Anti-Cancer Drugs, 3, pp. 331 335

A phase II study of sulofenur (LY186641) in gastric cancer

A Kamthan, JH Scarffe,[CA] J Walling, S Hatty, B Peters, R Coleman and JF Smyth

A Kamthan and JH Scarffe are at the Cancer Research Campaign, Department of Medical Oncology, Christie Hospital, Manchester M20 9BX, UK. Tel: 061 446 3000. Fax: 061 446 3352. J. Walling is at the Medical Department, Lilly Industries Ltd, Dextra Court, Chapel Hill, Basingstoke RG21 2SY, UK. S Hatty and B Peters are at the Lilly Research Centre, Windlesham, Surrey, UK. R Coleman and JF Smyth are at the ICRF Medical Oncology Unit, Western General Hospital, Edinburgh EH4 2XU, UK.

Sulofenur is a novel diarylsulfonylurea with proven anti-tumor activity in murine tumor models. In this phase II study in patients with advanced gastric or gastro-esophageal adenocarcinoma, 17 patients were treated with sulofenur orally at 700 mg/m^2 for 14 days every 3 weeks. No tumor responses were seen. The main toxicities were anemia, methemoglobinemia and abnormalities in liver function tests. These toxicities precluded dose escalation. However, plasma levels of sulofenur, and its hydroxy and keto metabolites were probably insufficient to exert anti-tumor effect in comparison with data from murine studies. Further structure–activity studies are warranted.

Key words: Anaemia, gastric adenocarcinoma, methemoglobinemia, sulofenur.

Introduction

Surgery currently represents the only curative treatment option for gastric and esophageal adenocarcinoma.[1] However, the majority of patients present with advanced disease and therefore are not candidates for surgical resection. The 5 year survival is less than 10%.[1] As yet there is no evidence that cytotoxic chemotherapy impacts on the survival of patients with gastric cancer.[2] Single agent response rates are of the order of 20% with 5-fluorouracil, adriamycin or mitomycin C.[1,3] Combinations of these agents will produce response rates of the order of 30–40%.[4,5] However, these responses have been only partial in nature and of relatively short duration. The median survival of patients with locally advanced or metastatic gastric cancer is 4–6 months. It is clear therefore that there is a need for new drugs with activity in gastric and gastro-esophageal adenocarcinoma.

Sulofenur, LY186641, *N*-(5-indanylsulfonyl)-*N'*-(4-chlorophenyl)urea, is a diarysulfonylurea which was developed as a potential anti-cancer drug by Eli Lilly (Indianapolis, IN, USA).[6,7] In pre-clinical studies sulofenur demonstrated significant activity against a wide range of murine tumor models and human xenografts including the colon 26 carcinoma and VCR 5 colon xenograft models.[7,8] Sulofenur has no hypoglycemic activity and has no effect on DNA, RNA or protein sythesis.[7] Its mechanism of action is unknown although recent data has suggested that it may localize inside mitochondria[8] and, at high doses, may uncouple oxidative phosphorylation.[11] Phase I studies defined the dose limiting toxicities as anemia and methemoglobinemia.[11–14] Phase II studies have been carried out in a variety of indications and we describe here the results of our phase II study in patients with gastric or gastro-esophageal adenocarcinoma.

Patients and methods

Patients with histologically proven inoperable or metastatic gastric or gastro-esophageal adenocarcinoma were entered into this study. The following were required for entry into the study: World Health Organization (WHO) performance status 0–2, life expectancy more than 3 months, adequate bone marrow reserve, measurable disease outside a previously irradiated area, normal glucose-6-phos-

[CA] Corresponding Author

phate dehyrogenase, methemoglobin less than 2%, serum bilirubin less than twice normal and serum transaminases less than 3 times normal, and informed consent. No patients received prior chemotherapy. Baseline evaluations included history and physical examination, tumor measurement by computerized tomography (CT) scan, ultrasound or by callipers, and a complete blood count, including differential and biochemical profile.

Sulofenur was given orally at 700 mg/m^2 for 14 days every 3 weeks for a minimum of two courses. Therapy was given on an out-patient basis and patients attended weekly for hematology, biochemistry and urinalysis. Tumor response and drug toxic effects were graded according to WHO criteria with assessment for response being made after two courses (NB methemoglobin was graded according to the scale shown in the legend for Figure 1). Dose reductions were made according to toxicity. No dose reductions were required for WHO grade I or II toxicity. In the case of a WHO grade III hemoglobin or grade III methemoglobin, the dose was reduced by 10%. For other grade III toxicities a 25% dose reduction applied. For grade IV hemoglobin or methemoglobin the dose was reduced by 20%. For other WHO grade IV toxicities the patient was removed from study. In addition, dose escalation was permitted up to 770 mg/m^2 if a patient showed minimal or no signs of toxicity during the first two courses. Repeat cycles of chemotherapy were only given if patients met the entry criteria before being retreated.

Methemoglobin was measured spectroscopically using the method of Evelyn *et al.*[14,15] Trough plasma levels of sulofenur its hydroxy and keto metabolites[14] were measured during the first course of therapy, samples were taken on day 0, day 7/8, day 14/15 and day 21/22, and were assayed by HPLC using a modification of the method of Taylor.[12,17]

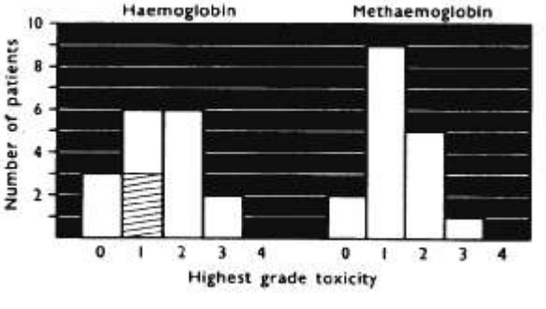

Figure 1. Frequency of worst hematologic toxicity by WHO grade for (a) hemoglobin. As no WHO toxicity grades exists for methemoglobin (b) the following scale was applied (% methemoglobin): grade 0, 0–2; grade 1, 2.1–10; grade 2, 10.1–20; grade 3, 20.1–30; grade 4, >30. Shaded bars represent the number of patients who had levels WHO grade 1 anemia prior to entry.

Results

Seventeen patients (15 males, two females) with a median age of 57 years (range 29–70) were entered into the study. One patient had a WHO performance status of 0, seven were WHO grade 1 and nine were WHO grade 2. Eleven patients had gastric and six gastro-esophageal adenocarcinoma. Grade of tumor differentiation was as follows: poorly, eight; moderate, six; well, one; unknown, two.

A total of 34 courses were administered with eight being subject to dose reduction (Table 1). Dose escalation to 770 mg/m^2 was attempted in one patient, but was not tolerated due to anemia.

Seven patients withdrew early, i.e. prior to receiving two courses. In three cases this was due to progressive disease and four were due to toxicity.

There were no responders. However, one patient had a mixed response with progression in the liver following four courses of sulofenur, but a small omental metastasis disappeared as measured by CT scanning and a celic lymph node was stable.

The major clinical toxicities associated with the use of sulofenur were fatigue and dyspnea. In addition, jaundice in two patients could be ascribed to sulofenur. Although 11 patients experienced some nausea and vomiting and four patients constipation, these were pre-existing and were considered to be symptoms of the disease rather than toxicity. Additionally there were single cases of WHO grade 2 diarrhoea, mucositis and fever, and three mild (WHO grade 1) infections in the absence of neutropenia. No alopecia was observed.

Dyspnoea occurred in nine patients. Of these, seven cases could be ascribed to methemoglobinemia and anemia, and in three cases symptoms were dose limiting, with one patient requiring i.v. methylene blue for symptom relief following a methemoglobin of 30%. Two days following withdrawal of sulofenur, his methemoglobin had dropped to 2.1%.

Of the remaining two cases of dyspnea, one was due to respiratory failure due to pulmonary metastases. The cause of the other case is unclear, although methemoglobinemia cannot be ruled out, since his methemoglobin levels were not measured

Table 1. Administered courses by patient

Patient no.	No. of courses	Cumulative dose (g)	Reason for dose reduction ($n = 8$)	Reason for early withdrawal ($n = 7$)
1	2	3.36		
2	1	1.68		progressive disease
3	4	6.16		
4	2	3.08		
5	2	3.24	patient error	
6	2	3.78		
7	1	1.68		jaundice
8	<3	3.50	patient error, methemoglobinemia	
9	<1	1.44	methemoglobinemia	progressive disease
10	2	3.78		
11	1	1.68	methemoglobinemia	methemoglobinemia
12	4	6.93		
13	<2	1.80	elevated alkaline phosphatase	elevated alkaline phosphatase
14	<1	0.72	dyspnea	syncope
15	<2	2.85	respiratory failure due to pulmonary mets	progressive disease
16	2	3.22	anemia	
17	2	3.78		

due to emergency admission to a non-trial hospital. This patient received only five doses of sulofenur when he became dyspneic and experienced a syncopal attack. An ECG was normal, but there was persistent hypoxia on blood gas analysis. A post-therapy chest X-ray showed pulmonary infiltrates although there was evidence of lymphangitis prior to sulofenur. The patient remained dyspneic despite normalization of methemoglobin levels shortly after discontinuation of therapy.

In total all but one patient experienced some degree of methemoglobinemia, levels are shown in Figure 2. As expected peak levels occurred whilst the patient was on therapy (days 7/8 and 14/15) with levels dropping to just above pre-treatment levels in the rest week (day 21/22). There was large inter- and intrapatient variability and again there was no evidence for a cumulative effect.

Thirteen of 17 patients experienced a fall in hemoglobin whilst on study (three patients had WHO grade 1 anemia on entry to the study) and 10 patients required transfusions. There was no evidence for cumulative toxicity (data not shown). No leucopenia or thrombocytopenia was observed.

Disordered liver function tests (LFTs) were also a feature of treatment. Five patients experienced rises in bilirubin and four rises in transaminases (Figure 3). Fourteen patients had elevated alkaline phosphatase although in nine cases this was raised prior to therapy. In two cases elevated LFTs caused

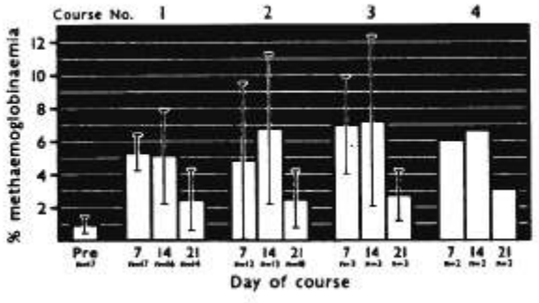

Figure 2. Mean percentage methemoglobinemia with time. Vertical bars are standard deviations about the mean where $n \geq 3$; where they are not shown, means are of two samples. Methemoglobin was routinely measured on day 7, 14 or 21. Additional measurements were carried out when clinically indicated, see text.

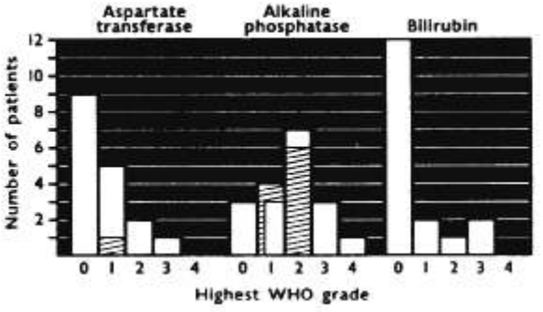

Figure 3. Frequency of worst LFTs by WHO grade for (a) aspartate transferase, (b) alkaline phosphatase and (c) bilirubin. Shaded bars represent the number of patients who had WHO 0 levels prior to entry.

early withdrawal from the study. One patient experienced a marked rise in bilirubin, alkaline phosphatase and aspartate transaminase following one course of sulofenur. The patient was jaundiced and pyrexial with no hepatomegaly and no sign of chronic liver disease, a CT showed no evidence of progressive disease. The histology of a liver biopsy was compatible with drug induced cholestatic jaundice. A second patient also experienced a marked rise in alkaline phosphatase and the patient died of hepatic failure which was ascribed to a combination of liver dysfunction induced by liver metastases and sulofenur.

Renal toxicity was mild, a grade 1 WHO rise in creatinine was seen in five patients, there were two cases of grade 1 hematuria, one of grade 1 proteinuria and one of grade 2 proteinuria.

The mean plasma levels of sulofenur, its hydroxy and keto metabolites were 0 pre-therapy; 187.4, 30.8, 44.2 μg/ml, respectively, on day 7/8; 147.1, 41.2, 41.41 μg/ml, respectively, on day 14/15; and levels of 20.6, 18.1 and 22.7 μg/ml, respectively, were still detectable on day 21/22. These data are shown in Figure 4. One patient had markedly higher plasma levels of the parent substance than did the other patients, with levels of 394.1 μg/ml at day 7 and 347.1 μg/ml at day 14. This was the patient referred to above whose omental metastases disappeared.

Discussion

Sulofenur is a new class of anti-tumor agent which produced documented responses in phase I studies, including a patient with refractory ovarian carcinoma.[12] It is of particular interest because it appears to have a unique mechanism of action and novel dose limiting toxicities. However, the current study failed to demonstrate any efficacy of the agent in patients with advanced gastro or gastro-esophageal adenocarcinoma.

It is debatable whether the plasma levels which were achieved were sufficient to exert anti-tumor effect. Sulofenur is known to be highly protein bound and indeed in *in vitro* sensitivity assays using colonic cell lines, cytotoxicity was enhanced by reducing the level of fetal bovine serum (FBS) from 10 to 0.5%.[9] *In vitro* studies using both colon and rhabdomyosarcoma[9] cell lines have demonstrated anti-tumor effect within the range obtained in the present clinical study. However, again these studies utilized FBS at 10%. In addition, data from mice suggests that a plasma level of 400 μg/ml is required to exert anti-tumor activity.[18] Interestingly the patient who had a mixed response had markedly higher levels of sulofenur than did the other patients. However, other studies have failed to demonstrate this relationship.[17] Attempts to raise plasma levels by increasing adminstered dose has produced unacceptable toxicity in the form of anemia and methemoglobinemia. Subsequent to completion of this study, P-CAM1, a metabolite of *p*-chloroaniline, was identified as a minor urinary excretion metabolite of sulofenur.[19,20] It is likely that *p*-chloroaniline or its metabolites may be responsible for the methemoglobin and/or hemolytic anemia.[21] These toxicities are unusual in an anti-cancer drug.

Other sulfonylureas have been shown to induce jaundice in some patients,[22–24] but animal models failed to demonstrate this toxicity of sulofenur. A review of the phase II database also failed to identify consistent risk factors. Liver metastasis did not predispose to deterioration in liver function.

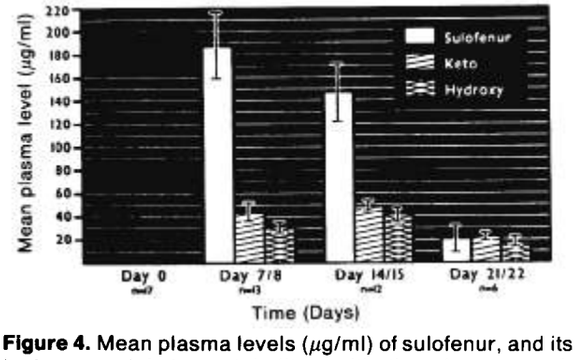

Figure 4. Mean plasma levels (μg/ml) of sulofenur, and its hydroxy and keto metabolites with time in course 1. Vertical bars show standard deviation about the mean. Data is not adjusted for dose. See Table 1.

Conclusion

Sulofenur is not emetogenic, neither does it cause myelosuppression or alopecia. Its toxicities do not overlap with established anti-cancer drugs. Unfortunately its lack of efficacy and dose limiting toxicity precludes its further clinical development in gastric or gastro-esophageal adenocarcinoma. However, the encouraging pre-clinical and phase I data are such that further structure–activity relationships are warranted in an attempt to reduce toxicity and allow higher doses of the drug to be given. Ideally a candidate analog needs to be identified which does not have the capacity to generate *p*-chloraniline metabolites and which does not have the propensity to cause liver damage. Further structure-activity studies are underway.

References

1. Arnott S. Gastrointestinal tract. In: Sikora K, Halnan KE, eds. *Treatment of cancer*, 2nd edn. London: Chapman and Hall 1990: 473–504.
2. Allum WH, Hallissay MT, Kelly KA. Adjuvant chemotherapy in operable gastric cancer. *Lancet* 1989; **ii:** 571–4.
3. O'Connell MJ. Current status of chemotherapy for advanced pancreatic and gastric cancer. *J Clin Oncol* 1985; **3:** 1032–9.
4. Bitran JD, Desser RK, Kozloff MF, *et al.* Treatment of metastatic pancreatic and gastric adenocarcinoma with 5 fluorouracil, adriamycin and mitomycin C (FAM). *Cancer Treat Rep* 1979; **63:** 2049–51.
5. Douglass HO, Jr, Lavin PT, Goudsmit A, *et al.* An eastern cooperative oncology group evaluation of combinations of methyl CCNU, mitomycin C, adriamycin and 5-fluorouracil in advanced measurable gastric cancer (EST2277). *J Clin Oncol* 1984; **2:** 1372–81.
6. Howbert JJ, Grossman CS, Crowell TA, *et al.* Novel agents effective against solid tumors: the diarysulfonylureas. Synthesis, activities and analysis of quantitiative structure–activity relationships. *J Med Chem* 1990; **33:** 2393–407.
7. Grindey GB. Identification of diarylsulfonylureas as novel anticancer drugs. *Proc Am Ass Cancer Res* 1988; **29:** 535.
8. Houghton PJ, Houghton JA, Myers L, *et al.* Evaluation of *N*-(5-indanysulfonyl)-*N*-(4-chlorophenyl)-urea against xenografts of pediatric rhabdomyosarcoma. *Cancer Chemother Pharmacol* 1989; **25:** 84–8.
9. Houghton PJ, Bailey FC, Houghton JA, *et al.* Evidence for mitochondrial localization of *N*-(4-methylphenylsulfony)-*N*-(4-chlorophenyl)urea in human colon adenocarcinoma cells. *Cancer Res* 1990; **50:** 664–8.
10. Houghton PJ, Sosinski J, Thakar J, *et al.* Diarylsulfonylurea's (DSU's): a two mechanism model for cytotoxicity. *Proc Am Ass Cancer Res* 1991; 2471.
11. Hainsworth JD, Hande KR, Satterlee WG, *et al.* Phase I clinical study of *N*-[(4-chlorophenyl)amino]carbonyl-2,3-dihydro-1H-indene-5-sulfonamide (LY186641). *Cancer Res* 1989; **49:** 5217–20.
12. Taylor CW, Alberts DS, Ketcham MA, *et al.* Clinical pharmacology of a novel diarylsulfonylurea anti-cancer agent. *J Clin Oncol* 1989; **7:** 1733–40.
13. Kuhn J, Craig J, Havlin K, *et al.* Phase I clinical and pharmacokinetic evaluation of oral LY186641 on a single q21d schedule. *Proc Am Soc Clin Oncol* 1988; **7:** 60.
14. Armand JP, Recondo G, Gouyette A, *et al.* Phase I and pharmacokinetic study of LY186641 administered daily for 2 weeks every 21 days. *Proc VI NCI EORTC Symp on New Drugs in Cancer Chemotherapy* 1989; 300.
15. Evelyn KA, Malloy MT. Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. *J Biol Chem* 1938; 126–662.
16. Van Kampen EJ, Zijlstra WG. Determination of hemoglobin and its derivatives. *Adv Clin Chem* 1965; **8:** 141–7.
17. O'Brien MER, Hardy J, Tan S, *et al.* A phase II study of sulofenur (a novel sulphonylurea, LY186641) in recurrent epithelial ovarian cancer, submitted.
18. Worzalla, ?. personal communication.
19. Ehlhardt WJ. Metabolism and disposition of the anticancer agent sulofenur in mouse, rat, monkey and human. *Drug Metab Disposit* 1991; **19:** 370–5.
20. Ehlhardt WJ, Howbert JJ. Metabolism and disposition of *p*-chloroaniline in rat, mouse and monkey. *Drug Metabol Disposit* 1991; **19:** 366–9.
21. Kiese M. *Methaemoglobinaemia: a comprehensive treatise.* Boca Raton, FL: CRC Press, 1974.
22. Wongpaitoon V, Mills PR, Russell RI, *et al.* Intrahepatic cholestasis and cutaneous bullae associated with glibenclamide therapy. *Postgrad Med J* 1981; **57:** 244–6.
23. Fraser BM, Steward W. Cholestatic jaundice following chlorpropramide self-poisoning *Clin Toxicol* 1978; **11:** 13.
24. Nakao NL, Gelb AM, Stenger RJ, *et al.* A case of chronic liver disease due to tolazamide. *Gastroenterology* 1985; **89:** 192–5.

(Received 6 May 1992; accepted 14 May 1992)