be a secondary genetic risk factor for developing bronchogenic carcinoma in male smokers.
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INTRODUCTION

ONLY A small percentage of smokers eventually develop lung cancer. This peculiar susceptibility could be due to a genetic predisposition based upon differences in enzymatic activation (or inactivation) of carcinogens, a possibility first suggested by Kellerman *et al.* [1] when they detected a higher inducibility of aryl-hydrocarbon hydroxylase in lymphocytes of patients with bronchogenic carcinoma than in smoker controls. This finding has not been universally accepted [2], but some years later Idle *et al.* [3] found an excess of extensive metabolisers (EM) of debrisoquine (DBQ) among patients with various types of cancer compared with controls. The same group reported a tendency towards higher rates of DBQ hydroxylation in two different series of lung cancer patients than in the general population

[4, 5]. Nevertheless, two other studies [6, 7] found no such difference.

Polymorphic oxidation of DBQ is a Mendelian trait. Poor metabolisers (PM) of DBQ are obligate homozygotes for the recessive allele, whereas EMs may be homozygotes or heterozygotes for the dominant allele [8]. The relevant enzymatic pathway is an isozyme of microsomal oxidative cytochrome P-450 called db1 or P450IID1 [9], which also oxidises many other endogenous and exogenous substrates [10].

Our aim was to study the relation between the genetically determined ability to oxidise DBQ and the risk for developing bronchogenic carcinoma.

SUBJECTS AND METHODS

84 patients (81 males, 3 females; mean age 61.6, S.D. 8.7) with histologically proven bronchogenic carcinoma were studied. There were 45 epidermoid, 28 mycrocytic and 1 great cell carcinomas, and 10 adenocarcinomas. All patients but 2 with adenocarcinoma were actual smokers or ex-smokers. None was receiving any drug known to interfere with DBQ metabolism [11] nor had been treated by surgery, chemotherapy or radiotherapy at the time of inclusion in the study. Liver and kidney functions were normal and no evidence of liver metastases was found. All patients gave informed consent for inclusion in the study.

The control group was 143 healthy male smokers (mean age 27.9, S.D. 10.6) who were not receiving any drug.

The oxidative phenotype of DBQ was established by giving a 10 mg Declinax tablet (kindly provided by Roche) at 2200, 2 h after a light dinner. All urine was collected over the following 8 h. Volume was measured and aliquots were stored at −20°C.

DBQ and 4-OH-DBQ in urine were measured with the flame ionisation gas-chromatographic method [12]. Metabolic ratio (MR) was calculated according to the formula [13]: (% dose excreted as unchanged DBQ) ÷ (% dose excreted as 4-OH-DBQ). The limit between PMs and EMs was established at a value for MR of 12.6 (log 1.1) [8].

Appropriate statistical analysis was done as follows: t test for independent variables and Kolmogorov–Smirnov test for two samples to analyse whether patients and controls had similar MR distributions. Since, in the general population (and in controls), the probability of being a PM is very low, we used the Poisson distribution to calculate the probability of observing a particular number of PMs among patients:

$$P(\epsilon = x) = \frac{\lambda^x}{x!} e^{-\lambda},$$

where λ = number of patients and x = probability for PM. The null hypothesis was rejected at $P < 0.05$.

RESULTS

4 patients (5%) and 10 controls (7%) with an MR over 12.6 were classified as PMs of DBQ ($P = 0.149$). The distribution of frequencies of values for MR of DBQ (Fig. 1) was also not significantly different.

Mean age was significantly lower in controls ($P < 0.001$). Daily consumption of tobacco was similar in cases and controls, although patients had given up smoking, at least for a few days, when included in the study.

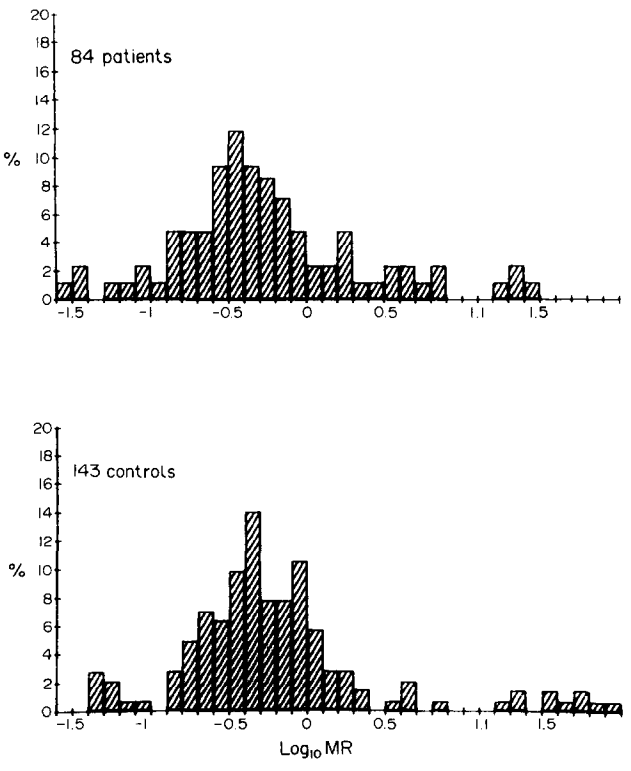

Fig. 1. Distribution of frequencies of $\log_{10}$ MR values of DBQ in 84 lung cancer patients and in 143 controls. Antimode (1.1) discriminates between extensive metabolisers (left) and poor metabolisers (right).

Only 1 of the 73 patients with epidermoid or microcytic carcinoma was classified as a PM of DBQ (Table 1). This frequency significantly differs from that found in the control group ($P = 0.031$), which indicates an excess of EMs among this cancer subgroup.

According to Speirs *et al.* [7], EM subjects can be further classified as very fast and intermediate metabolisers of DBQ. 63 patients (75%) and 110 controls (77%), with an MR under 1, were considered as very fast metabolisers, and 17 (20%) and 23 (16%), respectively, with MR between 1 and 12.6, as intermediate metabolisers. There was no difference in this distribution between both groups. All patients classified as intermediate metabolisers had epidermoid or microcytic carcinomas.

DISCUSSION

The distribution of the oxidative phenotype of DBQ in our patients with bronchogenic carcinoma was not different from

Table 1. Distribution of oxidative phenotype of DBQ metabolism among histological types of lung cancer

Histological type	No. of cases	EMs
Epidermoid	45	44
Microcytic	28	28
Adenocarcinoma	10	8
Great cell	1	0
Total	84	80

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Table 2. Studies on polymorphic oxidation of debrisoquine among lung cancer patients

Ref.	No. of cases	% EM		No. of controls	% EM	
4	245	98	(79)*	234	91	(28)*
5	104	98	(54)	104	91	(62)
6	270	93	(75)	270	89	(70)
7	82	90	(59)	†	–	–
16	89	99	‡	92	87	‡
Present series	84	95	(75)	143	93	(77)

*MR under 1.0.
†No control group.
‡Different limits between EM and IM were proposed.

that in the controls. Also, no differences were found in the relative frequencies of MR values. However, in patients with the two histological types of lung cancer (epidermoid and microcytic) more closely related to smoking [14], we found a significant excess of EMs.

Controls were younger than patients; cases and controls were nearly matched for sex and smoking habits. Nevertheless, neither age, sex nor smoking seems to influence the oxidative rate of DBQ [11].

The excess of EMs we found among our smoking-related lung cancer patients is in agreement with Ayesh *et al.* [4], mainly after reanalysis by Caporaso *et al.* [15], and also with Law *et al.* [5], but not with two negative reports [6, 7]. However, Caporaso *et al.* [16] have stated again that EMs of DBQ are at a significantly elevated risk of lung cancer (Table 2).

As did many other investigators in this field, we used the antimode proposed by Evans *et al.* [8] at 12.6. However, this limit has been questioned [17]. If we had used, for example, 4.48 [18], we would not have detected any excess of EM among smoking-related lung cancer patients. Therefore, our results must be interpreted cautiously. It seems preferable to analyse the distribution of frequencies of different categories of MRs [4]. Using this approach, Ayesh *et al.* identified an excess of patients with MRs lower than 1—or lower than 1.93 in a re-evaluation of their data [19]—78.8% vs. 27.8% in controls. They suggested that these "very fast metabolisers" [7] are true homozygotes for the dominant allele, having a definitely higher risk for developing smoking-related lung carcinoma. A case-control study [16] detected a very high risk for people with the EM (homozygous?) phenotype when compared with intermediate metabolisers (heterozygotes?) and PM subjects. In this study the MR values proposed as limits between the three phenotypes are different from the previous values, and they are not the same for whites and blacks.

Nevertheless, this excess of very fast metabolisers has not been confirmed in other studies, irrespective of the global results on the distribution of DBQ oxidative phenotype in lung cancer patients [5–7]. As Speirs *et al.* [7] have pointed out, such a difference seems to be due to a very low rate of very fast metabolisers among the control group proposed by Ayesh *et al.* [4] in relation to other studies. A similar, although lesser, excess of PM (13%) was found in the control group reported by Caporaso *et al.* [16]. After analysing current series (Table 2), we agree with some of the statements made by Speirs *et al.* [7], although these investigators do not provide a control group in their work.

It is generally accepted that many carcinogens must be activated to become proximate carcinogens before acting on DNA, and that this process takes place mainly in the oxidative microsomal P-450 system [2]. However, to date, no carcinogen has been found that follows the db1 oxidative route [20], and those compounds whose metabolic pathways have been identified are substrates for isozymes P-450 different from db1 [20, 21]. The suspected relation of the DBQ oxidative polymorphism with lung cancer risk could be due to a linkage disequilibrium with an oncogene, a tumour suppressor gene or genes encoding enzymes that do metabolise carcinogens [22], but none of these hypotheses has yet been supported.

Although oxidative polymorphism of DBQ might be related to the risk for developing lung cancer in male smokers, more studies are needed to establish if a very high oxidative rate of DBQ is a genetic risk factor and, if so, in which way. This could be useful to establish whether the debrisoquine/sparteine type polymorphism is related to the Mendelian inheritance suggested in the pathogenesis of lung cancer [23].

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Development and Characterisation of a Cyclophosphamide Resistant Variant of the BNML Rat Model for Acute Myelocytic Leukaemia

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A cyclophosphamide resistant subline (BNML/CPR) was developed *in vivo* in the BN rat acute myelocytic leukaemia (BNML) model. Full resistance was achieved after *in vivo* exposure of leukaemic animals to cyclophosphamide with, in total, 15 intraperitoneal injections of 100 mg/kg. The CPR line was cross-resistant to ifosfamide, but less so to mafosfamide. Continuous transplantation of the BNML/CPR line without a cyclophosphamide selection pressure resulted in the emergence of a subline (BNML/CPR>S) whose sensitivity to cyclophosphamide was similar to that of the parent BNML/S line. Both in the BNML parent line and in the BNML/CPR>S line, a 2p+ marker chromosome was present, whereas a 2p+q+ marker chromosome was characteristic for the BNML/CPR line. The mechanism of cyclophosphamide resistance can now be investigated in the BNML model at the DNA, at the mRNA and at the protein level.
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INTRODUCTION

RESISTANCE TO the cytostatic drugs for human cancer is still the major cause of failure to cure. One of the most widely used drugs is cyclophosphamide, which is incorporated in many treatment protocols for haematological malignancies, carcinomas and sarcomas [1].

Cyclophosphamide first has to be metabolised by the liver to yield the activated 4-hydroxy form. The next metabolite is aldophosphamide, which is metabolised to the cytotoxic phosphoramide mustard (PM) or to the deactivated carboxyphosphamide [1]. In deactivation, aldehyde dehydrogenase (ALDH) has an important role [2, 3].

Two enzyme systems may be involved in cyclophosphamide resistance—i.e. ALDH [4, 5] and glutathione-S-transferase and glutathione-dependent enzymes [6]. Increased intracellular ALDH has been correlated with reduced sensitivity to cyclophosphamide as well as to the activated metabolites [7]. Pretreatment with inhibitors of ALDH such as disulphiram resulted in restoration of the sensitivity for CP of CP resistant cell lines [8, 9]. However, others have reported that resistance to cyclophosphamide and to nitrogen mustards is correlated with increased levels of glutathione, glutathione-S-transferase or other glutathione-dependent enzymes [10, 11]. Other mechanisms, such as changes in drug transport and/or increased repair of cyclophosphamide-induced DNA lesions, may be involved. A prerequisite for studying resistance is the availability of a cyclophosphamide resistant cell line with well-defined characteristics. There are few cyclophosphamide-resistant animal tumour models available [6, 10, 12–15] and few *in vitro* cell lines of human origin [16–18].

In the rat, a model for acute myelocytic leukaemia (BNML) has proven suitable for preclinical research [19].

Here we describe the *in vivo* development of a cyclophosphamide resistant cell line from the BNML model (BNML/CPR),

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