Phase I pharmacodynamic study of time and sequence dependency of hydroxyurea in combination with gemcitabine: a California Cancer Consortium Trial

Abstract Preclinical studies in our laboratory have demonstrated that prior exposure to hydroxyurea increases the percentage of cells in S phase, enhancing the cytotoxicity of subsequent gemcitabine treatment in human oropharyngeal KB cells. To evaluate the clinical implications of this time- and sequence-dependent potentiation, we performed a phase I trial of hydroxyurea given over 24 h followed by a 30-min infusion of gemcitabine in weeks 1 and 2 of a 3-week cycle. The dose of hydroxyurea was fixed at 500 mg orally every 6 h for four doses starting 24 h before each dose of gemcitabine. The initial dose level of gemcitabine was 250 mg/m² on days 2 and 9, and this was escalated stepwise to 1000 mg/m² on days 2 and 9. Gemcitabine pharmacokinetics were determined on days 2 and 9 of the first cycle. Of 27 patients enrolled (12 female, 15 male), 24 were evaluable for response and 23 were evaluable for toxicity. Their median age was 56 years (range 27–76 years). Tumor types included lung, head and neck, pancreas, breast, colon, prostate, stomach, ovary, esophagus, germ cell, thyroid, gallbladder, and unknown primary. A total of 80 cycles of treatment were completed. One patient (unknown primary) had an objective partial response lasting 21 months, and 12 patients had stable disease. All observed dose-limiting toxicities were related to myelosuppression. The gemcitabine maximum tolerated dose was established at 750 mg/m² on days 2 and 9. Hydroxyurea had no effect on the plasma pharmacokinetics of gemcitabine. These results suggest that hydroxyurea followed by gemcitabine can be safely administered and has activity on this schedule. We are presently developing a phase II trial of this regimen for patients with platinum-resistant head and neck cancer.

Keywords Ribonucleotide reductase · Drug sequence · Gemcitabine · Hydroxyurea

Introduction

Gemcitabine (2,2'-difluorodeoxyctydine, dFdC) is a deoxyctydine (dC) analog that shows significant antitumor activity in solid tumors [1, 2]. For this prodrug to be cytotoxic, it must be converted to the triphosphate [3, 4]. Gemcitabine is phosphorylated by dC kinase to the monophosphate and further phosphorylated into the di- and triphosphates by other kinases intracellularly [4].
The incorporation of only one gemcitabine triphosphate (dFdCtP) molecule into the DNA strand can terminate DNA synthesis. After the active metabolite dFdCtP is added, only one more deoxynucleotide triphosphate molecule can be joined to the DNA strand before DNA elongation is prematurely terminated [5]. The proof-reading activity of DNA polymerase cannot correct this blockage, and the cell is arrested in S phase, leading to cell death by apoptosis [4, 5, 6, 7].

The competition between gemcitabine and dC limits phosphorylation of gemcitabine and its eventual incorporation into DNA [6]. However, gemcitabine potentiates its own incorporation by several mechanisms. Gemcitabine diphosphate decreases competition by lowering deoxynucleotide production through direct inhibition of ribonucleotide reductase, the enzyme that converts ribose nucleotides to deoxyribose nucleotides [7, 8, 9]. Also, dFdCtP in high concentrations can cause dCTP depletion by direct inhibition of CTP synthetase [9, 10, 11]. dCTP is a cofactor that determines the activity of dCMP deaminase, which deaminates both dCMP and gemcitabine monophosphate (dFdCMP) [12]. As a result of lowering dCTP levels and reducing the rate of deamination of dFdCMP, gemcitabine's intracellular half-life is greatly extended, increasing the likelihood of incorporation into DNA [13].

Gemcitabine has been used to treat solid tumors such as pancreatic cancer, small-cell lung cancer, advanced ovarian cancer, squamous cell carcinoma of the head and neck, bladder cancer, renal cell carcinoma, non-small-cell lung cancer, advanced gastric cancer, metastatic malignant melanoma, and advanced colorectal adenocarcinoma [14, 15, 16, 17]. The therapeutic activity of gemcitabine can be enhanced when it is combined with other anticancer drugs and it can also sensitize various human carcinoma cells in vitro to radiation even at noncytotoxic concentrations [18, 19, 20, 21, 22, 23, 24, 25, 26, 27]. Gemcitabine has been found to have synergistic effects with cisplatin if exposure to gemcitabine occurs 4 h before or after exposure to cisplatin [20]. This phenomenon is seen both in vivo and in vitro. While the mechanism is still unknown, high response rates have been observed with this combination in non-small-cell lung cancer and gemcitabine in combination with hydroxyurea has also been examined [19, 20, 21].

Hydroxyurea is an S phase-specific inhibitor of ribonucleotide reductase with a broad spectrum of preclinical and clinical antitumor activity [28, 29, 30]. As a standard first-line agent in chronic myelogenous leukemia [31], and as a radiosensitizer [32, 33, 34], large clinical experience has established the schedule-dependency of its biological effects. Antitumor effects and radio sensitization are greater with prolonged schedules of administration, which is characteristic of the majority of S phase-active agents. Ribonucleotide reductase is generally considered the “gate” between RNA and DNA precursor metabolism, because it is the only enzyme that converts ribonucleotides (normally present at two logs greater concentration than deoxyribonucleotides) to deoxyribonucleotides. As an inhibitor of ribonucleotide reductase, hydroxyurea depletes DNA precursors without affecting RNA and protein synthesis, causing G1/S cell cycle arrest and cytotoxicity via “unbalanced growth”. Lately, in vitro studies investigating the interaction between gemcitabine and hydroxyurea we have shown that the combination has an enhanced effect when gemcitabine is administered 8 h following hydroxyurea (unpublished data). The decrease in colony formation ability and incorporation of [3H]gemcitabine both peak when gemcitabine treatment is initiated after 8 h exposure to hydroxyurea. A decrease in RRM2 subunit mRNA, protein, and activity is noted after between 4 and 8 h of hydroxyurea exposure. The subsequent depletion of the dCTP pool allows increased incorporation of dFdCtP into DNA, resulting in higher levels of cytotoxicity than either treatment alone. This phase I study was conducted to validate the preclinical observations and results summarized here.

**Patients and methods**

**Patient selection**

Between February 1998 and October 1999, 27 patients were entered into this phase I trial. All patients had histologically verified advanced malignancies, unresponsive to previous chemotherapeutic regimens, or for which no “standard” chemotherapeutic regimen existed. Patients were required to have a Karnofsky performance status of ≥60%, age ≥18 years, an expected survival of at least 2 months, adequate renal function defined by serum creatinine ≤2.0 mg/dl or 24-h creatinine clearance of ≥50 ml/min, adequate bone marrow function defined by an absolute neutrophil count (ANC) ≥1200/dl and a platelet count ≥100,000/µl, and adequate hepatic function defined by a serum bilirubin ≤3.0 mg/dl with aspartate aminotransferase and alanine aminotransferase within five times the upper limit of normal. Prior radiation or chemotherapy must have been completed at least 4 weeks before beginning treatment on this protocol. There was no limit to the number of prior courses of chemotherapy. Pregnant female patients were excluded. All patients gave their voluntary informed consent and signed a consent document that had been reviewed and approved by the City of Hope National Medical Center Institutional Review Board. This trial was also approved by the Cancer Therapy Evaluation Program of the National Cancer Institute (NCI).

**Pretreatment evaluation**

All patients had a complete history and physical examination, including documentation of weight, Karnofsky performance status, evaluation for the presence of measurable or evaluable disease, baseline laboratory blood tests, chest radiograph, electrocardiogram, urinalysis, pregnancy test if indicated, and computed tomographic scans of the chest, abdomen, and pelvis as needed to document measurable or evaluable disease. Patients with measurable disease were required to have radiographic procedures for analysis of measurable disease repeated after two cycles of therapy.

**Treatment plan**

Hydroxyurea was administered orally at 500 mg every 6 h for 4 doses on days 1 and 8 of each cycle. Gemcitabine was administered as a 30-min infusion 6 h after the fourth dose of hydroxyurea (i.e., on days 2 and 9). Patients were required to have a platelet count