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James R. Rigas · Prudence A. Francis
Vincent A. Miller · William P. Tong
Nancy Roistacher · Mark G. Kris · John P. Orazem
Charles W. Young · Raymond P. Warrell, Jr.

Clinical and pharmacology study of chloroquinoxaline sulfonamide given on a weekly schedule

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Abstract The in vitro human tumor colony-forming assay identified chloroquinoxaline sulfonamide (CQS) as an active agent at human plasma concentrations of > 100 μg/ml. In the initial phase I trial of CQS given every 28 days, peak plasma concentrations > 500 μg/ml were associated with reversible dose-limiting hypoglycemia and occasional cardiac arrhythmias. Therefore, we evaluated whether a weekly schedule of treatment might minimize the drug-associated toxicity while maintaining potential therapeutic concentrations. CQS was given intravenously over 1 h once per week for 4 weeks to 12 patients, beginning at a dose of 2,000 mg/m^2. All patients underwent monitoring for cardiac arrhythmias and hypoglycemia. Plasma drug levels were measured following each dose. Mild hypoglycemia was the most common adverse effect. A median nadir plasma glucose concentration of 56 mg/dl was observed at a weekly dose of 2,500 mg/m^2. Two patients experienced cardiac dysrhythmia while on study. Continuous electrocardiographic monitoring failed to identify any significant infusion-related arrhythmia. The median CQS plasma concentration measured 24 h following a 2,000-mg/m^2 dose of CQS was > 100 μg/ml, and the cumulative area under the concentration x time curve (AUC) determined at concentrations of ≥ 100 μg/ml was similar to that observed with the every-28-day schedule. The weekly schedule described herein appears to maximize the plasma AUC with an acceptable margin of safety. The recommended phase II dose and schedule for CQS is 2,000 mg/m^2 given once per week. Although severe hypoglycemia is unlikely, glucose monitoring is appropriate for 6 h following CQS administration.

Key words Chloroquinoxaline sulfonamide · Pharmacokinetics · Chemotherapy · Phase I trial

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J.R Rigas (✉) · P.A. Francis · V.A. Miller · M.G. Kris
Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, 1275 York Avenue, New York, NY 10021, USA

J.R. Rigas · C.W. Young · R.P. Warrell, Jr.
Developmental Chemotherapy Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY, USA

N. Roistacher
Cardiology Service, Division of General Medicine, Department of Medicine, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY, USA

J.P. Orazem
Department of Biostatistics, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY, USA

W.P. Tong
Core Pharmacology Laboratory, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY, USA

Introduction

Chloroquinoxaline sulfonamide (CQS; NSC-339004; benzenesulfonamide (4-amino-N[5(on 8)-chloro-2-quinoxalinyl]) is the first halogenated heterocyclic sulfanilamide to enter into clinical anticancer drug testing. This agent was identified as a potentially active antitumor agent in the new drug screening program developed by the United States National Cancer Institute's (NCI) Developmental Therapeutics Program following introduction of the human tumor colony-forming assay [9]. In this in vitro assay, CQS at concentrations of ≥ 10 μg/ml demonstrated antitumor activity in human lung, breast, melanoma, ovary, kidney, and colon tumors [8]. The drug also demonstrated colony inhibition in 6 of 11 common human tumors.

In an initial phase I study, we gave CQS intravenously every 28 days at doses ranging from 18 to 4,870 mg/m^2 [6]. Transient hypoglycemia associated with hyperinsulinemia at peak plasma CQS concentrations of > 500 μg/ml was the limiting adverse effect at a dose of 4,870 mg/m^2. Infusion-related supraventricular tachyarrhythmias were observed in four patients at CQS doses of $> 4{,}000$ mg/m^2. Cumulative toxicity, mucositis, and significant myelosuppression were not observed. Plasma elimination followed a two-compartment model with a prolonged terminal elimination phase ($t_{1/2\beta}$, 52 ± 6 h).

These finding suggested that cumulative CQS exposure over time could be maintained and that drug-associated reactions due to high peak levels might be minimized if a more frequent dosing schedule were employed. This trial was designed to test this hypothesis and to characterize further the pharmacology and adverse-effect profile associated with the administration of CQS on a weekly schedule.

Patients and methods

Patient selection

Eligibility requirements included histologic documentation of cancer, an age of ≥ 18 years, a Karnofsky performance status of ≥ 50, no radiation therapy or chemotherapy in the preceding 21 days, no use of sulfonamide or sulfonylurea medications in the preceding 7 days, a leukocyte count of $\geq 3{,}500/\mu l$, a platelet count of $\geq 100{,}000/\mu l$, a serum bilirubin level of ≤ 1.5 mg/dl, a serum creatinine value of ≤ 1.5 mg/dl or creatinine clearance of ≥ 50 ml min^{-1} 1.7 m^{-2}, and a normal erythrocyte level of glucose-6-phosphate dehydrogenase. Patients with diabetes mellitus, supraventricular tachyarrhythmia, leukemia, hemolytic anemia, glucose-6-phosphate dehydrogenase deficiency, or sulfonamide allergies and lactating or pregnant women were excluded. Written informed consent was obtained and the study was approved by this center's institutional review board.

Study design

All patients were hospitalized for glucose and cardiac monitoring, and all underwent a complete history and physical examination. Initial laboratory studies included complete blood counts, serum glucose determination, and a multichannel biochemical screening profile. These studies were repeated before each subsequent dose of CQS. Standard criteria for response [5] and the NCI Common Toxicity Criteria were used. The prescribed dose of CQS was infused over 1 h every 7 days for a total of four doses. Individuals whose disease was stable or responding received additional cycles consisting of four weekly doses of CQS followed by a 2-week rest period until there was evidence of disease progression or unacceptable toxicity. At least three patients were entered at each dose level. The starting dose was 2,000 mg/m^2. At levels where toxicity was observed, an additional three patients were studied. Dose escalation for individual patients was not permitted. The maximum tolerated dose (MTD) was defined as the dose that produced reversible grade 2 toxicity in $\geq 70\%$ of patients or grade 3 toxicity in $\geq 30\%$ of patients. CQS was supplied as a yellow lyophilized powder by the Division of Cancer Treatment, NCI, Bethesda, Maryland. The drug in each vial containing 500 mg of CQS with 750 mg of *N*-methylglucamine was reconstituted with 9.2 ml of sterile water for injection (USP) at a pH of 9.5–10.5. Doses were resuspended in 150 ml of 5% dextrose or 0.9% NaCl.

Glucose and cardiac monitoring

All patients were assessed for cardiac arrhythmias during the initial four doses of CQS. CQS was given on a medical cardiac telemetry unit with continuous electrocardiographic monitoring using the Hewlett-Packard 78720 Arrhythmia Monitoring System (Hewlett-Packard, Inc., Andover, Mass.). Electrocardiographic monitoring began at least 2 h prior to CQS administration and continued for at least 24 h following the infusion. All patients were monitored for hypoglycemia with each dose of CQS and were hospitalized for glucose monitoring during the initial four weekly infusions of CQS. Fingerstick glucose concentration was measured by Chemstrip bG with Accu-Chek II/III (Boehringer Mannheim Co., Indianapolis, Ind.) every 2 h for 8 h, then every 4 h for 16 h after the infusion of CQS. Patients who had a fingerstick glucose concentration of < 40 mg/dl or who developed symptoms of hypoglycemia had a plasma venipuncture glucose measurement and received 50 ml of 50% dextrose. Symptomatic patients with a plasma venipuncture glucose concentration of < 60 mg/ml or asymptomatic patients with plasma venipuncture glucose concentrations of < 40 mg/dl received a continuous infusion of 10% glucose. Patients were not pretreated with intravenous glucose, although increased oral carbohydrate intake was encouraged. Individuals who received all four weekly doses of CQS without developing hypoglycemia received subsequent treatment courses in the ambulatory clinic with fingerstick glucose monitoring every 2 h for 6 h.

Pharmacokinetics studies

Pharmacokinetics studies were performed during the first course on three patients treated at the 2,000-mg/m^2 dose level. Heparinized blood samples were obtained pretreatment and at 1, 2, 4, 6, and 24 h on the 1st day of treatment, then prior to each weekly dose and at 2 and 24 h after each drug infusion. Blood samples were immediately separated by centrifugation and the plasma was stored at -70°C.

Analytic methods

Erythrocyte glucose-6-phosphate dehydrogenase (oxidoreductase, EC 1.1.1.49) activity was determined quantitatively by a spectrophotometric method (Sigma Diagnostics, St. Louis, Mo.). Plasma levels of CQS were analyzed by high-performance liquid chromatography (HPLC) with UV detection using the modified method of McCormack et al. (see [6]). Separation was performed by direct injection of plasma by the filled-loop method with a Rheodybne 7520 microinjection valve and a 1-μL loop onto a 5-μm Zorbax CN cartridge column (4 × 80 mm). The mobile phase was 80% HPLC-grade water containing KH_2PO_4 (50 m*M*) with 20% acetonitrile. Triethylamine (0.02%) was added to the mobile phase, and the final pH of the mobile phase was adjusted to 6.1. The flow rate was 1 ml/min and detection was evaluated at 254 nm. The retention time for CQS was 8 min. This HPLC method has the sensitivity to detect CQS concentrations of 5 μg/ml, and the standard CQS concentration curve generated for concentrations ranging from 10 to 160 μg/ml has a squared correlation coefficient of 0.99.

Pharmacokinetic calculations

Plasma CQS concentrations were determined by peak-area ratios of the internal standard versus the compound. Pharmacokinetic

parameters were estimated using computerized software (MKMODEL, version 4.42; Biosoft, Ferguson, Mo.). The plasma CQS concentrations measured at 2 and 24 h were used to estimate the area under the concentration × time (AUC) value. This limited sampling model was established from the data obtained from the complete pharmacokinetic evaluation with a correlation coefficient of 0.91 (6).

Results

Clinical study

In all, 82 doses of CQS were given to 12 patients (Table 1). All 12 patients were evaluable for toxicity. Of 22 treatment courses (4 weekly doses), 18 (81.8%) were deemed adequate to assess the toxicity. All six patients treated at the 2,000-mg/m^2 dose level completed the 4-week treatment course as compared with only two patients treated at the 2,500-mg/m^2 level. The four incomplete courses at the 2,500-mg/m^2 dose were due to disease progression (one patient), atrial arrhythmias (two patients), and thrombocytopenia (one patient).

Hypoglycemic effects

Transient, mild hypoglycemia following CQS infusion was the most common non-hematologic reaction noted. Hypoglycemia (plasma venipuncture glucose concentration, 40–64 mg/dl) occurred in 2 of 58 (3.4%) treatment courses of CQS given at a dose of 2,000 mg/m^2 and in 3 of 24 courses (12.5%) given at 2,500 mg/m^2 (Table 2). All episodes of hypoglycemia occurred within 4 h of the infusion. The median nadir plasma venipuncture glucose values were 69 and 56 mg/dl for patients receiving 2,000 and 2,500 mg/m^2, respectively. Grade 3 or greater hypoglycemia (plasma venipuncture glucose level, $\leq$ 39 mg/dl) was not observed. Two patients experienced symptoms of lightheadedness, diaphoresis, and perioral numbness that were not associated with hypoglycemia. Five patients received ten subsequent treatment courses in the ambulatory chemotherapy clinic. Three patients received cumulative doses of $\geq$ 30,000 mg/m^2 of CQS without developing progressive fasting hypoglycemia or glucose intolerance.

Table 1 Characteristics of patients receiving weekly CQS

	Number
Patients	12
Median age (years)	65
Range	49–81
Male:Female	5:7
Karnofsky performance status:	
80–100	11
60–70	1
Primary cancer:	
Non-small-cell lung cancer	9
Colorectal carcinoma	3
Prior chemotherapy	11
Prior radiation	1

Cardiac effects

In all, 38 infusions of CQS were monitored by continuous electrocardiography, and 8 patients developed cardiac dysrhythmias. Four patients had asymptomatic sinus tachycardia (heart rate, > 100 beats/min) while being monitored. Two episodes of asymptomatic nonsustained supraventricular tachyarrhythmia (heart rate, 150–185 beats/min) were recorded; one occurred 2 h following the initial 2,500-mg/m^2 infusion of CQS and the other, 8 h prior to the fourth 2,500-mg/m^2 weekly infusion. Two additional patients (one treated at 2,000 mg/m^2 and one, at 2,500 mg/m^2) were found to have clinically insignificant three- to four-beat atrial arrhythmias recorded prior to and following the administration of CQS. One patient was hospitalized for increasing shortness of breath associated with intermittent episodes of atrial fibrillation (grade 3) requiring treatment (3 days following his initial 2,500-mg/m^2 infusion of CQS). One patient receiving verapamil for asymmetric septal hypertrophy developed symptomatic bradycardia (grade 4) following drainage of a pericardial effusion. This event occurred 6 h following this patient's second 2,500-mg/m^2 infusion of CQS.

Miscellaneous effects

Hematologic toxicities encountered following weekly CQS administration were mild. Grade 2 leukopenia

Table 2 Hypoglycemia and cardiac dysrhythmias encountered following weekly CQS administration

Dose (mg/m^2)	Number of patients	Number of doses	Number of patients exhibiting toxicity							
			Hypoglycemia NCI grade				Dysrhythmia NCI grade			
			1	2	3	4	1	2	3	4
2,000	6	58	0	2	0	0	3	0	0	0
2,500	6	24	1	3	0	0	3	0	1	1

Table 3 Weekly CQS toxicity summary

Toxicity	Highest NCI toxicity grade for each patient				
	0	1	2	3	4
Anemia	0	6	4	2	0
Diarrhea	9	2	1	0	0
Fever	7	1	4	0	0
Hypoglycemia	6	1	5	0	0
Leukopenia	7	2	3	0	0
Nausea	8	3	1	0	0
Thrombocytopenia	8	3	1	0	0

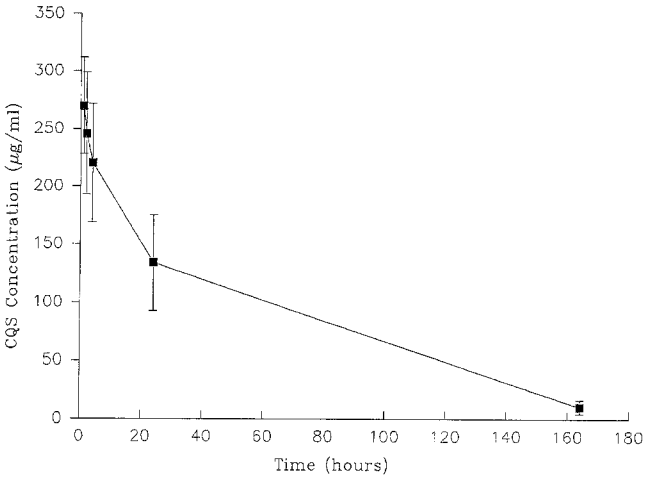

Fig. 1 Plasma concentration (mean ± SD) versus time plot of the elimination of CQS for 3 patients following the initial 1-h infusion of 2,000 mg/m^2

occurred in three patients who received 2,500 mg/m^2. Two of the three patients who developed grade 2 leukopenia following at least four doses of CQS had clinically significant metastatic liver disease from adenocarcinoma of the colon. The other patient who developed prolonged leukopenia and thrombocytopenia following one 2,500-mg/m^2 dose of CQS had a history of significant prior irradiation exposure. Disease-related anemia without evidence of hemolysis, diarrhea, fever, without infection, and nausea were also observed (Table 3). In 1 patient, rigors, fever, and cyanosis were observed following the 11th and 12th infusions of 2,500 mg/m^2 doses of CQS. These episodes were not associated with cardiac dysrhythmia, infection, or hypoglycemia.

Antitumor response

Major tumor regressions were not observed. One patient with non-small-cell lung cancer (NSCLC) experienced a greater than 50% reduction in his tumor that lasted only 3 weeks. Three additional patients (one with adenocarcinoma of the colon and two with NSCLC) achieved disease stabilization lasting 4–5 months.

Pharmacokinetic results

The plasma elimination of CQS over time as recorded for three patients receiving 2,000 mg/m^2 of CQS is presented in Fig. 1. The pharmacokinetic parameters determined for these patients were similar to those observed for the administration of CQS every 28 days with the exception of the prolonged terminal elimination half-life ($t_{\frac{1}{2}}\gamma$) of 90 h (Table 4). As a result of this delayed elimination, a median plasma CQS concentration of 14 μg/ml (range, 4–18 μg/ml) was measured immediately prior to the second, third, and fourth weekly doses of CQS (Fig. 2). These residual CQS concentrations were not associated with alterations in the pharmacokinetic parameters or observed toxic reactions. The mean plasma CQS concentrations measured 24 h following each weekly 1-h infusion at a dose of 2,000 mg/m^2 of CQS exceeded 100 μg/ml (Fig. 2). We then compared the plasma CQS exposure for concentrations greater than those used in the NCI human tumor cell colony-forming assays (100 μg/ml) with the plasma levels obtained in both the weekly and the every-28-day dosing schedules. In this analysis, we estimated the AUC($t_{0\rightarrow<100\,\mu g/ml}$) from the completion of the 1-h infusion (t_0) to the time at which the plasma CQS concentration fell below 100 μg/ml ($t_{<100\,\mu g/ml}$) using the 2- and 24-h CQS concentrations. The AUC($t_{0\rightarrow<100\,\mu g/ml}$) calculated for the four weekly 2,000-mg/m^2 infusions of CQS was 10,528 μg h ml^{-1} as compared with the AUC($t_{0\rightarrow<100\,\mu g/ml}$) of 8,981 μg h ml^{-1} achieved by infusion of the recommended phase II dose (4,060 mg/m^2) every 28 days (Table 4).

Table 4 Pharmacokinetic parameters of weekly and monthly CQS

Dose (mg/m^2)	Number of patients	$t_{1/2}\beta$ (h)	$t_{1/2}\gamma$ (h)	AUC($t_{0\rightarrow<100\,\mu g/ml}$) (μg h ml^{-1})	ΣAUC($t_{0\rightarrow<100\,\mu g/ml}$) (μg h ml^{-1})	CL_{tb} (ml h^{-1} m^{-2})	Vd_{as} (1/m^2)
2,000	3	27	98	2527	10,528	134	8.2
4,060[a]	4	29	–	8981	–	264	10.5

[a] Pharmacokinetic parameters determined for the phase II dose of CQS (4,060 mg/m^2) as given on an every-28-day schedule [6]

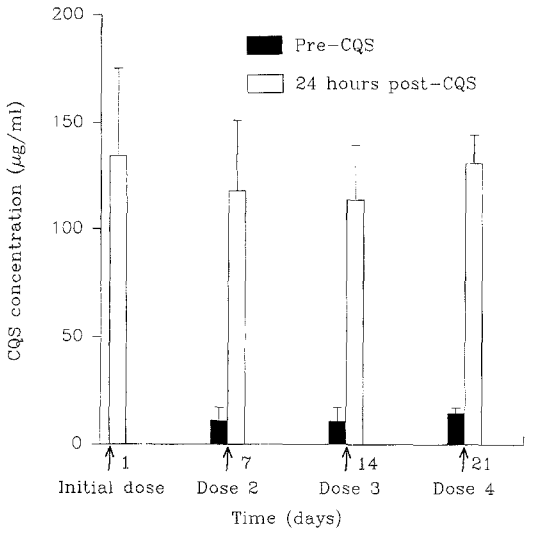

Fig. 2. Concentrations of CQS (mean ± SD) measured prior to (■) and 24 h (□) following each 2,000-mg/m^2 intravenous dose of CQS given weekly for 4 weeks to 3 patients

Discussions

This phase I trial demonstrates that the administration of CQS on a weekly schedule can maintain cumulative drug exposure over time while minimizing the dose-limiting toxicity of hypoglycemia. The long terminal elimination phase ($t_{\frac{1}{2}}\gamma$, 90 h), avid protein binding, and lower peak plasma concentrations permitted the safe administration of four weekly doses of 2,000 mg/m^2 of CQS. Relative to the in vitro concentrations of CQS used in the preclinical colony-forming assay, we found that the $AUCt_{0\rightarrow<100\,\mu g/ml}$ was somewhat higher for the four weekly infusions as compared with monthly treatment at that schedule's maximally tolerated dose (4,060 mg/m^2).

Hypoglycemia was the most common adverse effect encountered. However, less than 4% of the infusions given at 2,000 mg/m^2 were complicated by plasma glucose concentrations of 40–64 mg/dl and none fell below 39 mg/dl. All drug-associated adverse effects were more common in patients who received 2,500 mg/m^2 of CQS. Clinically important leukopenia or thrombocytopenia was not encountered. Two patients experienced cardiac dysrhythmia (that was possibly disease-related) following treatment with 2,500 mg/m^2. One of these patients developed intermittent atrial fibrillation several days following the initial infusion, and another patient developed symptomatic bradycardia following an invasive pericardial procedure. Neither of these arrhythmias was detected by continuous cardiac monitoring. In over 1,000 patient-hours of cardiac monitoring, we recorded only 2 episodes of asymptomatic nonsustained atrial tachyarrhythmia in 34 monitored infusions (5%). The clinical significance of these atrial arrhythmias in this patient population is doubtful. This arrhythmia is the most common ectopic tachyarrhythmia recorded on ambulatory electrocardiography monitoring, with the prevalence in young adults being 2%–5% [3]. These arrhythmias are more frequently recorded in older populations and may be even more common in patients with cancer [7]. Thus, our data do not indicate that continuous cardiac monitoring is required or useful during treatment with CQS.

Clinically, a target human plasma concentration of ≥ 100 μg/ml of CQS had been suggested by the preclinical studies. These in vitro studies identified CQS as an active antitumor agent at a concentration of ≥ 10 μg/ml in a colony-forming assay [9]. This minimal target concentration was derived from the free drug concentrations employed in studies performed in media containing 10% fetal calf serum (FCS). Thus, a human plasma total CQS equivalent of the inhibitory concentration (corrected for differences in protein binding) would be at least 100 μg/ml over a minimum of 24 h [10]. In this study, CQS concentrations in plasma for three patients who received 2,000 mg/m^2 of CQS exceeded 100 μg/ml at 24 hours. In addition, the sum of AUCs for the time when the concentrations were ≥ 100 μg/ml ($AUCt_{\rightarrow<100\,\mu g/ml}$) was somewhat greater for the four weekly infusions as compared with the monthly schedule, suggesting that the former schedule might be preferable from the standpoint of both safety and potential efficacy.

In the human tumor colony-forming assay, CQS displayed a distinctive pattern of antitumor activity that suggested a novel mechanism of action for this drug. CQS does not intercalate with DNA or induce alterations in folate homeostasis [1]; although, in vitro inhibition of DNA replication and G_0/G_1 cell-cycle arrest have been documented [2, 4], the importance of these effects are uncertain. In this study, CQS again showed modest anticancer activity in NSCLC, similar to our previous finding [6]. The drug was generally well tolerated, did not cause significant myelosuppression, and lacked cumulative adverse effects. These attributes suggest that further clinical evaluation of CQS is appropriate in other diseases employing this weekly schedule with individual CQS dosing to target 24-h plasma concentrations of ≥ 100 μg/ml. We have initiated such a study in patients with NSCLC.

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