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SHORT COMMUNICATION

Relationship between chemoresistance of lung tumours and cigarette smoking

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It is well documented that chemical carcinogenesis results in tumours that are resistant to the cytotoxic and growth inhibitory effects of various carcinogens [Carr, 1987]. Interestingly, there exists a remarkable parallel between the biochemical changes with carcinogen resistance and multidrug-resistance. The most frequently reported alteration in multidrug resistant cells, namely the overexpression of the 170 kDa membrane glycoprotein, is also found in both preneoplastic and neoplastic lesions producd by carcinogens [Thorgeirsson *et al.*, 1987; Fairchild *et al.*, 1987; Burt & Thorgeirsson, 1988; Burt *et al.*, 1988; Gottesman, 1988; Volm *et al.*, 1990]. Since human lung carcinoma is predominantly caused by cigarette smoking [Doll & Peto, 1981] the question arises whether lung tumours of smokers tend to be chemoresistant more frequently than tumours occurring in nonsmokers. To answer this question we determined the resistance of human non-small cell lung carcinomas and compared these results with the cigarette smoking habits of the patients.

One hundred and sixty patients with previously untreated non-small cell lung carcinomas were entered into this investigation (Table I). The morphological classification of the carcinomas was based on the WHO recommendations [World Health Organization, 1981]. All patients were staged at the time of surgery. Staging (pTNM) was performed according to the guidelines of the American Joint Committee for Cancer Staging and End Results Reporting [Carr & Mountain, 1977]. Eleven per cent of the smokers smoked fewer than 10 cigarettes, 19%, 10 to 19, 38%, 20 to 29, 12%, 30 to 39, and 19% more than 40 cigarettes daily.

Most of the patients were treated by surgical procedures alone, or by combined surgical and radiation therapy. For this reason we used an *in vitro* short-term test for determining the resistance of the tumours to drugs. The short-term test for predicting resistance to chemotherapy has been described previously [Volm *et al.*, 1979; Group of Sensitivity Testing of Tumours (KSST), 1981]. Its basic feature is measurement of changes in the incorporation of radioactive nucleic acid precursors into cell suspensions made from fresh tumour biopsies after addition of doxorubicin. The suspensions were incubated with different concentrations of doxorubicin for 3 h. Subsequently, the acid-insoluble radioactivity was measured by scintillation counting. The test threshold between sensitive and resistant tumours was derived from an earlier clinical study [Group of Sensitivity Testing of Tumours (KSST), 1981]. Although we cannot separate tumour cells and stromal cells within the tumour cell suspensions, in general, resistant tumours are predictable with a high accuracy in the *in vitro* short-term test. In a co-operative study [Group of Sensitivity Testing of Tumours (KSST), 1981; Volm *et al.*, 1983] conducted by nine different hospitals, results of the short-term test were compared with results of chemotherapy in patients. If the alternative evaluations (progression or remission) are compared with the *in vitro* results, 56 of the 57 tumours that were resistant in the test were clinically progressive (98%) and 40 of 58 tumours that tested sensitive showed clinical remission (69%). There was also good agreement between the *in vitro* test results and survival. Similar results were obtained in subsequent studies [Volm *et al.*, 1985*a*,*b*, 1988]. These results have recently been confirmed by Khoo *et al.* (1989) and Auner *et al.* (1989).

As expected, the lung tumours in the present study responded very differently in the *in vitro* test system. Forty-one (25%) out of 160 tumours were classified as sensitive and 119 tumours (75%) as resistant. In Table II the relationship between test results *in vitro* (sensitive/resistant) and smoking (nonsmokers/smokers) of all analysed non-small cell lung carcinomas are presented. A significant relationship between smoking and response of the tumours to doxorubicin *in vitro* was found ($P = 0.002$). Carcinomas of smokers tended to be resistant more frequently (81%) than carcinomas of nonsmokers (53%). Similar results were obtained when the analysis was restricted only to those patients with epidermoid lung carcinomas ($P = 0.001$). Of the tumours of smokers 91%, and of the tumours of non-smokers 50% were resistant. In contrast to these data there exists no relationship

Table I Patient characteristics

Clinical characteristics	*No. of patients*
Age	
<40	5
40–49	18
50–59	75
60–69	47
⩾70	15
Sex	
male	142
female	18
Histology	
Epidermoid Ca	88
Adeno Ca	49
Large cell Ca	23
Stage	
I	33
II	17
III	110
Smoking habits*	
Nonsmokers	32
Smokers	127

*One case of large cell carcinoma could not be categorised.

Table II Relationship between resistance and smoking habits of patients with non-small lung carcinomas

	Test results	*Nonsmokers n (%)*	*Smokers n (%)*	*P*
All tumours	sensitive	15 (47)	24 (19)	0.002
	resistant	17 (53)	103 (81)	
Epidermoid Ca	sensitive	7 (50)	7 (9)	0.001
	resistant	7 (50)	67 (91)	
Adeno Ca	sensitive	6 (38)	13 (39)	
	resistant	10 (62)	20 (61)	n.s.

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between resistance and smoking for adenocarciomas of the lung. This may be expected because adenocarcinomas are said to be less frequently associated with smoking than are epidermoid lung carcinomas [Gould & Warren, 1989]. We further analysed the patients with regard to the number of cigarettes smoked and to cessation of smoking and could not find any influence of these factors (data not shown).

Until now, the mechanisms for the resistance of lung tumours are unknown and may be multifactorial. It can be speculated that, as a detoxifying transport system, the P-glycoprotein might be increased with other known detoxifying systems such as glutathione transferase, cytochrome P-450 isoforms and topoisomerase II. Whereas Lai *et al.* (1989) demonstrated only a weak expression of the multidrug-resistance (MDR) gene in 14 out of 24 human lung tumours, Radosevich *et al.* (1989) found P-glycoprotein expressing cells in 100 out of 131 non-small cell lung carcinomas by immunohistochemical techniques. We recently investigated the intrinsic resistance of a panel of human epidermoid lung cancer xenografts grown in nude mice [Volm *et al.*, 1989*b*] and found a correlation between expression of P-glycoprotein and degree of resistance. Carmichael *et al.* (1988) measured glutathione levels in 30 human lung cancer lines and found lower levels in cell lines derived from small cell lung cancer specimens compared to non-small cell lung cancer. Non-small cell lung cancers were found to have increased activity of 4 detoxification enzymes (glutathione transferase, glutathione reductase, γ-glutamyl transpeptidase, superoxide dismutase) compared to small cell lung tumours. These differences in glutathione levels and detoxification enzyme levels may also prove to be important causes for intrinsic drug resistance often seen in patients with non-small cell lung cancer. Zijlstra *et al.* (1987) demonstrated that the resistance in a doxorubicin-resistant human lung carcinoma cell line was multifactorial with decreased intracellular doxorubicin levels, increased DNA repair, and altered doxorubicin-topoisomerase interaction. Investigations are continuing in our laboratory to determine which mechanisms of resistance of lung tumours are active.

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