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Phase I trial of chloroguinoxaline sulfonamide, with correlation of its pharmacokinetics and pharmacodynamics

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Abstract To define a maximum tolerable dose, chloroquinoxaline sulfonamide (CQS) was given as a 1-h infusion every 28 days to cancer patients for whom no effective standard therapy was available. Doses were escalated in cohorts of at least three patients each. Plasma for characterization of the pharmacokinetics of free and total CQS was obtained during and after the initial infusion and, when possible, during and after subsequent infusions of CQS if the dose had been reduced. A total of 101 courses of CQS in 55 patients were evaluated. Dose levels ranged from 18 to 3,700 mg/m^2. The dose-limiting toxicity was hypoglycemia, first recognized at the 3,700-mg/m^2 dose. When dose-limiting hypoglycemia was recognized, patients were entered at successively lower doses, with close monitoring of plasma glucose and insulin concentrations being done in 26 patients. Grade 1–3 hypoglycemia occurred within 4 h of the termination of CQS infusion and cleared by 24 h. Symptomatic hypoglycemia was more frequent at doses of CQS above 1,000 mg/m^2. Concomitant administration of 5% glucose did not ameliorate the hypoglycemia associated with CQS doses of $>1,000$ mg/m^2. The total calorie intake, percentage of ideal body weight, or percentage of weight lost did not explain the incidence or severity of hypoglycemia in 12 patients in whom these data were obtained. Cardiac tachyarrhythmias occurred in 7 patients who received CQS at doses of $\geq 1,000$ mg/m^2, and tachyarrhythmia was associated with hypoglycemia in 3 patients. Other toxicities were sporadic, but the frequency of toxicity was higher at CQS doses of $\geq 1,000$ mg/m^2. These toxicities included fever, rash, lightheadedness, leukopenia, thrombocytopenia, alopecia, diarrhea, nausea, and vomiting. All toxicities were reversible. Mean peak plasma [CQS] and AUC increased with dose, with a suggestion that peak plasma [CQS] plateaued at higher doses. The decline in plasma [CQS] was fitted to a three-compartment, open linear model. The terminal half-life ranged from 28 to 206 h. Total body clearance ranged from 44 to 881 ml/h with no evidence of saturation. Urinary excretion of the parent compound in 24 h averaged $<5\%$. CQS not bound to plasma protein (free CQS) comprised 1%–17% of total plasma CQS and was not related to dose. A relationship was defined between the magnitude of hypoglycemia and CQS pharmacokinetic parameters. The percentage of decrease in plasma [glucose], i.e., $(predose\ [glucose]\text{-}nadir\ [glucose]/predose\ [glucose]) \times 100$, correlated with both free and total peak plasma [CQS]. The relationship was described by the Hill equation: $Effect = (Emax)(peak)^H/(peak_{50})^H + (peak)^H$, where the maximal effect ($Emax$) equals the maximal possible percentage of decrease in plasma [glucose] equals 100%, $peak_{50}$ is the peak total [CQS] at which E is half-maximal (326 mg/l), and H is the Hill constant, a measure of the sigmoidicity of the relationship (1.06). The relationship fit the data precisely with a mean absolute error (MAE) of 10.42 and was unbiased with a mean error (ME) of -0.06. The recommended phase II dose of CQS is 1,000 mg/m^2. Because the magnitude of hypoglycemia

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after CQS administration is related to peak plasma [CQS], repetitive CQS doses of $\leq 1{,}000$ mg/m^2 would probably be tolerated better than single large doses of equivalent intensity.

Key words Phase I · Pharmacokinetics · Chloroquinoxaline · Sulfonamide

Abbreviations *CQS* Chloroquinoxaline sulfonamide · *AUC* area under the plasma concentration × time curve · CLTB total body clearance · MAE mean absolute error · ME mean error · DNA deoxyribonucleic acid · BCNU carmustine [*N*, *N*-bis(2-chloroethyl)-N-nitrosourea] · ECOG Eastern Cooperative Oncology Group · WBC white blood cell count · PLT platelet count · ALT alanine aminotransferase · AST aspartate aminotransferase · PT prothrombin time · PTT partial thromboplastin time · EKG electrocardiogram · D5*W* 5% dextrose in water · HPLC high-performance liquid chromatography · BEE basal energy expenditure

Introduction

Chloroquinoxaline sulfonamide (CQS, NSC 339004) is a benzenesulfonamide that showed selective activity against solid tumors in vitro in a tumor colony-forming assay where a [CQS] of 10 mg/l produced response rates of 40% for tumors of the breast, lung, and ovary as well as melanoma [8]. However, at least 3 times this concentration was necessary to inhibit the growth of P388 leukemia. Studies of xenografts of ovarian (Ovcar-3) and lung (H82 small-cell lung cancer) cancers in nude mice demonstrated a minimal response at nontoxic doses [8]. Preclinical pharmacokinetics studies revealed > 90% protein binding of CQS [8]. Animal toxicology studies revealed that dogs were more susceptible to the toxic effects of CQS due, in part, to an extremely long half-life of 45–132 h [8]. Toxicities noted in dogs after single-dose trials were bloody diarrhea, hematopoietic toxicity, lymphoid tissue toxicity, and pancreatic, adrenal, and testicular toxicity [8].

The mechanism of action of CQS is unknown at present. Branda et al. [1] did not find that CQS interfered with folate homeostasis, as had been reported for sulfaquinoxaline. Likewise, studies have not shown any interaction of CQS with DNA [1]. The mechanism of action of CQS is currently under investigation.

On the basis of encouraging in vitro results obtained at drug concentrations achieved by CQS doses that were nontoxic to animals, a phase I trial of CQS was begun using a 1-h infusion schedule every 28 days. The initial dose level was 18 mg/m^2, which was equivalent to one-tenth of the toxic low dose in dogs.

Patients and methods

Patient selection

Eligible patients included those aged ≥ 18 years who had solid tumors for which standard therapy was not deemed effective. Patients had recovered from all toxicity of prior treatment and had not received radiation or chemotherapy for at least 4 weeks (> 6 weeks for drugs associated with prolonged myelosuppression, such as mitomycin C or BCNU. An ECOG performance status of < 3 and a life expectancy of at least 12 weeks were required. Laboratory requirements included a WBC of ≥ 3,500/μl, a PLT of ≥ 100,000/μl, a level of bilirubin < 1.5 mg/dl, serum ALT and AST values ≤ 2-fold the upper limit of normal, a serum creatinine level of ≤ 1.5 mg/dl or creatinine clearance of ≥ 50 ml/min, a serum total protein value of ≥ 5 g/dl, and a serum albumin level of ≥ 3 g/dl. All patients signed informed consent forms approved by the Institutional Reveiw Board at the University of Maryland at Baltimore and the Cancer Therapy Evaluation Program, National Cancer Institute (Bethesda, Md, USA). Patients with the following characteristics were excluded from participation: pregnancy of breast feeding, an allergy to sulfa drugs, a history of seizure disorder, a primary brain tumor or brain metastasis, and a history of glucose-6-phosphate dehydrogenase deficiency or insulin-dependent diabetes mellitus.

Baseline and follow-up studies

Prior to entry onto study, all patients had a complete history and physical examination and, where possible, tumor measurement. Baseline laboratory studies included the following determinations: CBC, PLT, differential WBC, PT, PTT, urinalysis, serum urea nitrogen, creatinine, creatinine clearance, calcium, phosphate, bilirubin, ALT, AST, electrolytes, uric acid, albumin, and total protein. In addition, all patients had a baseline electrocardiogram (EKG) and chest radiograph done at entry. Follow-up studies included weekly toxicity evaluation, physical examination, and determinations of CBC, PLT, differential WBC, serum urea nitogen, electrolytes, glucose, liver function, and phosphorus.

After hypoglycemia was noted as a toxicity, we monitored serum glucose concentrations prior to drug administration. Fingerstick glucose determination as well as serum glucose determinations were done at 30 min into the infusion, at the end of infusion, at 15 min postinfusion, and then at the time at which blood was withdrawn for pharmacokinetics studies. Serum insulin concentrations were measured prior to drug administration, at 1 h and 24 h after completion of the CQS infusion, as well as at the time of any blood glucose concentration of ≤ 60 mg/dl and prior to administration of supplemental parenteral glucose. Patients who demonstrated symptoms of hypoglycemia and/or had a blood glucose determination of < 50 mg/dl received 50 ml of 50% dextrose as an intravenous bolus and were started on a continuous infusion of 10% dextrose until 24 h after completion of the CQS infusion. All baseline studies except the chest radiograph, EKG, and determination of creatinine clearance were repeated prior to each subsequent course.

For patients with measurable tumors, tumor measurement was assessed after at least every two courses. A complete remission was defined as the disappearance of all evidence of malignancy for a period of at least 4 weeks. A partial remission was defined as a decrease of ≥ 50% in the sum of the products of the largest perpendicular diameters of all tumor lesions lasting for at least 4 weeks, with no other tumor progression. Progressive disease was defined as an increase of ≥ 25% in the sum of the products of the largest perpendicular diameters of any lesion, the appearance of any new lesion, and all clearly progressive skeletal involvement manifested by increasing numbers of lytic lesions. Toxicity was graded according to the Common Toxicity Criteria of the National Cancer Institute (National Institutes of Health, Bethesda, Md.). The

maximum tolerable dose was defined as the dose producing hypoglycemia of at least grade 2 in at least two of up to six patients treated at that dose.

Drug administration

CQS was supplied as a yellow, lyophilized powder by the Division of Cancer Treatment, National Cancer Institute. Each drug vial contained 500 mg of CQS with 750 mg of *N*-methylglucamine. Drug was reconstituted with 9.2 ml of sterile water for injection (USP). The appropriate dose of CQS was diluted in 150 m*l* of 0.9% NaCl and was given intravenously over 1 h with an IVAC 599 (IVAX, Inc., USA) infusion pump. The initial dose was 18 mg/m^2. Doses were escalated in cohorts of at least three patients each to 36, 60, 90, 216, 270, 338, 439, 1,360, 2,000, 2,600 and 3,700 mg/m^2. Accelerated dose escalation occurred at between 90 and 216 mg/m^2 and at between 439 and 1,360 mg/m^2 because of the lack of toxicity reported from other centers studying CQS. Once hypoglycemia was noted, dose deescalation occurred from 3,700 mg/m^2 downward to 2,600, 2,000 and 1,600 mg/m^2. When hypoglycemia was noted at 1,600 mg/m^2, CQS was given concurrently with 5% dextrose in water (D5W) at 100 ml/h for 6 h beginning at the same time as the CQS infusion. When hypoglycemia occurred despite this modification, doses were reduced again to 1,000 mg/m^2 with concurrent D5W so as to define a nontoxic outpatient dose of drug, i.e., a dose that would not result in a nadir plasma glucose concentration of < 50 mg/l and would not require aggressive glucose supplementation. Drug courses were repeated every 28 days unless the patient had not recovered from drug toxicity, had progressive disease, or refused further treatment. Prophylactic antiemetics were not given. Antiemetics were used if nausea and/or vomiting occurred.

Pharmacokinietics

Blood was withdrawn from a peripheral vein into heparinized tubes prior to drug infusion; at 30 min into the infusion; at the end of the infusion; at 5, 15, and 30 min after the end of the infusion; and at 1, 2, 4, 8, 12,18, 24, 48, 96, 144, and 168 h after the end of the infusion. If possible, samples were also obtained on days 14, 21, and 28 after the initial infusion. Plasma was separated from whole blood by centrifugation at 2,000 *g* at 4°C and was stored at -20°C until analysis. At doses of $\geq 1,000$ mg/m^2, free CQS was separated from protein-bound CQS by passage of plasma over Centrifree ultrafiltration devices (Amicon Corporation, Danvers, Mass. USA). Urine was collected in 4-h fractions for the first 24 h after dosing. The volume of each fraction was measured, and an aliquot was removed from each sample and frozen at -20°C until analysis.

Quantitation of CQS in plasma and urine was accomplished by isocratic high-performance liquid chromatography (HPLC) according a modification of the method of Branda et al. [1]. Briefly, 500 µl of plasma was mixed with 500 µl of 3.3×10^{-5} *M* acetophenone internal standard and 1 ml of acetonitrile. Excess ammonium sulfate was added to precipitate protein, and samples were centrifuged at 15,000 *g* for 15 min. The supernatant was placed into autosampler vials containing microinserts (Sun Brokers, Inc., Wilmington, N.C., USA).

The HPLC system consisted of a Waters 510 pump and a WISP 710 autosampler (Waters, Inc., Milford, Mass. USA) connected to an Alltech (Alltech Associates, Deerfield, Ill. USA) econosphere C_{18} column (250 mm $\times$ 4.6 mm inside diameter; particle size, 5 µm. A Brownlee (Brownlee Labs, Santa Clara, Calif. USA) RP-18 guard column was used. Detection was accomplished with a Waters Model 440 UV Absorbance Detector set at 254 nm. Integration was accomplished with a SpectraPhysics SP 4270 (SpcetraPhysics, Inc., Piscataway, NJ, USA) integrator. The mobile phase was 35% acetonitrile: 65% HPLC-grade water containing 50 m*M* KH_2PO_4 and 10 m*M* heptane sulfonic acid pumped at a flow rate of 1 ml/min.

Under these conditions, the limit of detection was 0.5 mg/l, and the retention times for CQS and the internal standard were approximately 6.3 and 8.6 min, respectively. The standard curve for CQS was linear between concentrations of 0 and 1,000 mg/l. Endogenous substances did not interfere with detection of CQS or the internal standard.

CQS concentrations were determined by comparing the ratio of the areas of the CQS peak to internal standard peak in the patient sample with concomitantly performed standard curve. All samples were run in duplicate. The decline in plasma total CQS concentration was modeled as a three-compartment, open linear model with PCNONLIN (Statistical Consultants, Inc., Lexington, Ky., USA) software. The AUC of non-protein-bound CQS was calculated as dose/AUC.

Dietary assessment

Eight patients receiving 1,600 mg/m^2 and four patients receiving 1,000 mg/m^2 CQS were asked to keep a food diary for the 3 days prior to their scheduled treatment. In all, 8 of 12 patients kept records prior to 2 courses, for a total of 20 records. Completed diaries were reviewed with the patient for accuracy and completeness. Calorie intake was calculated with the Nutritionist IV program (N-Squared Computing, Salem, Ore., USA), and the total required calorie intake was calculated as BEE $\times$ 1.3 (Harris-Benedict equation). The proportion of actual intake to required intake was calculated.

Results

Clinical trial

Altogether, 57 patients received 105 courses of CQS; 101 courses delivered to 55 patients were evaluable. One patient was only partially assessable for toxicity, as he developed hives while receiving the CQS infusion at a dose of 3,700 mg/m^2, and the infusion was stopped after 50 ml fo CQS had been infused; the course was terminated, but the patient did receive an evaluable second course of CQS. Two patients were inevaluable: one, due to early death from disease progression on day 6 of the first course of CQS (439 mg/m^2); and the other, due to early death from disease progression on day 19 of the first course of CQS (90 mg/m^2). The patients' characteristics are shown in Table 1. The CQS doses, number of patients per dose, and number of courses per dose are shown in Table 2. Two patients who received CQS doses of 3,700 mg/m^2 received dose reductions to 2,600 mg/m^2 for subsequent courses because of myelotoxicity in one patient and sediment in the urine in the other. A third patient, who received CQS at a dose of 1,600 mg/m^2, had the dose reduced to 1,200 mg/m^2 because of hypoglycemia.

Response

One patient had a partial response lasting for 7 months. This patient had a squamous-cell carcinoma of the head and neck, which metastasized to the lungs. He was

Table 1 Patients characteristics (*SCLC small-cell lung cancer*, NSCLC non-small-cell lung cancer)

Patients entered	57
Evaluable:	55
Males	36
Females	19
Median age (range)	57 (34–77) years
Median performance status (range)	1 (0–2)
Tumor type:	
Head/neck	8
Colorectum	11
NSCLC	9
Pancreas	4
Stomach	4
Melanoma	3
Esophagus	3
Multiple primary	2
Unknown primary	2
Other (Renal, Hepatoma, Mesothelioma, Thyroid, Prostate, Ovary, Breast, Pseudomyxoma peritonei, SCLC: 1 each)	
Prior therapy:	
Radiation only	8
Chemotherapy only	23
Both	21
Neither	3

Table 2 COS Dose escalation

COS dose (ng/m^3)	Patients (*n*)	Courses (*n*)
18	3	4
36	3	4
60	3	6
90	3	4
216	3	4
270	3	4
338	3	3
439	4	14
1,000	8	15
1,360	3	6
1,600	7	10
2,000	2	2
2,600	7	20
3,700	5	9
Totals	57	105

initially treated at 3,700 mg/m^2 and was also the only patient to have experienced grade 4 leukopenia. Subsequently, doses were given to this patient at the 2,600-mg/m^2 level.

Toxicity

Table 3 summarizes the toxicity observed during the trial. The hematologic toxicity was mild. Grade 3 thrombocytopenia was seen in only one patient at a CQS dose of 3,700 mg/m^2. The same patient, described above as a responder, developed grade 4 leukopenia during treatment with the 3,700-mg/m^2 dose, which recurred as grade 2 leukopenia when the dose was lowered to 2,600 mg/m^2. At this dose, an additional two patients had grade 2 leukopenia and two patients had grade 1 leukopenia. One patient each had grade 2 leukopenia at doses of 1,000 and 2,000 mg/m^2.

The nonhematologic toxicities are also listed in Table 3. In retrospect, many of these toxicities may have been secondary to hypoglycemia. Hypoglycemia was dose-limiting in this trial. The degree of hypoglycemia was documented only at CQS doses of $\geq$1,000 mg/m^2, as frequent serum glucose sampling was not performed at lower doses. Nadir serum glucose concentrations generally occurred within the first 4 h after the CQS infusion, and in the majority of patients the nadir serum glucose concentration occurred within 2 h of the end of the CQS infusion (Table 4). When plasma glucose concentrations below 50 mg/dl (fingerstick determination) occurred, as they did in ten patients, and administration of D50W was required, patients recovered to near-normal glucose concentrations by 24 h. However, patients required supplemental D10W given at 125 ml/h in addition to bolus D50W to maintain near-normal plasma glucose concentrations (Table 4). Grade 1 or 2 hypoglycemia (plasma glucose, 40–64 mg/dl) was observed in 15 of 27 assessable courses of CQS at or above doses of 1,000 mg/m^2, and grade 3 hypoglycemia was noted in one patient at the 3,700-mg/m^2 dose. Seven patients had nadir plasma glucose concentrations of $\leq$ 50 mg/dl. Recovery of plasma glucose concentrations occurred in all patients by 24 h. In patients whose glucose nadir was $>$ 50 mg/dl, recovery to pretreatment plasma glucose concentrations took place within 8 h except in two patients, both of whom remained asymptomatic. However, the plasma insulin concentration was elevated disproportionately relative to the plasma glucose concentration in several patients (Table 4).

Four patients who received CQS at 3,700 mg/m^2, two patients who received CQS at 2,000 mg/m^2, and one patient who received CQS at 1,000 mg/m^2 had grade 1 cardiac sinus tachyarrhythmias. One additional patient, who received CQS at a dose of 1,000 mg/m^2, had a grade 3 sinus tachyarrhythmia. However, this patient had a ventilatory-perfusion nuclear scan consistent with a high probability for pulmonary embolus. The tachyarrhythmias were associated with hypoglycemia in three cases and with a probable preexisting condition in one patient. No patient who received CQS at a dose of $<$1,000 mg/m^2 had cardiac tachyarrthymia. One possible and one likely pulmonary embolus were noted in two patients with advanced disease who received CQS at a dose of 1,000 mg/m^2. In addition, phlebitis at the site of infusion was noted in two patients: one receiving a CQS dose of 1,600 mg/m^2 and one treated at a dose of 3,700 mg/m^2.

Table 3 Toxicities associated with administration of COS

Dose (mg/m^2)	Patients/courses (n)	Number of events	
		Grade 1–2	Grade 3–4
18	3/4	fever (3)	
36	3/4	Anemia (1)	0
		Fever (1)	
60	3/6	0	0
90	3/4	0	0
216	3/4	Anemia (2)	0
		Dry mouth (1)	
		Lightheadedness (1)	
270	3/4	Fever (1)	0
338	3/3	Diarrhea (1)	0
		Fever (2)	
439	4/14	Azotemia (2)	0
1,000	8/15	Anemia (2)	Arrhyfthmia
		Arrhythmia (1)	
		Fever (2)	
		Grade 2 hypoglycemia (1)	
		Grade 1 hypoglycemia (4)	
		Hypokalemia (1)	
		Lightheadedness (1)	
		Grade 2 leukopenia (1)	
1,360	3/6	Dyspnea (1)	0
		Lightheadedness (1)	
		Pain (1)	
1,600	7/10	Anemia (2)	0
		Dry mouth (1)	
		Fever (3)	
		Grade 2 hypoglycemia (4)	
		Grade 1 hypoglycemia (1)	
		Hypertension (1)	
		Hypotension (1)	
		Infection (1)	
		Irritability (1)	
		Libido (1)	
		Lightheadedness (1)	
		Mucositis (1)	
		Pain (2)	
2,000	2/2	Arrhythmia (2)	0
		Grade 2 hypoglycemia (2)	
		Grade 2 leukopenia (1)	
		Rash (2)	
2,600	7/20	Azotemia (1)	Azotemia (1)
		Diarrhea (1)	
		Grade 2 hypoglycemia (1)	
		Lightheadedness (1)	
		Rash (1)	
3,700	5/9	Anemia (2)	Hypoglycemia (1)
		Arrhythmia (4)	
		Diarrhea (1)	Thrombocytopenia (1)
		Hives (1)	
		Grade 2 hypoglycemia (4)	
		Grade 1 hypoglycemia (1)	Leukopenia (1)
		hyperglycemia (1)	
		Hypokalemia (3)	Abdominal cramp (1)
		Hypotension (1)	
		Infection (3)	
		Grade 2 leukopenia (2)	
		Grade 1 leukopenia (2)	
		Lightheadedness (2)	
		Milky urine (1)	
		Mucositis (2)	
		Myalgia (1)	
		Phlebitis (1)	
		Pruritis (1)	
		Rash (2)	
		Diaphoresis (3)	

Table 4 Hypoglycemia associated with CQS

Patient	Dose (mg/m^2)	Nadir glucose (mg/dl)	% Decrease glucose	Nadir time (h)[a]	Recovery (h)[a,b]	Insulin (unit/ml)[c]	Peak [CQS], mg/l (total/fre)	Supplemental glucose[d]
1	3,700 (course 1)	46	70	1.25	5.5	36	472/38	Yes
	2,600 (course 2)	50	54	1.25	5	78	371/24	Yes
2	3,700 (course 1)	94	58	13	19	75	574/14	No
	2,600 (course 2)	113	38	2	3	92	392/–	No
3	3,700	29	81	3.5	13	164	435/29	Yes
4	3,700 (course 1)	155	54	19	24	48	359/21	No
	(course 2)	93	35	13	24	–	541	No
5	2,600	51	55	1.25	3	42	230/12	Yes
6	2,600	54	58	1.3	5	407	405/11	Yes
7	2,000	44	61	1.25	5	63	331/23	Yes
8	2,000	49	59	1.08	7	135	364/14	Yes
9	1,600	68	46	3	7	700	515/36	No
10	1,600	44	59	1.5	5	700	284/6.5	Yes
11	1,600	63	61	1.5	3	–	319/9.5	No
12	1,600[e]	56	20	2	9	52	132/9	Yes
13	1,600[e]	61	55	1.5	5	300	234/–	No
14	1,600[e] (course 1)	50	48	1.2	5	363	21/18	Yes
	1,200[e] (course 2)	63	32	1.5	2	100	207/	No
15	1,600[e]	115	26	2	3	–	285/7	No
16	1,000[e]	66	10	7	9	151	153/3	No
17	1,000[e]	63	30	1.25	2	166	192/0.11	No
18	1,000[e]	66	38	2	3	64	265/4.5	No
19	1,000[e]	84	24	2	3	21	116/2	No
20	1,000[e]	99	31	0.5	1	–	197/4	No
21	1,000[e]	64	31	5	7	24	153/3	No
22	1,000[e]	83	43	13	19	7	170/3	No
23	1,000[e]	91	12.5	1.25	1.5	50	175/0.7	No

[a]Hours after the start of CQS infusion
[b]Recovery to 80 mg/dl or greater
[c]Associated with glucose nadir
[d]Bolus D50W + D10W drip to ~ 18–20 h
[e]D5W 100 cc/h6 h concurrent with the start of CQS administration

Infectious toxicities were uncommon. One patient, described above as a responder, who received CQS at doses of 3,700 and 2,600 mg/m^2, developed grade 4 leukopenia, thrombocytopenia, mucositis, tracheitis, a rectal lesion, and oral thrush. A second patient, who received CQS at a dose of 1,600 mg/m^2, developed a grade 2 infection consisting of the herpetic lip lesion. At the 3,700-mg/m^2 dose, two patients developed turbid, orange urine during the period immediately after the CQS infusion. This was found to be due to precipitation of large amounts of CQS in the urine of these patients.

Grade 1–2 sporadic toxicities occurred throughout the dose range and included: chest pain (one patient each at CQS doses of 18 and 60 mg/m^2); dysesthesias (one patient each at CQS doses of 36 and 2,000 mg/m^2); fatigue (one patient each at CQS doses of 36, 1,000 and 2,000 mg/m^2 and two patients each at CQS doses of 216, 1,600 and 3,700 mg/m^2); emesis (one patient each at CQS doses of 36, 216, 1,600, and 3,700 mg/m^2); nausea (one patient each at CQS doses of 36, 1,000, 1,600, 2,000, 2,600, and 3,700 mg/m^2); abdominal cramping (one patient each at CQS doses of 60, 1,000 and 3,700 mg/m^2); alopecia (one patient each at CQS doses of 1,360 and 3,700 mg/m^2); flushing (one patient at a CQS dose of 1,000 mg/m^2 and two patients each at CQS doses of 2,000 and 3,700 mg/m^2); constipation (one patient at a CQS dose of 216 mg/m^2); change in taste (one patient each at CQS doses of 36 and 3,700 mg/m^2); hypochloremia (one patient at a CQS dose of 439 mg/m^2); hyponatremia and hypophosphatemia (one patient each at a CQS dose of 3,700 mg/m^2); headache (one patient each at CQS doses of 1,600 and 2,000 mg/m^2 and two patients at a CQS dose of 3,700 mg/m^2); elevations in liver enzymes (three patients at a CQS dose of 1,000 mg/m^2 and one patient each at CQS doses of 1,360, 1,600 and 3,700 mg/m^2); anorexia (two patients each at CQS doses of 1,000 and 2,600 mg/m^2 and one patient each at CQS doses of 1,360, 1,600 and 2,000 mg/m^2); and weakness (one patient each at CQS doses of 1,000, 1,360, and 1,600 mg/m^2). Dry or red eyes were noted in three patients (one patient receiving CQS dose of 1,360 mg/m^2 and two patients treated at CQS doses of 1,600 mg/m^2).

Grade 3 and 4 toxicities not described above all occurred at or above the 1,000-mg/m^2 dose and included one patient each with abdominal pain, elevated creatinine clearance, and hypoglycemia.

Grade 1–2 fever was observed in 12 of 57 patients throughout the dose range. The fever developed within 8 h of CQS infusion and cleared by 24 h after the infusion. However, fever was not observed at CQS doses of $\geq 2{,}000\ mg/m^2$. Grade 1–2 dyspnea was observed in six patients at CQS doses of $\geq 1{,}000\ mg/m^2$.

Pharmacokinetics

Figure 1 depicts the peak total plasma CQS concentration as a function of the CQS dose. The AUC also increased with dose (data not shown). There was a suggestion of a plateau of peak total plasma CQS concentration with increasing dose. Tables 5 and 6 give the mean values and ranges of pharmacokinetic parameters obtained for total and non-protein-bound CQS, respectively. Plasma CQS concentrations declined in a triexponential fashion. The terminal half-life of total CQS ranged from 28 to 206 h. CLTB ranged between 44 and 881 ml/h, with no evidence of saturation. In a number of patients, there was a rebound in CQS plasma concentrations at between approximately 18 and 24 h, raising the possibility of enterohepatic circulation [4].

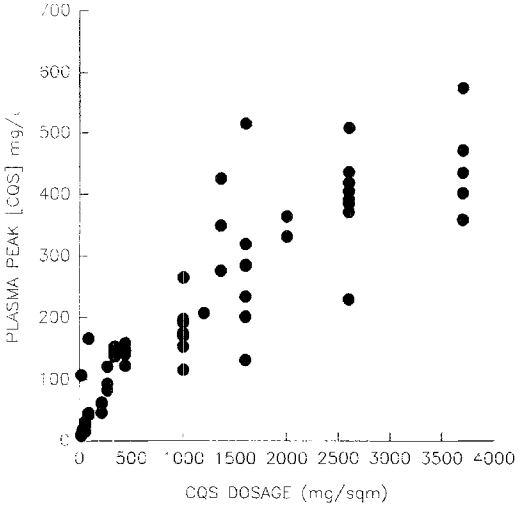

Fig. 1 Relationship of the CQS dose to the peak plasma CQS concentration

Intrapatient variability in CQS pharmacokinetics was investigated in a limited number of patients, as can be seen in Table 4. Only one patient (who received CQS at a dose of $3{,}700\ mg/m^2$) had repeat pharmacokinetic assessments in more than one course at the same dose. This patient had peak CQS plasma concentrations of 359 and 541 mg/l and CQS AUCs of 12,515 and 23,669 mg/l^{-1} h in courses 1 and 2, respectively. Three patients had repeat pharmacokinetic assessments after a dose reduction. In two of these patients, who received an initial CQS dose of $3{,}700\ mg/m^2$ and a subsequent 30% dose reduction to 2,600 mg/m2, pharmacokinetic parameters were lower during the second course by 32% and 21% (CQS peak concentration) and by 22% and 38% (CQS AUC). The third patient, who had a CQS dose reduction from 1,600 and $1{,}200\ mg/m^2$ for course 2, had similar peak concentration and AUC values for both courses. The percentage of decrease in plasma glucose concentration corresponded to the decrease in peak CQS concentration for two of these three patients.

Non-protein-bound CQS was measured in all patients treated at or above the $1{,}000\text{-}mg/m^2$ dose. There was no definite increase in the peak plasma non-protein- bound CQS concentration or AUC with increasing dose, and wide interpatient variability was seen. Non-protein-bound CQS represented 1.6%–17% of the peak total CQS concentrations in plasma. Between 1% and 5% of the CQS was excreted in urine as the parent compound during the initial 24-h period after drug administration.

Three possible metabolites of CQS were observed. The retention times of these metabolities were 2.5, 3.7,

Table 5 Mean (range) pharmacokinetic parameters of total COS

DOS age (mg/m)	*n*	Peak [COS] (mg/l)	$t_{1/2}\ \alpha$ (h)	$t_{1/2}\ \beta$ (h)	$t_{1/2}\ \gamma$ (h)	AUC ($mg\,l^{-1}$ h)	CLTB (ml/h)
18	3	9 (8.2–10.7)	1.2 (0.5–1.7)	5.3 (2.7–8)	94 (76–108)	277 (146–384)	142 (74–248)
36	3	16 (14–19)	1.1 (0.01–2.3)	31 (1.5–86)	68 (61–174)	479 (378–583)	160 (124–213)
60	3	24 (15–31)	0.8 (0.01–1.2)	4 (1.5–8.2)	72 (53–120)	917 (357–1,585)	158 (73–267)
90	3	84 (41–166)	1.3 (0.1–2.3)	3.6 (1.8–5.9)	50 (46–56)	1,165 (1,061–1,245)	147 (138–161)
216	3	56 (46–63)	0.8 (0.6–0.9)	12 (5–24)	127 (72–206)	5,670 (3,748–8,154)	84 (44–123)
270	3	99 (82–121)	0.5 (0.3–0.7)	2.5 (2.1–2.8)	52 (47–54)	2,608 (2,501–2,710)	156 (147–169)
338	3	145 (138–153)	0.5 (0.1–0.8)	3.5 (2.6–4.2)	52 (47–55)	4,237 (3,392–5,477)	147 (125–169)
439	4	142 (122–158)	0.5 (0.2–0.9)	7 (2–16)	73 (49–95)	5,208 (4,059–7,750)	140 (95–188)
1,000	8	178 (116–265)	0.1 (0.01–0.17)	5 (1.9–8.9)	54 (38–81)	6,692 (5,033–9,525)	299 (166–391)
1,360	3	350 (276–425)	0.5 (0.03–1.5)	1.6 (0.04–3.4)	47 (42–51)	12,659 (11,019–14,894)	189 (148–227)
1,600	6	281 (132–319)	0.2 (0.04–0.4)	8 (3–18)	73 (31–121)	8,421 (4,321–11,207)	360 (217–570)
2,000	2	348 (331;364)	0.1 (0.07;0.21)	4.8 (3.1;6.4)	30.5 (28;33)	8,071 (6,412;9,730)	447 (395;499)
2,600	6	394 (230–508)	0.5 (0.01–2.4)	13 (5–30)	56 (35–87)	13,357 (5,903–21,775)	413 (211–881)
3,700	5	448 (359–574)	0.7 (0.1–3)	19 (2–45)	52 (28–94)	20,026 (12,515–35,052)	386 (186–597)

Table 6 Mean (range) pharmacokinetic parameters of non-protein-bound (free) CQS

DOSE (mg/m^2)	n	Peak [CQS] (mg/l)	AUC ($mg\,l^{-1}\,h$)	CLTB (l/h)
1,000	8	2.5 (0.7–4.5)	61 (6–309)	79 (6–367)
1,360	3	40 (16–44)	145 (62–285)	25 (8–40)
1,600	7	14 (6–36)	88 (9–478)	91 (4–349)
2,000	2	18 (14;23)	45 (31;59)	124 (54;124)
2,600	7	30 (11–86)	163 (19–605)	68 (8–269)
3,700	5	30 (14–46)	581 (47–2,111)	53 (3–161)

and 5.6 min, respectively. None of these materials co-chromatographed with *N*-acetyl-CQS (unpublished data).

Pharmacokinetic-pharmacodynamic relationships

Hypoglycemia

Figure 2 depicts the relationship between the percentage of decrease in plasma glucose concentrations, defined as

$$(Predose[glucose] - nadir[glucose]/predose[glucose]) \times 100\,,$$

and the peak plasma total [CQS]. These data were fit to the Hill equation model, i.e.,

$$Effect = (Emax)(peak)^H/(peak_{50})^H + (peak)^H\,,$$

with the ADAPT program [2]. In this model, *Emax* is fixed at the maximal possible decrease in [glucose] = 100%, $peak_{50}$ represents the peak plasma total [CQS] at which the effect is 50% of Emax, and *H* is the Hill constant, a measure of the "sigmoidicity" of the relationship. The value of $Peak_{50}$ for our population was 326 mg/l, and the value of H was 1.06. The relationship fit the data relatively precisely with an MAE of 10.42 and was unbiased, as reflected in the ME of − 0.06. The CQS AUC did not correlate well with the magnitude of hypoglycemia (data not shown). Changes in plasma insulin concentration likewise did not correlate well with the peak CQS concentration.

The magnitude of change in plasma insulin concentration, i.e.,

$$(Maximal[insulin] - baseline[insulin]/baseline[insulin]) \times 100\,,$$

did not correlate with the percentage of change in plasma glucose (Fig. 3).

Myelosuppression

We evaluated the relationships between the percentage of decrease in WBC and the CQS dose, peak concentration, and AUC (data not shown). There was no relationship between the percentage of decrease in WBC and the CQS peak concentration. At least a 30% reduction in WBC was noted in each of the patients treated at CQS doses of 3,700 mg/m^2. There may have been a trend toward an association of the percentage of decrease in WBC and the CQS AUC, but due to the small number of patients with higher CQS AUCs, definite conclusions cannot be drawn. Of seven patients who experienced leukopenia of grade ≥ 2, four experienced only very brief episodes, which occurred on days 1–3 after treatment and resolved by day 7.

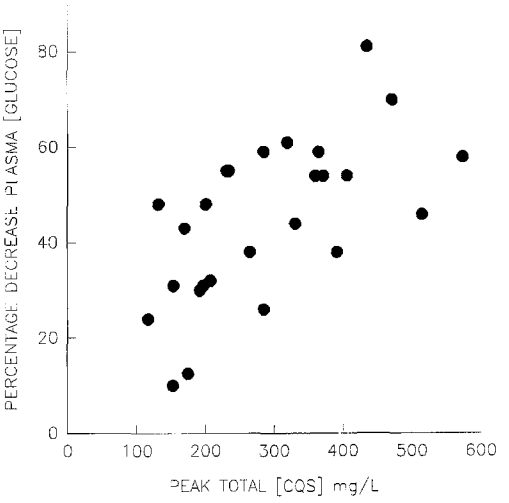

Fig. 2 Relationship of the peak plasma total (protein-bound and non-protein-bound) CQS concentration to the percentage of decrease in plasma glucose (pretreatment [glucose] − nadir [glucose]/pretreatment [glucose]) × 100

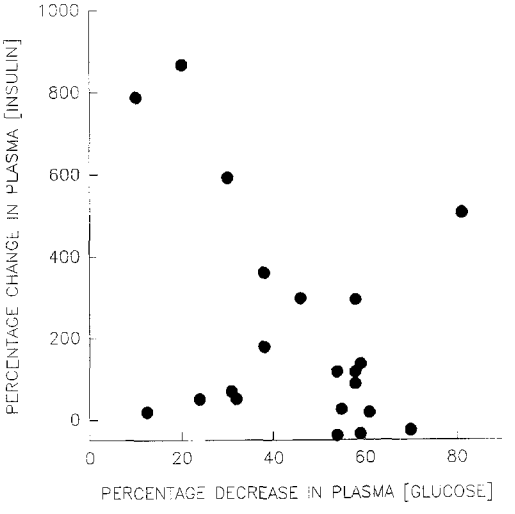

Fig. 3 Relationship of the percentage of decrease in plasma [glucose] to the percentage of change in plasma [insulin]

Nutritional analyses

Dietary data were obtained for 12 patients prior to at least 1 course of CQS and in 8 patients prior to 2 courses of CQS. No relationship between the calorie intake for the 3 days prior to CQS administration, expressed as a percentage of the calculated caloric need, and the incidence or severity of hypoglycemia was detected. The patients took in a median of 78% of the needed calories (range, 33%–270%). Most patients remained within 5% of the baseline weight. Only one patient lost >10% of body weight while on study. The percentage of ideal body weight maintained by these patients ranged between 67% and 152% (median, 98%). It was not possible to discern a relationship between either the percentage of weight lost or the ideal body weight and the incidence or severity of hypoglycemia.

Discussion

On the schedule used in the current study, the dose-limiting toxicity of CQS was hypoglycemia. The aim of our study was to define a CQS dose that would require neither inpatient monitoring nor intensive monitoring of plasma glucose concentration. An acceptable glucose nadir was felt to be approximately 50mg/dl. This corresponds to a grade 2 hypoglycemia according to the toxicity criteria employed (grade 2 = plasma glucose concentration of 40–54 mg/dl). By these criteria, the maximum tolerable dose was 1,600 mg/m^2. The only dose at which it was possible to give CQS to all patients in the cohort without supplemental D50W bolus and D10W drip was the 1,000-mg/m^2 dose. This conclusion is based on careful monitoring of glucose during the CQS infusion.

Our maximum tolerable dose differs from that defined in the study by Rigas et al. [7]. That group found a maximum tolerable dose of approximately 4,000 mg/m^2 on the same administration schedule. However, eight of nine patients receiving CQS doses of $\geq$4,000 mg/m^2 had plasma glucose concentrations of $\leq$ 40 mg/dl, which would not have been acceptable toxicity by our criteria. In addition, several patients required supplemental boluses of glucose followed by 24–28 h of glucose infusion as treatment for this hypoglycemia. Rigas et al. do not give specific information on the incidence or severity of hypoglycemia in patients treated at lower doses of CQS. In addition to the above mentioned points, the difference in magnitude of the maximum tolerable dose in our patients as compared with those treated by Rigas et al. may have several explanations. Differences in baseline nutrition between the two patient cohorts may be a factor. However, the performance status of our patient population was similar to that in the Rigas study, and the nutritional intake as assessed by dietary history for the 3 days prior to drug administration, the percentage of recent weight change, and the percentage of ideal body weight did not correlate with the incidence or severity of hypoglycemia. Only a subset of our patients were studied with respect to caloric intake. More of an influence of the baseline nutritional status upon hypoglycemia after CQS administration might have been detected by expanding the data collection across the entire dose range.

Above CQS doses of 2,000 mg/m^2, the correlation between CQS peak concentration and CQS dose appears to be nonlinear. Nonlinear equations fitted to this data appear to define this relationship marginally better than linear equations, with r^2 values being 0.82 and 0.78, respectively. Closer inspection of the data reveals that this nonlinearity occurs at higher CQS doses on and that both linear and nonlinear equations fit the data equally well between CQS doses of 0 and 2,000 mg/m^2. The reasons for a less than linear increase in peak CQS concentration with increasing dose could include more rapid drug clearance (such as drug-induced clearance) or could include such physical reasons as precipitation or drug adhering to administration materials. In this study, CQS was noted to precipitate in urine at the 3,700-mg/m^2 dose, and some drug may have precipitated with protein during preparation for plasma assay. This nonlinearity above CQS doses of 2,000 mg/m^2 may at least partially explain why some patients did not have severe hypoglycemia even at high doses of CQS (Tables 3, 4) and may perhaps explain why Rigas et al. [7] were capable of giving higher doses of CQS. Interpatient variability would allow some patients to achieve CQS peak concentrations in the range where only a 50% decrease in glucose concentration would be expected. Thus, the glucose nadir may not be in the hypoglycemic range if the patient's initial serum glucose concentration was high.

Although hypoglycemia was first documented at the 3,700-mg/m^2 dose, in retrospect, many patients had symptoms that were consistent with a hypoglycemic state. These symptoms included abdominal pain; changes in blood pressure; blurred vision; diaphoresis; dry mouth; cardiac tachyarrhythmias; electrolyte disturbances, particularly hypokalemia [6] ; headache; lightheadedness; mood changes; nausea; and vomiting. Most of these toxicities occurred at CQS doses of $\geq$1,000 mg/m^2.

As expected from the time of onset of hypoglycemia, the percentage of decrease in plasma glucose concentration correlated with the peak plasma CQS concentration. We were incapable of ameliorating the hypoglycemia associated with doses of > 1,000 mg/m^2 by concomitant administration of an infusion of D5W. Based on the relationship of our data as fitted with the Hill equation, a decrease of 50% from the initial plasma glucose concentration would be expected at a peak plasma CQS concentration of 326 mg/l. This

peak concentration would likely be reached at a dose of between 1,000 and 1,360 mg/m^2 (Table 5). However, interpatient variability is expected to be at least 20% and, therefore, some patients may well tolerate more CQS and some may tolerate less. If the CQS concentration achieved were critical, a dose-individualization algorithm might be helpful in attaining the desired peak concentration. Our limited intrapatient data indicate that dose reduction correlated with a lower peak plasma CQS concentration and a lower percentage of decrease in plasma glucose concentration after CQS administration. Because the dose-limiting toxicity of CQS is associated with the peak concentration, weekly repeated administration of doses not associated with hypoglycemia would achieve a higher AUC overall and might achieve higher activity. In vitro studies noted antitumor activity of CQS at concentrations of 10 mg/l.

Our data indicate that approximately 10% of the total CQS plasma concentration is not protein-bound. CQS concentrations remain above 100 mg/l for approximately 24 h after a 1,000-mg/m^2 dose and, thus, non-protein-bound CQS concentrations in plasma remain above 10 mg/l for about 24 h. More frequent administration of CQS at intervals approximating the terminal half-life may enable the achievement of relatively constant plasma CQS concentrations at about the effective concentration in vitro. A gamma half-life of between 28 and 206 h was observed in our study. Repeated dosing of CQS at weekly intervals may result in a slow accumulation of the plasma CQS concentration over time. This would not likely lead to severe hypoglycemic toxicity if the CQS concentration remained under 326 mg/l. However, if continued, repeated administration could lead to transient hypoglycemia on the day of administration. As shown in Table 4, only mild hypoglycemia was observed at the 1,000-mg/m^2 dose, and most patients (seven of eight) recovered from this toxicity within 9 h of the start of the CQS infusion. The remaining patient did not have significant hypoglycemia. Therefore, it would be safe to give a CQS dose of 1,000 mg/m^2 in an outpatient setting with concomitant D5W drip.

The mechanism of action of the hypoglycemia was suggested by the finding of high insulin concentrations in plasma while plasma glucose concentrations were below normal. Sulfonylureas, which are structurally similar to CQS and other sulfonamides, are oral hypoglycemic agents that cause insulin release from pancreatic islet cells. Insulin release, as well as the action of sulfonylureas, appears to be dependent on binding of the agent to cell-surface receptors on the pancreatic beta cell [5, 9]. This binding results in reduced conductance of an ATP-sensitive K^+ channel, which results in calcium influx and insulin secretion [5, 9]. Presumably, there is enough of a structural relationship between CQS and sulfonylureas that CQS, at high doses, can act similarly to sulfonylureas. Recent work has shown that sulfonylureas can block ingress of CQS into L1210 leukemia cells as well [3]. In addition, cardiac cells have been shown to have specific binding sites for sulfonylureas [9]. We, as well as other investigators [7] have noted cardiac tachyarrhythmias in patients receiving CQS. This toxicity was only grade 1 in the majority of our patients. Whether these dysrhythmias are secondary to the hypoglycemia itself or are induced by CQS cannot be discerned from our data.

It is not surprising that the magnitude of glucose decrease observed in our trial does not have a relationship to the magnitude of insulin increase observed (Fig. 3). We did not control oral intake on this trial, and the plasma concentrations of insulin were measured infrequently as compared with the frequency of measurements of plasma glucose concentrations. These data were obtained in patients without a history of diabetes mellitus. Extrapolation to patients with diabetes mellitus or abnormalities in glucose homeostasis cannot be made.

We observed one response in a generally nonresponsive tumor, even when doses were decreased for toxicity. It is interesting that this patient was the only one who had severe myelosuppressive toxicity. Due to the limited sample size, we cannot comment further on any possible relationship between myelotoxicity and response. However, this patient also experienced excessive toxicity when treated with 5-fluorouracil after removal from the CQS study.

CQS represents an interesting compound that was brought into trial specifically because it showed activity in vitro against solid-tumor cells that was much greater than its activity against leukemia cells. The toxicity of this compound has been difficult to predict, with the exception of hypoglycemia. However, our data imply that hypoglycemia can be predicted if the peak concentration is known. Phase II trials is appropriate tumors may be more appropriately done using doses of 1,000 mg/m^2 every 5–7 days rather than a monthly infusion.

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