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ORIGINAL ARTICLE

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Phase II trial of chloroquinoxaline sulfonamide (CQS) in patients with stage III and IV non-small-cell lung cancer

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Abstract *Purpose*: Chloroquinoxaline sulfonamide (CQS) was one of the first agents identified by the human tumor colony-forming assay (HTCFA) as possessing antitumor activity in non-small-cell lung cancer (NSCLC). Prior phase I studies had suggested that plasma concentrations equivalent to those showing efficacy in the HTCFA could be reliably attained in humans. This phase II study assessed the antitumor activity of CQS while using an adaptive control pharmacokinetic modelling system to attain targeted plasma levels of this novel compound. *Methods*: A group of 20 patients with stage III or IV NSCLC received CQS as a 1-h weekly infusion at an initial dose of 2 g/m^2. In all patients, 24-h plasma concentrations of CQS were measured. Patients with levels <100 μg/ml had dose increases determined by their 24-h levels and pharmacokinetic parameters obtained from two prior phase I trials of this agent. These individuals had 24-h CQS levels repeated after their second weeks' treatment and doses were readjusted if the target concentration was not reached. Antitumor response assessment was made every 6 weeks. *Results*: Of the 20 patients, 18 attained the target plasma concentration, and 16 of these achieved this initially or with just one dose adjustment. No major objective antitumor responses were observed (major response rate 0%, 95% CI 0–17%). CQS was well tolerated with hypoglycemia being the most clinically significant toxicity. *Conclusions*: When given on this schedule CQS is inactive in NSCLC despite the fact that the target concentration was achieved in 90% of patients. The ability of the HTCFA to identify active agents remains unproved.

Key words CQS · Phase II trial · NSCLC · HTCFA

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Introduction

Chloroquinoxaline sulfonamide (CQS, 4-amino-N[5(on 8)-chloro-2-quinoxanyl]), a halogenated sulfanilamide, was one of the first agents identified by the human tumor colony-forming assay (HTCFA) as possessing antitumor activity [11]. In this in vitro assay, "continuous" exposure to CQS at concentrations of >10 μg/ml was efficacious in human lung cancers [9]. In 1989, in our initial phase I trial, CQS was given at doses ranging from 18 to 4870 mg/m^2 every 4 weeks [9]. Even at the highest doses, dose-limiting hypoglycemia was mild, characteristically occurred within 4 h of administration and correlated with peak plasma concentrations of CQS of >500 μg/ml [9]. Given this, we sought to determine whether CQS dose intensity could be maintained or increased while minimizing hypoglycemia by dosing more frequently.

In our second phase I trial we gave CQS intravenously over 1 h once weekly [10]. The median 24-h plasma CQS concentration following a 2 g/m^2 dose was >100 μg/ml, no hypoglycemia more than grade 2 was seen, and greater dose intensity was achieved [10]. At higher doses, more frequent hypoglycemia resulted [10].

Thus, a dose of 2 g/m^2 given weekly was chosen for further study. With the specific activity against human lung cancer observed in vitro, and our ability to deliver CQS safely at doses that resulted in concentrations comparable to those proven effective in the HTCFA, we undertook this phase II trial in patients with previously untreated, advanced non-small cell lung cancer (NSCLC).

Methods

Patient population

From October 1993 to August 1994, 21 patients with pathologically confirmed NSCLC were enrolled in this trial. One patient was found to be ineligible because of renal insufficiency and was never treated. All had unresectable, clinical stage III or IV disease as defined by the current International Staging System [7]. All had a Karnofsky performance status of at least 60%. Patients were required to have either bidimensionally measurable or evaluable indicator lesions on physical examination, chest roentgenogram (CXR) or computed tomography (CT) scan [3, 4, 6]. Specifically excluded as indicator lesions were malignant effusions, bone metastases and previously irradiated lesions. Patients who had undergone previous surgery for their lung cancer which had recurred were eligible. No prior chemotherapy was allowed. Patients were not permitted to have received radiation therapy to major bone marrow areas within 3 weeks prior to study entry. Patients with a history of hemolytic anemia, insulin-dependent diabetes mellitus, sulfa allergy or recent sulfa use were excluded. Individuals with untreated or clinically evident brain metastases, unstable cardiac disease, or prior malignancy other than basal cell carcinoma of the skin were also excluded. Laboratory requirements for eligibility included leukocyte count $\geq$4000/ml, platelet count $\geq$160 000/ml, bilirubin $\leq$1.0 mg/dl, creatinine $\leq$1.5 mg/dl or creatinine clearance $\geq$50 ml/min per 1.73 m^2, and a normal level of glucose-6-phosphate dehydrogenase (G-6-PD). Written informed consent was obtained from all patients, and the protocol and consent were approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center.

Treatment plan

General

Before therapy, all patients had a complete history and physical examination, complete blood count with differential (CBC), glucose, liver and renal function tests, CXR and electrocardiogram (ECG). Additional scans were obtained only if clinically indicated.

Patients initially received CQS at a dose of 2 g/m^2 intravenously (IV), infused over 1 h, every week for 4 weeks followed by a 1-week rest period and subsequent disease reevaluation (every 6 weeks). CQS was administered in the Adult Day Hospital of our institution. Individuals who experienced disease stabilization continued to receive CQS until there was evidence of disease progression or unacceptable toxicity. CQS was supplied by the Division of Cancer Treatment of the National Cancer Institute as a yellow, lyophilized powder which was reconstituted with 9.2 ml sterile water to yield a 50 mg/ml concentration of drug with a pH of 9.2. Prior to infusion, CQS was further diluted in 250 ml 5% dextrose.

Blood glucose and CBC were checked prior to all treatments and ECGs were performed and reviewed weekly on treatment days during the first two cycles and on each reevaluation day thereafter. Patients had finger-stick measurements for blood glucose checked every 2 h for 6 h after CQS infusion for the first two treatment cycles and for any episodes clinically consistent with hypoglycemia. Patients found to have peripheral finger glucose <55 mg/dl (grade 2 hypoglycemia) with symptoms of hypoglycemia or <40 mg/dl (grade 3 hypoglycemia) without symptoms of hypoglycemia had an intravenous plasma or serum glucose drawn and received 50 ml (one ampule) of 50% glucose. If the serum or plasma glucose level was <55 mg/dl with symptoms or <40 mg/dl without symptoms of hypoglycemia, the individual was admitted to the hospital for intravenous glucose administration until the glucose was >55 mg/dl off intravenous glucose. Patients experiencing symptomatic grade 2 hypoglycemia or asymptomatic grade 3 hypoglycemia received a 25% dose reductions. Toxic effects were graded according to the Common Toxicity Criteria of the National Cancer Institute (USA).

Pharmacokinetic modelling

All patients had plasma CQS levels measured 24 h following the initial CQS infusion. Patients not encountering drug-related toxicity requiring dose modification following their first treatment and having plasma CQS concentrations <100 μg/ml received subsequent doses which were calculated to predict a target CQS concentration of 100–130 μg/ml at 24 h. The predicted dose for an individual was calculated using pharmacokinetic parameters established in our two phase 1 trials [9, 10] and the individual's 24-h plasma CQS concentration using the Abbottbase Pharmacokinetic System [1]. The specific parameters incorporated included: $t_{1/2}$ β 27 h, $t_{1/2}\gamma$ 98 h, CL_{tb} 134 ml/h · m^2, and Vd_{as} 8.2 l/m^2.

In those requiring dose increases, a CQS concentration was again measured following the next CQS infusion and if the plasma CQS concentration remained <100 μg/ml the CQS dose was again adjusted and the protocol repeated. There was no predetermined CQS dose above which additional increases were not to be made. Concentrations of CQS were analyzed by HPLC using the modified methods of Branda et al. [2]. This 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 [10]. Published response criteria were used [3, 4, 6].

Results

Patient characteristics

Of the 21 patients, 20 were entered and are included in the response and toxicity analysis. Nineteen patients completed one cycle of therapy. One patient died of disease progression during the first cycle before response could be formally assessed but was included in the analysis as a non-responder. Pretreatment characteristics of the patients are presented in Table 1.

Pharmacokinetic modelling

Of the 20 patients, 13 attained a 24-h CQS concentration of ≥100 μg/ml after their initial 2 g/m^2 dose (range 102–148 μg/ml, median 112 μg/ml). In the remaining seven patients, levels ranged from 63 to 93 μg/ml (median 79 μg/ml, mean 77 μg/ml). Of these, three patients attained target levels with one dose adjustment (range of dose increases 4.3–45.2%), one patient required two dose adjustments (dose increase 63%), and one patient required six dose adjustments (dose increase 41%). Two patients, both of whom were removed from study for

Table 1 Patient characteristics

Number of patients	20
Stage III-B	7
IV	13
Female:male	13:7
Age (years)	
Median	58
Range	27–68
Karnofsky performance status	
80–90%	17
70%	3
Weight loss ≥6%	4
Serum LDH >200	9
Bone metastases	5
Adenocarcinoma	19
Squamous cell carcinoma	1
Prior therapy	
None	12
Surgery	6
Radiotherapy	4

progression of disease after one cycle, failed to reach the desired 24-h level in four attempts.

Response and survival

No major objective responses were seen. The median survival for all patients was 7.2 months, and 45% of patients survived 1 year.

Toxicity

The 20 patients received 196 courses of CQS. The median number of doses of CQS was 12 (range 2–24). A summary of the maximum grades of toxicities for each patient is presented in Table 2. In all, CQS was well tolerated. Clinically relevant toxicity observed with CQS when given at this dose and on this schedule was essentially limited to hypoglycemia. Cardiac dysrhythmia consisted of asymptomatic premature atrial or ventricular contractions noted on the ECGs obtained weekly, prior to treatment, during the first two cycles. One patient appeared to develop a rash several days after receiving CQS on two separate occasions. Thereafter, she received pretreatment with corticosteroids, diphenhydramine, and cimetidine and experienced no such further reaction.

Table 2 Highest NCI toxicity grade values are percent of patients, (n = 20)

Toxicity	0	1	2	3	4
Anemia	25	40	35	0	0
Hypoglycemia[a]	55	20	15	5	5
Cardiac dysrhythmia	70	30	0	0	0
Headache	75	25	0	0	0
Abdominal pain	85	15	0	0	0
Thrombocytopenia	85	15	0	0	0
Neutropenia	90	5	5	0	0

[a]Serum glucose (mg/dl) grade 0 >64, grade 1 55–64, grade 2 40–54, grade 3 30–39, grade 4 <30

Discussion

In the mid-1980s NCI screens for identifying agents with antitumor activity were expanded from two in vivo murine leukemia models, L1210 and P388, to include the in vitro testing of fresh human tumors through selective cloning in primary culture [8]. A primary goal of this undertaking was to identify agents with activity in the more common solid tumors and the avoidance of the continued bias of preferentially selecting agents with activity in leukemias. Two agents from an initial screen of more than 300 were felt to be of sufficient interest to warrant clinical development [11]. Dihydrolenperone, a butyrophenone structurally similar to haloperidol, showed some evidence of selective cytotoxicity in lung cancer cell lines [5]. However, while dihydrolenperone was an active drug in the HTCFA at a concentration of 10 μg/ml, the achievable plasma concentrations in humans were 2 to 3 log orders lower and no antitumor activity was observed [5]. CQS, on the other hand, showed excellent colony inhibition in 6 of 11 common tumor types including 20 of 26 lung tumors when tested at a concentration of 10 μg/ml [9] and with continuous drug exposure, 10–21 days on average. When this free drug concentration from the HTCFA, which employs 10% fetal calf serum (FCS) as growth medium, was corrected for differences in protein binding it was estimated that a human plasma equivalent would be at least 100 μg/ml over a minimum of 24 h [12].

CQS was thus chosen for phase II study because of preclinical activity observed in NSCLC, a favorable toxicity profile, an ambulatory schedule of administration, and antitumor effects seen in two phase I trials at our institution. This study sought to assess whether the in vitro activity of CQS seen in the HTCFA could be confirmed in vivo. A secondary goal was to assess the value of employing an adaptive control pharmacokinetic model to individualize CQS dosing to achieve a plasma concentration of ≥100 μg/ml at 24 h.

While assessment of antitumor response was the primary endpoint, it was important to prospectively target optimal drug concentrations so that inadequate dosing would not be an issue in our attempt to confirm in patients the antitumor effects seen in the HTCFA. The hypothesis that clinically relevant concentrations were obtainable was confirmed in 90% of patients, 16 of whom attained the target plasma level on the initial or second treatment. There was no difference in survival between the 13 patients who attained the target plasma concentration on initial dosing and the 7 who required dose adjustments, although such analyses are hindered by small numbers of patients and the inherent flaws of subgroup analysis. Two patients failed to ever reach the desired 24-h CQS concentration. The first of these had an initial concentration of 70 μg/ml and despite a

subsequent dose increase of 56%, her 24-h level fell to 50 μg/ml after the third dose. This patient's course was complicated by recurrent pulmonary emboli requiring heparin. It is plausible that heparin may have accelerated CQS degradation or enhanced renal excretion. A protein binding effect of heparin is less likely since total CQS concentration was measured by our HPLC assay.

CQS is a well-tolerated novel agent with an unclear mechanism of action. Hypoglycemia was readily managed and cardiac arrhythmias were asymptomatic and probably more reflective of the comorbidities of the studied patients than inherent toxicity of CQS [10]. CQS does not appear to intercalate with DNA or induce alterations in folate metabolism; although in vitro inhibition of DNA replication and G0/G1 cell-cycle arrest have been noted [9]. Use of an adaptive control mechanism to attain target CQS concentrations was successful in all but two patients and no increased toxicity was noted as a result of dose increases. However, despite achievement of plasma CQS levels inhibitory against lung cancer in the HTCFA, CQS was not active against NSCLC in patients.

This trial marks the second time (dihydrolenperone being the first) where a drug identified by the HTCFA has been found not to be helpful clinically. One possible explanation for this discrepancy is that our trial assured relevant CQS concentrations for at least 24 h while exposure in the HTCFA was longer. This hypothesis is currently being tested in a phase I trial of CQS given as a continuous infusion over 7 days (S. Grunberg, personal communication). Nonetheless, the ability of the HTCFA to select effective therapies has yet to be established and its role in drug development remains investigational. Further study of CQS at this dose and schedule cannot be recommended; however, use of similar pharmacokinetic methods is feasible and can ensure that each patient treated on a phase II trial actually receives clinically relevant doses of the study drug. This is particularly important in trials of drugs that fail to show activity as it helps eliminate the concern that lack of activity was caused by inadequate drug delivery rather than an inherent lack of antitumor effect in patients.

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