Investigational New Drugs

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A phase II study of chloroquinoxaline sulfonamide (CQS) in patients with metastatic colorectal carcinoma (MCRC)

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Summary

Purpose: Phase II multicenter study investigated the efficacy and toxicity of the novel halogenated derivative of sulfaquixonaline Chloroquinoxaline Sulfonamide (CQS) in metastatic colorectal cancer. *Experimental design:* Eligible patients with metastatic or recurrent colorectal cancer received CQS at a dose schedule of 2000 mg/m^2 over an hour weekly for 4 weeks every 42 days. Treatment was continued until unexpected toxicity or disease progression. *Results:* A total of seventeen patients were enrolled on this study. 94% of all patients enrolled had prior treatment. Sixteen patients were evaluable for response with fifteen patients showing evidence of disease progression and one patient with prolonged stable disease. One patient had non-evaluable disease. Following this interim analysis, the drug was considered ineffective and the study was terminated early. The most frequent adverse event was anemia. No patients discontinued the treatment because of toxicity. *Conclusion:* CQS, when given at a dose of 2000 mg/m^2 weekly for 4 weeks every 42 days to patients with metastatic colorectal cancer, does not result in significant tumor regression.

Introduction

Adenocarcinoma of the colon and rectum is a major health problem in the United States with approximately 150,000 cases estimated to occur in the year 2005 [1]. One-third of those patients will die from their disease every year [1]. The overall survival in colorectal cancer is 62% with only about 8–9% surviving 5 years with metastatic disease [2]. Chemotherapy has been the mainstay of therapy in patients with metastatic disease with modest effects on survival.

The investigational drug Chloroquinoxaline Sulfonamide (CQS) is a halogenated derivative of sulfaquixonaline, an antifungal agent used in the control of coccidiosis in animals [3]. The mechanism of action of this compound is largely unknown until recently, although unlike sulfaquixonaline, it does not interfere with folate homeostasis [4]. *In vitro*, CQS acts as a topoisomerases (topo) IIα and IIβ poison, thus inhibiting DNA replication [5]. CQS was found to induce arrest of mitogen stimulated lymphocytes in G_0/G_1 stage of the cell cycle [5–7]. The antitumor activity of CQS was first discovered in the human tumor colony-forming assay, with inhibitory effects against human lung, colon, breast and other tumor types. *In vivo* studies of CQS using H82 small cell lung tumor implanted subcutaneously into athymic mice resulted in tumor regression when the drug was given via intraperitoneal and subcutaneous routes for five days [4].

Three phase I studies with CQS were conducted simultaneously looking at either a one hour intravenous infusion every 28 days or a one hour infusion weekly for 4 weeks every 42 days [8–10]. Dose limiting toxicities included hypoglycemia and supraventricular tachycardias were seen with doses higher than 4000 mg/m^2 given every 28 days. There were seven minor responses seen in patients with non-small cell lung cancer (six) and colon cancer (one). Using a weekly schedule, the MTD was determined to be 2000 mg/m^2/wk [8]. In this study there were a total of three minor responses in patients with non-small cell lung cancer (two) and colorectal cancer (one). In one study, it was shown that when correcting for protein binding, the equivalent target human plasma CQS concentration derived from the human tumor

colony-forming assay would be at least 100 μg/ml during a minimum of 24 h [8].

Based on the evidence of preclinical antitumor activity and the presence of antitumor activity in colon cancer in both phase I studies, a phase II study of CQS in patients with metastatic colorectal cancer was conducted.

Patient and methods

Patient selection

Patients entered on this study had metastatic colorectal cancer and measurable disease. Patients were allowed up to one prior chemotherapy, and in addition could have one biologic therapy. Patients were also allowed to have had adjuvant therapy only if the recurrence was at least six months after receiving therapy. Additional inclusion criteria were ECOG performance status of $\leq$2, normal organ and marrow function as defined by leukocytes of $>$3,000/ml, absolute neutrophil count of $>$1,500/ml platelets $>$100,000/ml , total bilirubin within normal institutional limits , AST and/or ALT $<$ 2.5 X institutional upper limit of normal and creatinine within normal institutional limits.

Treatment

CQS was given using the schedule of 2000 mg/m^2 over an hour weekly for 4 weeks every 42 days. Based on phase I data, patients on this study were carefully monitored for cardiac arrhythmias and disturbances in glucose metabolism. Patients had an ECG strip prior to and after their weekly treatment during the first cycle and then the first day of each subsequent cycle. In addition, blood glucose was checked prior to each treatment and then by fingerstick every 2 h post treatment for 4 h for the first cycle and for any episode clinically consistent with hypoglycemia.

Efficacy assessment

Patients were evaluated at baseline and every 12 weeks for response. Tumor measurements were done by CT scanning and responses were assessed using RECIST criteria [11].

Safety assessment

Toxicity was assessed using the NCI Common Toxicity Criteria (CTC) version 2.0

Pharmacokinetic analysis

We performed limited pharmacokinetics based on the results of the phase I studies [8–10]. Pharmacokinetic analysis was performed at 0, 2 and 24 h following the first dose of CQS administration. Samples were obtained from 3 patients. Serum analysis of CQS levels was performed using the HPLC method previously described [3, 8]. Essentially, various amounts of CQS were added to 0.5 ml of blank human plasma to result in concentrations ranging between 500 and 4000 ng/ml and R-XK469 {(2-[4-(7-Chloro-2-quinoxaliny) oxy] phenoxy propionic acid)} was used as the internal standard, to result in a concentration of 1 μg/ml. These solutions were used to construct a calibration curve. For plasma samples 0.5 ml was used with the concentration of internal standard used kept constant at 1 μg/ml. Plasma proteins were precipitated by use of 2.5 ml of acetonitrile. After centrifugation at 1500 g, the supernatant was removed and evaporated under a stream of nitrogen. The residue was reconstituted in 200 μl of mobile phase and a 50 μl aliquot was analyzed for CQS by HPLC. The mobile phase consisted of 30% methanol containing 20 mM ammonium nitrate and 0.2% acetic acid. The flow rate was 1 ml/min and the components were detected by UV at 330 nm. HPLC was typically run for 30–40 min. Under the stated condition, the typical retention times were 20.8 min for R-XK469 and 24.15 min for CQS, with no other major peaks in the chromatogram up to 40 min elution time. No attempt was made to locate and identify CQS metabolites. The assay was linear from 0.5 to 4000 μg/ml. of CQS using 0.5 ml plasma. The within day reproducibility was evaluated at 0.5, 1 and 2 μg/ml concentrations with shown percent CV values of 3.9,5.5 and 9.2 respectively ($n = 6$). The corresponding accuracy percent values 96.3, 90.9 and 85.6 respectively.

Statistical considerations

This was a minimax two-stage design of Simon with the primary endpoint being overall response rate. In this design we considered CQS to be uninteresting or ineffective if the true response probability was less than 20%.The two-stage design establishes that if 3 or fewer responses are seen in the first 19 response-evaluable patients, the study is terminated early and CQS is deemed ineffective for this patient population.

Results

Patient characteristics

The majority of patients (94%) had received prior chemotherapy (Table 1). The most commonly used regimens were 5FU and CPT-11. The rest of the characteristics are listed in Table 1.

Objective response

Sixteen patients were evaluable for response with fifteen patients showing evidence of disease progression and one patient with prolonged stable disease. One patient had non-

Table 1. Patients characteristics

Gender	
Male	59% (10/17)
Female	41% (7/17)
Race	
White	82% (14/17)
Black	12% (2/17)
Asian	6% (1/17)
Site of disease	
Colon	65% (11/17)
Rectum	35% (6/17)
Performance status	
0	59% (10/17)
1	35% (6/17)
2	6% (1/17)
Prior therapy	94% (16/17)
Participating sites	
Cleveland Clinic	8/17
Ohio State Univ	3/17
University of MI	3/17
Central Baptist	3/17

evaluable disease. Following this interim analysis, the drug was considered ineffective and the study was terminated early.

Safety

Toxicities from this drug at the dose tested were very mild.. Most common toxicities included anemia (24%), constipation (18%), hypoglycemia, leukopenia, flushing, stomatitis, sensory neuropathy, headache and fatigue (12%). Only 1 patient had grade 3 toxicity with urticaria. No patients had grade 4 toxicity on this study. For a full list of toxicities , please refer to Table 2.

Pharmacokinetic analysis

Three patients were studied and the results are shown in Figure 1. Essentially, serum CQS levels were consistently below the therapeutic target of 100 μg/ml in the first 24 h after the CQS dose administered.

Table 2. Observed toxicities with CQS

	Grade % (N)				
Toxicity	All grades	Grade 1	Grade 2	Grade 3	Grade 4
Anemia	24% (4)	24% (4)			
Constipation	18% (3)	18% (3)			
Hypoglycemia	12% (2)		12% (2)		
Sensory Neuropathy	12% (2)	12% (2)			
Flushing	12% (2)	12% (2)			
Stomatitis	12% (2)	12% (2)			
Fatigue	12% (2)	6% (1)	6% (1)		
Headache	12% (2)	12% (2)			
Leucopenia	12% (2)		12% (2)		
Lymphopenia	6% (1)	6% (1)			
Urticaria	6% (1)			6% (1)	
Nausea	6% (1)		6% (1)		
Hyperglycemia	6% (1)	6% (1)			
Ataxia	6% (1)		6% (1)		
Fever	6% (1)	6% (1)			
Musculoskeletal pain	6% (1)	6% (1)			
VZV reactivation*	6% (1)		6% (1)		
DVT^	6% (1)		6% (1)		

*Varicella zoster reactivation
†Deep venous thromobosis

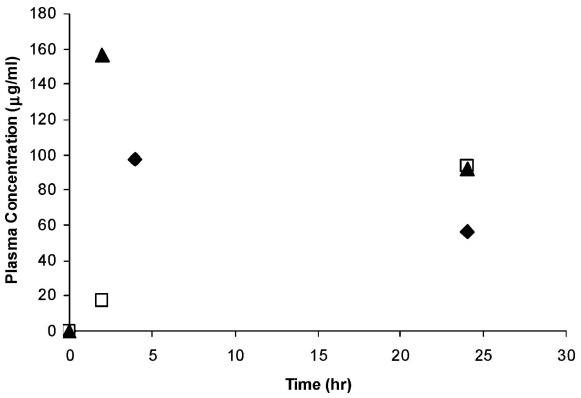

Figure 1. Pharmacokinetics of CQS at 0, 2 and 24 hours after the administration of the first dose of CQS. The limited sampling was performed on only 3 patients.

Discussion

This phase II study of the novel compound Chloroquinoxaline Sulfonamide (CQS)–a halogenated derivative of sulfaquixonaline- in patients with advanced colorectal cancer was initiated based on promising *in vitro* and *in vivo* anticancer activity as well as some anticancer activity in a prior phase I study [8]. In this study we showed that CQS given at 2000 mg/m^2 over an hour weekly for 4 weeks every 42 days was well tolerated but lacked clinical activity in patients with metastatic colorectal cancer.

The lack of efficacy in this study might possibly be related to one or more of the following: (1) All 3 patients included in the pharmacokinetic analysis failed to achieve the target serum level of 100 μg/ml in the first 24 h. However, this was a limited pharmacokinetic analysis (as intended by the

study design), and data from all patients were not available. However, a previously published study looking at CQS with the same dose/schedule in patients with non-small cell lung cancer showed no activity of the drug despite that the target concentration being achieved in 90% of the patients following dose readjustments in a number of those patients. (2) The fact that most patients had been exposed to prior therapy. It is well known that second line therapy in colorectal cancer is largely ineffective with response rates of less than 10% [12, 13]. Our study was designed to consider a response rate of at least 20% as interesting, approximately double the response rate seen with "active" therapies in the second-line setting [12, 13]. Perhaps this response rate of interest was too ambitious, resulting in an underestimation of the activity of this drug. (3) As noted before, CQS inhibits both topo IIα and IIβ [5]. Published *in vivo* data suggests a good rationale for targeting topo II in colorectal cancer based on a higher level of expression of topo IIα gene in tumors relative to normal tissue [14]. However, this study suggested that the sequential chemotherapy targeting topo I and topo II enzymes by modulating topo IIα expression by topo I inhibitors might be more effective in colon cancer, in terms of their relationship between topo I and topo IIα expression in tumor cells [14]. Perhaps, an improved strategy might be to use this drug is in sequence with a topo I inhibitor such as irinotecan, a drug with activity in colorectal cancer.

In conclusion, CQS given intravenously at 2000 mg/m^2 over an hour weekly for 4 weeks every 42 days was well tolerated but lacked noticeable activity in patients with metastatic colorectal cancer. This lack of activity might be related to one on more element as noted above, however, further study of CQS at this dose and schedule cannot be recommended.

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