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Short Communication

Phase II Study of Liarozole in Advanced Non-small Cell Lung Cancer

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The aim of this phase II study was to investigate the efficacy and tolerability of liarozole, a novel benzimidazole derivative, in non-small cell lung cancer (NSCLC). Liarozole 300 mg twice daily orally was evaluated in 14 patients with stage IIIB and IV NSCLC. 8 patients had received prior treatment with chemotherapy and/or radiotherapy. WHO toxicity grading and response criteria were used. Liarozole was well tolerated. Grade 2 toxicities included alopecia (1 patient), dermatological toxicity (5 patients), dry mouth (2 patients) and nausea and vomiting (2 patients). Leukocytosis was seen in 5 patients, including 2 cases with an elevated white cell count pretreatment. Liarozole was discontinued in 1 patient who developed intolerable progressive pruritis associated with an erythematous rash. No objective tumour response was seen, all 14 patients developing progressive disease within 4 months of commencing treatment. Liarozole was well tolerated but was ineffective as single agent therapy in the management of NSCLC. The side-effect profile was compatible with inhibition of retinoic acid degradation. © **1998 Elsevier Science Ltd. All rights reserved.**

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INTRODUCTION

MORE THAN half a million new cases of lung cancer are diagnosed worldwide each year. Non-small cell lung cancer (NSCLC) accounts for approximately 80% of these and is the leading cause of cancer related death. Despite surgery, radiotherapy and chemotherapy, the overall prognosis for NSCLC patients is poor, with a 5-year survival in the region of 12% [1]. Therefore, novel approaches to treatment are necessary.

Liarozole (R 75.251; 5-[(3-chlorophenyl)(1*H*-imidazole-1-yl)methyl]-1*H*-benzimidazole) is a novel azole derivative. Like other azole derivatives, such as ketoconazole, liarozole inhibits several cytochrome P-450-dependent enzymes, including aromatase, 17α-hydroxylase/17,20-lyase, 11-hydroxylase and 4-hydroxylase [2, 3]. *In vitro* growth inhibition studies show that liarozole has minimal or absent cytostatic/cytotoxic effects on human solid tumours, including the breast MCF-7, prostatic DU145 and LNCaP and mouse F9 teratocarcinoma cell lines [4, 5], but inhibits retinoic acid degradation [5]. In contrast with the lack of *in vitro* growth inhibition, liarozole is a potent inhibitor of androgen-dependent and androgen-independent, moderately and poorly differentiated, prostatic tumours *in vivo* [6]. Results of initial studies in patients with hormone refractory prostate cancer have been encouraging. Objective response rates of 19–30%, associated with a significant improvement in symptoms, have been seen in patients treated with liarozole 300 mg twice daily orally [7].

Recent work has provided some insight into the anti-tumour activity of liarozole. At 40 mg/kg/day, liarozole has been shown to inhibit the xenograft tumour growth of, and the development of bone metastases from the human prostate cancer cell line PC-3 ML-B^2 in SCID mice. The inhibitory effect was potentiated by all-*trans*-retinoic acid 0.75 mg/kg/day where a significant increase in intratumoural retinoic acid was seen compared to that detected in untreated animals. Liarozole also inhibited type IV collagenase secretion by the cell line *in vitro* and synthesis *in vivo* [8].

Angiogenesis is essential for tumour growth beyond 1–2 mm in diameter and plays a central role in tumour metastasis [9]. Inhibition of matrix metalloproteinases such as collagenase IV results in inhibition of angiogenesis [10]. Retinoic acid and related analogues such as fenretinide may

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inhibit angiogenesis, endothelial cell motility and tubule formation [11]. Liarozole 75–300 mg orally increases plasma all-*trans* retinoic acid concentrations for up to 6 hours after administration in healthy human subjects [12]. Taken together these findings suggest that liarozole inhibits tumour growth *in vivo* at least in part through the inhibition of angiogenesis.

Angiogenesis, as assessed by blood vessel counts, is the most important prognostic factor in operable NSCLC, high blood vessel counts predicting nodal involvement at surgery and a poor outcome [13]. As such, liarozole, a potential anti-angiogenic agent with activity in the treatment of solid tumours such as prostate cancer, may have a role to play in the management of NSCLC. This phase II study was designed to evaluate the efficacy and tolerability of liarozole in the treatment of NSCLC.

PATIENTS AND METHODS

Patients aged ≤ 75 years with histologically or cytologically proven, radiologically measurable or evaluable NSCLC, a life expectancy ≥ 60 days and ECOG performance status ≤ 2 were considered eligible for the study and were only included after giving their informed written consent. Pretreatment investigations included a complete clinical history and physical examination, radiological evaluation of the tumour, a full blood count, urea and electrolytes, liver enzymes and bone chemistry and an electrocardiogram (ECG). Patients were excluded from the study if they had renal and/or liver function > 1.5 times the upper limit of normal, had received chemotherapy within the previous 4 weeks, were receiving concomitant treatment with endocrine agents, chemotherapy or retinoids, had evidence of impaired adrenal function or hypokalaemia or had a second concomitant malignancy. Vitamin A/retinoic acid therapy was prohibited whilst on study.

Liarozole 300 mg twice daily, orally (Janssen Pharmaceutica, Belgium) was administered for a maximum of 6 months or until the development of progressive disease. Liarozole was reduced by 50% to 150 mg twice daily orally if grade 3 toxicities attributable to the agent were observed. In view of potential interaction with antacids or H_2-antagonists, these were to be taken, if required, at least 1 h prior to the administration of liarozole, or in the evening after treatment if a sustained released form was to be used. Follow-up evaluations were carried out every 2 weeks during the first 2 months of therapy and every month thereafter. Formal staging of measurable disease was performed every 3 months together with an ECG. The study was conducted according to the Declaration of Helsinki and approved by the Central Oxford Research Ethics Committee.

Table 1. *Patients' characteristics*

Characteristics	No. of patients
Total number	14
Male: female	5:9
Median age (years, range)	59 (51–74)
ECOG performance status	
0	2
1	8
2	3
3	1
Histology	
Squamous cell carcinoma	6
Adenocarcinoma	5
Large cell carcinoma	2
Poorly differentiated carcinoma	1
Stage of disease	
IIIB	2
IV	12
Previous treatment	
Chemotherapy only	3
Radiotherapy only	3
Chemotherapy and radiotherapy	2
Evaluable for response	14
Measurable	12
Evaluable	2

Statistical analysis

The study design chosen to evaluate the potential efficacy of liarozole was Gehan's two stage design. If no responses are seen in the first 14 patients the study is terminated as statistically the drug has a $> 95\%$ probability of having a response rate of $< 20\%$ [14].

RESULTS

Patients

14 patients, 5 male and 9 female, age range 51–74 years (median 59.7 years) were recruited to the study. 8 patients had received previous treatment. The patients' characteristics are outlined in Table 1.

Response and survival

Of the 14 patients recruited to the study, 12 had measurable and 2 evaluable disease. 2 patients had stable disease lasting 3 and 4 months. The others had progressive disease documented within 2 months of commencing liarozole. At the patient's request and because of the relatively slow rate of tumour growth, liarozole was continued to a total of 25 weeks in the patient who developed progressive disease after 4 months of treatment. The median survival for all patients was 3 months (range 1–16 months). Although the numbers were small, there was no notable difference in outcome between the 8 pretreated and 6 therapy naïve patients (median survival 69 days versus 81 days, respectively).

Toxicity

The agent was well tolerated with few significant side-effects (Table 2). Grade 3 skin toxicity was experienced by 1 patient. The patient developed pruritis which worsened with time becoming intense and continuous. This was associated with an erythematous skin rash and nail dystrophy. The symptoms settled on discontinuation of the treatment. Grade 2 toxicities included alopecia (1 patient), dermatological toxicity with a dry, flaky and pruritic skin rash (5 patients),

Table 2. *Number of patients developing grade* 1, 2, 3 *or* 4 *toxicities (WHO criteria)*

	Toxicity grade			
	1	2	3	4
Alopecia	1	1	0	0
Fatigue	1	0	0	0
Pain	2	0	0	0
Skin	1	5	1	0
Dry mouth	2	2	0	0
Diarrhoea	1	0	0	0
Nausea and vomiting	0	2	1	0

dry mouth (2 patients) and nausea and vomiting (2 patients). A rise in the total white cell count above the normal range was seen in 3 patients and of granulocytes alone in 1 patient. 2 patients presenting with elevated white cell counts had a subsequent rise on treatment. No notable non-tumour related biochemical abnormalities were seen.

DISCUSSION

The results indicate that single agent liarozole is well tolerated but is inactive as a single agent in the treatment of patients with advanced, stage IIIB and IV NSCLC. In keeping with both the experimental data [3–6, 8] and clinical studies in patients with prostate cancer [7], the side-effect profile observed in our patients is consistent with increased tissue levels of retinoic acid resulting from liarozole treatment. The side-effects seen included cutaneous problems, such as dryness and flaking of the skin, dry mucosae, nail dystrophy and alopecia, nausea and vomiting and leukocytosis. Other side-effects associated with treatment with retinoids (which include natural vitamin A (retinol) and its esters and synthetic analogues), but not seen in our patients, include headaches and dizziness, chills and sudden fevers [15].

Vitamin A or retinol is essential for the normal growth, maintenance and differentiation of epithelia, including human bronchial epithelial cells. The effects of retinoic acid are mainly mediated by its nuclear receptors, the retinoic acid receptors (RARs) and retinoid X receptors, that in turn regulate target gene expression by binding to specific retinoic acid response elements. Normal human tracheobronchial epithelial cells show a dramatic induction of RAR-β mRNA and significant growth inhibition after exposure to retinoids [16, 17]. Retinoids are currently being evaluated as cancer chemopreventive agents because of their activity in *in vivo* experimental systems, including skin, bladder, lung, breast and oral cavity carcinogenesis. In clinical trials, several retinoids have achieved significant activity in the reversal of head and neck, skin and cervical premalignancy and in the prevention of second primary cancers associated with head and neck, skin and NSCLC tumours [15, 18]. Liarozole has been shown to enhance the chemopreventive activity of retinoic acid and β-carotene [19]. Furthermore, with regards to NSCLC, recent work has shown that rapid catabolism of all-*trans*-retinoic acid is seen in patients with squamous and large cell NSCLC phenotypes as compared with control subjects [20]. From these data it might be anticipated that retinoids would have a direct antiproliferative effect on NSCLC tumours.

Recent work has shown that retinoic acid treatment has no growth inhibitory effects in the majority of human lung cancer cell lines. Indeed in two of 15 NSCLC cell lines evaluated in one study, retinoic acid treatment resulted in growth stimulation [16]. The *RAR-β* gene is localised in a chromosomal region frequently deleted in lung cancer cells. The absence of RAR-β expression has been reported in a number of NSCLC tumour cell lines [21]. Furthermore, the majority of cell lines tested fail to show induction of RAR-β by retinoic acid [16, 17]. In transfection experiments in epidermoid lung tumour derived cells, all clones expressing RAR-β were less tumorigenic in nude mice than were the untransfected controls. Furthermore, increased tumour latency and a reduced growth rate were seen. These data support the contention that RAR-β functions as a tumour suppressor gene in epidermoid lung tumorigenesis [21]. The lack of expression of RAR-β mRNA and its abnormal regulation by retinoic acid is also seen in other solid tumours, including breast cancer [22].

A recent phase II study has evaluated all-*trans*-retinoic acid in the treatment of metastatic NSCLC. Little antitumour activity was seen. Two partial responses were documented in the 28 patients treated, lasting 7 and 13 months, respectively. Toxicities included cutaneous side-effects, headache and myalgia. A significant number of patients developed elevations of hepatic transaminases or hyperlipidaemia and 3 patients had treatment related leukocytosis [23]. These findings are consistent with those seen in our study.

The use of anti-angiogenic agents alone, in combination with other anti-angiogenic and/or biological agents or in combination with cytotoxic agents in the treatment of solid tumours is currently under investigation. The combination of retinoids with 1, 25-dihydroxyvitamin D_3 and/or interferon-α2a has shown enhanced anti-angiogenic activity in HPV-harbouring and non-HPV-harbouring tumour cell lines [24, 25]. The anti-angiogenic activity of retinoids is mediated through the RAR-α receptor [25]. Based on the encouraging results seen in patients with advanced squamous cancer of the skin and cervix a phase II study evaluating 13-*cis*-retinoic acid and interferon-α2a in patients with advanced squamous cell lung cancer has been performed. Only one partial response was seen in the 21 patients enrolled to the study, while 8 patients came off the study due to side-effects, including fatigue and anorexia [26].

This report demonstrates that liarozole, a potential anti-angiogenic agent, is well tolerated in patients with advanced NSCLC. Experimental evidence has shown that anti-angiogenic agents, including the fumagillan derivative, TNP-470 [27], and the gelatinase inhibitor, CT1746 [28], may enhance the antitumour activity of cytotoxic agents. Phase II studies should be considered evaluating liarozole in combination with cytotoxic agents in the treatment not only of NSCLC but of other solid tumours, including prostate cancer.

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