Cancer Chemother Pharmacol (1993) 31: 419–422

Cancer Chemotherapy and Pharmacology

© Springer-Verlag 1993

Short communication

Phase II trial of the novel sulphonylurea sulofenur in advanced breast cancer

Denis C. Talbot[1], Ian E. Smith[1], Marianne C. Nicolson[1], Trevor J. Powles[1], Diane Button[1], Jackie Walling[2]

[1] Breast Unit, Royal Marsden Hospital, London, SW3 6JJ, and Sutton, UK
[2] Medical Department, Lilly Industries Limited, Dextra Court, Basingstoke, Hants RG21 2SY, UK

Received 26 May 1992/Accepted 12 October 1992

Summary. A total of 18 women with advanced breast cancer were treated with sulofenur [LY186641; *N*-(5-indanylsulfonyl)-*N'*-(4-chlorophenyl)-urea], a diarylsulfonylurea that has broad-spectrum activity against a number of murine mammary tumour xenografts. The dosage chosen on the basis of pre-clinical and phase I studies was 700 mg/m^2 given orally once daily for 14 days, with treatments being repeated every 3 weeks. There was no response. All patients experienced at least grade 1 anaemia, and two patients developed symptomatic methaemoglobinaemia. Two patients developed grade 4 rises in serum liver-function values along with histological changes consistent with drug-induced toxicity. The mean plasma concentrations of 176 μg/ml were lower than the levels required to exert anti-tumour effect in the mouse model.

Introduction

Breast cancer is the commonest malignancy in women, with over 25,000 new cases being recorded annually in the United Kingdom [3]. The majority of patients develop metastatic disease for which there is no cure, although response rates of 50%–60% can be achieved with chemotherapy. Thus, a key aim of treatment with chemotherapy is to provide palliation. The use of many currently available cytotoxic drugs is limited by myelosuppression, and the development of active anticancer drugs whose myelotoxicity is limited may result in improved treatment.

Sulofenur [LY186641; *N*-(5-indanylsulfonyl)-*N'*-(4-chlorophenyl)-urea, Eli Lilly, Indianapolis, Ind., USA; Fig. 1] is a diarylsulfonylurea that has broad-spectrum activity against a number of murine tumour models, including the C3H mammary tumour and the MX1 human mammary xenograft [7]. The mechanism of action of diarylsulfonylureas is not fully understood. They are equally cytotoxic to proliferating and quiescent cells, they do not appear to affect DNA, RNA or protein synthesis [7] and they have cell-cycle specific effects as measured by flow cytometry [2]. Diarylsulfonylureas accumulate in mitochondria [11] and may exert cytotoxicity through uncoupling of oxidative phosphorylation [12]. Anti-tumour activity appears greater when the drug is given in repeated low doses by the oral route rather than by intermittent high-dose systemic treatment [17]. Phase I studies have demonstrated that the maximum tolerated dose is 900 mg/m^2 p.o. given daily for 14 days every 3 weeks. The dose-limiting toxicity was haemolytic anaemia and methaemoglobinaemia [1, 8, 15, 17].

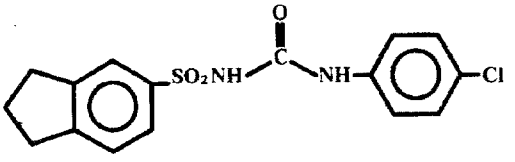

Fig. 1. Structure of sulofenur [LY186641; *N*-(5-indanylsulfonyl)-*N'*-(4-chlorophenyl)-urea]

The objectives of the present phase II study were to determine the rate of objective response to and the tolerability of sulofenur given orally at a dose of 700 mg/m^2 daily for 14 days every 3 weeks to patients with advanced breast cancer.

Patients and methods

Study design. This trial was an open, single-centre phase II study of sulofenur in patients with advanced breast cancer. Patient accrual to a total of 45 was planned, depending on the response rates obtained after the accrual of 15 and 30 patients, respectively, giving the study a 90.7%

Correspondence to: D. C. Talbot, University of Oxford, ICRF Clinical Oncology Unit, Churchill Hospital, Headington, Oxford OX3 7LJ, UK

Table 1. Sulofenur in advanced breast cancer – previous treatment

ET	CT (number of patients)		
	No previous CT	1st-line CT	1st- and 2nd-line CT
Tamoxifen	0	4	2
Tamoxifen and 2nd-line ET	1	5	3
Tamoxifen, 2nd- and 3rd-line ET	1	1	1

All treatments were given sequentially. CT, Cytotoxic chemotherapy; ET, endocrine therapy

power at a type-1 error of 5.7% to detect a response rate of 40% for sulofenur.

Patients. Patients with histologically or cytologically proven advanced breast cancer were entered into the study after they had given their informed consent. All patients had received previous endocrine therapy and up to two prior chemotherapeutic regimens. Criteria for entry into the study included the following: a WHO performance status of 0–2; a life expectancy of >3 months; adequate bone marrow reserve; measurable disease outside a previously irradiated area; a blood methaemoglobin level of <2%; a normal serum glucose-6-phosphate dehydrogenase value; a serum bilirubin level of less than twice the normal value; and serum transaminase levels of less than three times the normal values.

Sulofenur was given orally at a dose of 700 mg/m^2 once daily for 14 days every 3 weeks [1] on an out-patient basis. Patients attended the out-patient department weekly for clinical review, assessment of toxicity, urinalysis and measurement of biochemical and haematological profiles. Response and toxicity were evaluated according to WHO criteria [18]. Plasma levels of sulofenur and of its hydroxy and keto metabolites were measured by high-performance liquid chromatography (HPLC) using a modification of the method described by Taylor et al. [17]. Blood samples were taken at 24 h after the 7th, 14th and 21st days of the first course of sulofenur. Patients gave their informed consent to participate according to the guidelines of the Royal Marsden Hospital Ethics Committee.

Results

A total of 18 women (median age, 52 years; range, 35–67 years) with a good WHO performance status (0, 11; 1, 5; 2,2) were entered into the study. In all, 14 patients were post-menopausal, 1 was pre-menopausal and 3 were peri-menopausal (less than 2 years since their last menstrual cycle). All were evaluable for toxicity and 17 were evaluable for response (meningeal disease at the time of entry into the study excluded 1 patient from the evaluation of response). All patients had advanced breast cancer resistant to tamoxifen. In all, 16 (89%) of the patients had also received previous chemotherapy, including standard combinations of drugs (Table 1). Most patients had at least 2 sites of disease, with the anatomical locations being as follows: skin/soft tissue, 12; breast, 8; liver, 6; lymph nodes, 5; lung/pleura, 5; bone, 4; stomach/ovary/peritoneum, 4; and chest wall, 3. A total of 48 courses of sulofenur were given (median number of courses per patient, 2; range, 1–5).

Table 2. Frequency of the worst haematological toxicity encountered according to grade

Toxicity	WHO grade				
	0	1	2	3	4
Haemoglobin	0	7(1)	9	1	1
Methaemoglobin[a]	0	14	4	0	0
Total white blood cells	11	4(2)	3	0	0
Platelets	16	2	0	0	0

Data represent the numbers of patients experiencing toxicity. Figures in parentheses indicate the number of patients whose WBC was <4 × 10^9/l at entry or whose haemoglobin level was <11 g/100 ml at entry

[a] As no WHO toxicity score exists for methaemoglobin, the following grading scale was used:

Grade	0	1	2	3	4
% Methaemoglobin	0–2	2.1–10	10.1–20	20.1–30	>30

Response

No response was observed. All patients eventually progressed during treatment or withdrew from the study because of unacceptable toxicity.

Haematological toxicity

Table 2 summarises the haematological toxicity attributable to sulofenur. All patients experienced methaemoglobinaemia and anaemia. Overall, 11 patients received a total of 44 units of transfused blood (range, 2–11 transfusions per patient). Two patients developed symptomatic methaemoglobinaemia requiring i.v. infusion of methylene blue; the symptoms resolved and methaemoglobin levels normalised within 2 h of methylene blue infusion. Of three patients who developed grade 2 neutropenia, one had cytological confirmation of progressive disease in the bone marrow. No significant thrombocytopenia was seen except in the patients known to have bone marrow disease.

Non-haematological toxicity

Two patients (11%) developed grade 4 rises in serum liver-function values after completing their first cycle of sulofenur. Liver ultrasound examination and viral serology were normal in both cases. Liver biopsies were reported as showing evidence of the recent onset of hepatitic changes consistent with drug-induced toxicity in one patient and of mixed hepatitic and cholangiolytic appearances, with eosinophilic infiltration and cholestasis likely to be drug-induced in the second patient. Sulofenur treatment was discontinued and liver function returned to normal in both patients.

Two patients (11%) required anti-coagulation for treatment of deep femoral venous thrombosis. Venous thrombosis was not considered to be directly related to breast cancer. Blood-sugar levels remained within normal limits in all patients. Other non-haematological toxicities encountered included diarrhoea (WHO grade 1) in 1 patient

Table 3. Plasma levels of sulofenur and of its hydroxy and keto metabolites during the first course of chemotherapy with sulofenur

	Day 7 (9 patients)			Day 14 (9 patients)			Day 21 (2 patients)		
	S	H	K	S	H	K	S	H	K
Mean plasma levels (μg/ml)	176.0	42.9	56.67	126.1	48.7	53.5	8.1	16.7	20.9
Standard deviation (μg/ml)	81.5	8.6	16.64	74.2	22.5	22.0	N/A	N/A	N/A

S, Sulofenur; H, hydroxy metabolite; K, keto metabolite; NA, not applicable

(5%), nausea (WHO grade 1) in 1 patient (5%) and reversible paraesthesia in the toes (WHO grade 1) in 1 patient (5%). Alopecia did not occur.

Of 48 evaluable courses, 10 were delayed (8 because of anaemia, 1 due to chest infection and 1 because of abnormal liver-function values). In all, 11 courses were given at reduced doses (methaemoglobinaemia, 7; anaemia, 3; deterioration in renal function, 1).

Pharmacokinetics

The mean plasma levels of sulofenur and of its hydroxy and keto metabolites are shown in Table 3. The compounds were not detected on day 0. The mean peak plasma level of sulofenur was 176 μg/ml on day 7. On day 21, when patients had been off therapy for 7 days, the levels had fallen but remained within detectable limits. There was marked inter-patient variability.

Discussion

Sulofenur is the first sulfonylurea to show experimental evidence of anti-tumour activity. It is likely to have a novel mechanism of action and has an unusual toxicity profile. Despite the evidence of broad anti-tumour activity found in pre-clinical testing, no response was achieved in 17 evaluable patients with advanced breast cancer in the present phase II study.

The mean plasma level of the parent compound attained in this study was 176 μg/ml, which is consistent with data obtained in other studies using the same schedule [13]. Although the plasma concentrations achieved in the present study are similar to the concentrations of sulofenur that had anti-tumour activity in vitro in rhabdomyosarcoma cell lines in the presence of 10% foetal bovine serum (FBS) [10], it does appear that rhabdomyosarcoma is uniquely sensitive to the drug. Indeed, when freshly explanted breast tumours were exposed to sulofenur in the presence of 10% FBS, the 50% growth-inhibitory concentration (IC_{50}) was 540 μg/ml [17], and it has been demonstrated in vitro that a reduction in the concentration of FBS results in an increase in the cytotoxicity of sulofenur. The plasma levels required to produce an anti-tumour effect in vivo in mice are of the order of 400 μg/ml (Worzalla, personal communication). Taken together, these data suggest that the lack of anti-tumour effect observed in the current study might be explained not only by there being insufficiently high plasma levels but also by a high degree of protein binding.

The major symptomatic toxicities were due to haemolytic anaemia and methaemoglobinaemia as reported in phase I studies [1, 8, 15, 17] and phase II trials in ovarian [16] and gastric [13] cancer. Methaemoglobinaemia was rapidly reversed by the infusion of methylene blue as is standard practice for the treatment of sulfonylurea-induced methaemoglobinaemia. In phase I studies a positive correlation was found between parent-drug plasma concentrations and 24-h methaemoglobin levels; no relationship between the sulofenur area under the concentration-time curve (AUC) and the 24- or 48-h methaemoglobin values was noted [9]. In previous studies, reversal of toxicity was achieved by withdrawal of the drug or by i.v. methylene blue infusion at 1–2 mg/kg.

Following completion of the study, 2-hydroxychloroanaline sulfate, one of the metabolites of *p*-chloroaniline, was identified as a minor urinary metabolite of sulofenur [4, 5]. *p*-Chloroaniline is a known inducer of methaemoglobinaemia and anaemia [14]. We were concerned by the observation that two patients entered into the trial developed severe acute hepatitis associated with grade 4 increases in liver-function values shortly after completing the first course of sulofenur. Histological evidence indicated the cause to be drug-related, and both patients completely recovered following the discontinuation of sulofenur. Cholestatic jaundice is a recognised side effect of the sulfonylureas [6]. Three cases of reversible, possibly drug-induced, cholestatic jaundice were seen in the phase I study, and deteriorations of liver-function values were reported in other phase II studies [13, 16]. There does not appear to be any predictor for patients who are at risk of developing hepatotoxicity during sulofenur administration.

We felt that further escalation of the sulofenur dose would have increased the frequency of serious adverse events to unacceptable levels and that it was therefore unwarranted. However, since the drug has shown anti-tumour activity in mice, further structure-activity studies would be helpful in assessing the clinical potential of this interesting class of drugs.

Acknowledgements. The authors are grateful to Dr. B. Portman (Consultant Histopathologist, Liver Unit, Kings College Hospital, London) for his expert histological review of the liver biopsy specimens and to Mrs. J. Holborn for her help in the preparation of the manuscript.

References

1. Armand JP, Recondo G, Gouyette A, Belerahdek M, Bonnay M, Chabot G, L'Homme C (1989) Phase I and pharmacokinetic study of LY186641 administered daily for 2 weeks every 21 days. Proceedings, 4th NCI EORTC Symposium on New Drugs in Cancer Chemotherapy, Amsterdam 1989, p 300
2. Boder GB, Beasley FW, Grindey GB (1989) Mechanism of action studies with LY186641. Proc Am Assoc Cancer Res 30: 554
3. Cancer Registry (1990) Cancer statistics: registrations 1985. Series MBI 18, England and Wales, 1985. HMSO, London
4. Ehlhardt WJ (1991) Metabolism and disposition of the anti-cancer agent sulofenur in mouse, rat, monkey and human. Drug Metab Dispos 19: 370
5. Ehlhardt WJ, Howbert JJ (1991) Metabolism and disposition of *p*-chloroaniline in rat, mouse and monkey. Drug Metab Dispos 19: 366
6. Fraser BM, Steward WK (1977) Cholestatic jaundice following chlorpropramide self poisoning. Clin Toxicol 11: 13
7. Grindey GB (1988) Identification of diarylsulfonylureas as novel anticancer drugs. Proc Am Assoc Cancer Res 29: 535
8. Hainsworth JD, Hande KR, Satterlee WG, Kuttesch J, Johnson DH, Grindey G, Jackson LE, Greco FA (1989) Phase I clinical study of *N*-[(4-chlorophenyl)-amino]carbonyl-2,3-dihydro-1H-indene-5-sulfonamide (LY186641). Cancer Res 49: 5217
9. Hande KR, Kuttesch J, Hamilton M, Satterlee W, Jackson L, Grindey G, Hainsworth JD (1990) Kinetics of *N*-[(4-chlorophenyl)amino]carbonyl-2,3-dihydro-1H-indene-5-sulfonamide (LY186641) in humans. Cancer Res 50: 3910
10. Houghton PJ, Houghton JA, Myers L, Cheshire P, Howbert JJ, Grindey GB (1989) Evaluation of *N*-(5-indanylsulfonyl)-*N'*-(4-chlorophenyl)-urea against xenografts of pediatric rhabdomyosarcoma. Cancer Chemother Pharmacol 25: 84
11. Houghton PJ, Bailey FC, Houghton JA, Murti KG, Howbert JJ, Grindey GB (1990) Evidence for mitochondrial localisation of *N*-(4-methylphenylsulfonyl)-*N'*-(4-chlorophenyl)urea in human colon adenocarcinoma cells. Cancer Res 50: 664
12. Houghton PJ, Sosinski J, Thaker J, Houghton JA, Grindey GB (1991) Diarylsulphonylureas (DSUs): a two-mechanism model for cytotoxicity. Proc Am Assoc Cancer Res 32: 407
13. Kamthan A, Scarffe JH, Walling J, Hatty S, Coleman R, Smyth JF (1992) A phase II study of sulofenur (LY186641) in gastric cancer. Anti Cancer Drugs 3: 331
14. Kiese M (1974) Methaemoglobinaemia: a comprehensive treatise. CRC Press, Cleveland, Ohio
15. Kuhn J, Craig J, Howlin K, Brown T, Beougher K, Van Hoff D, Satterlee W, Hamilton MJ (1988) Clinical phase I clinical and pharmacokinetic evaluation of oral LY186641 in a single 21 day schedule. Proc Am Soc Clin Oncol 7: 60
16. O'Brien MER, Hardy J, Tan S, Walling J, Peters B, Hatty S, Wiltshaw E (1992) A phase II study of sulofenur (a novel sulphonylurea LY186641) in recurrent epithelial ovarian cancer. Cancer Chemother Pharmacol 30: 245
17. Taylor CW, Alberts DS, Ketcham MA, Satterlee WG, Holdsworth MT, Plezia PM, Peng YM, McCloskey TM, Roe DJ, Hamilton M, Salmon SE (1989) Clinical pharmacology of a novel diarylsulfonylurea anticancer agent. J Clin Oncol 11: 1733
18. WHO (1979) Handbook for reporting results in cancer treatment. Offset publication 48. Geneva