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Gemcitabine

Gemcitabine (2',2'-difluorodeoxy-cytidine, dFdC) is an analog of deoxycytidine. It is transformed to the active triphosphate (dFdCTP) after intracellular phosphorylation. Gemcitabine monophosphate is inserted into the DNA and inhibits DNA elongation as a false nucleotide. In contrast to other antimetabolites, an additional, altered nucleotide is inserted behind dFdC inhibiting repair mechanisms (masked chain termination).

By this, repair enzymes (exonucleases) of the DNA are inhibited and repair mechanisms are prevented. This factor, as well as enzymic inhibition of gemcitabine diphosphate, lead to high intracellular concentrations of gemcitabine and enforce the cytostatic effect. Gemcitabine is mostly inserted into DNA but partly also into RNA. Competition at the receptor with the nucleoside deoxycytidine phosphate (dCTP) leads to a competitive inhibition of DNA polymerases.[1]

The effects of gemcitabine on cellular metabolism therefore include:

- inhibition of ribonucleotide reductase with lowering/disruption of deoxynucleotide de nove synthesis (mostly dCTP);
- multiplication of effect of deoxycitidine kinase by inhibition of the negative feedback on this enzyme, leading to enhanced phosphorylation of gemcitabine;
- inhibition of the enzyme responsible for the elimination of gemcitabine (deoxycitidine monophosphate deaminase). By exhausting the dCTP pool and increasing the intracellular concentration of dFdCTP, the positive feedback mechanism of the enzyme is further inhibited;
- inhibition of cytidine triphosphate synthetase (CTP-synthetase) leading to a further exhaustion of the dCTP pool and inhibition of RNA synthesis.

Pharmacokinetics

After infusion of 1,000 mg/m^2 of gemcitabine over 30 min, maximal concentrations of 10-40 µg/mL are observed. The extracellular half-life is approximately 30 minutes. Gemcitabine is metabolized to the cytostatically inactive metabolite 2'desoxy-2',2'-difluorouridine (dFdU) at a rate of 91-98%. Metabolization occurs in liver, kidneys, blood and other tissues via cytidine deaminases. After infusion of 1,000 mg/m^2, 92-98% of the dose is recovered in urine within one week. The excretion of the original substance and dFdU via the urinary tract is 99% with less than 1% being eliminated in the feces.

Cytostatically active metabolites of gemcitabine are not detectable in plasma or urine. The plasma protein binding of gemcitabine is only 10%.

So far, gemcitabine has been found to demonstrate a broad spectrum of activity in solid tumors, including pancreatic, ovarian, breast, lung and bladder cancers. In hematopoietic malignancies, gemcitabine has shown a high level of activity as a single agent in relapsed or refractory Hodgkin's disease and some degree of efficacy in aggressive and indolent non-Hodgkin's lymphoma.

T-cell disorders

Concerning cutaneous T-cell lymphoma (CTCL) , we conducted a phase II trial in 44 consecutive, previously treated patients with mycosis fungoides (MF) (30 cases) and peripheral T-cell lymphoma unspecified (PTCLU) (14 cases) with exclusive skin involvement. Gemcitabine was given to all patients on days 1,8, and 15 of a 28-day schedule at a dose of 1,200 mg/m^2 for a total of three cycles. Of the 44 patients, five (11.5%) achieved complete responses, 26 (59%) partial responses, and the remaining 13 showed no benefit from the treatment. Two of the complete responses were histologically confirmed. The complete and partial response rates were the same far patients with MF and those with PTCLU, respectively. No difference in terms of overall response rate was observed between relapsed and refractory patients. The median durations of complete response and partial response were 15 months and 10 months, respectively. This report has confirmed our preliminary data on 13 patients,[2] who are also included here with a longer follow-up; in this study, gemcitabinetreated patients had a higher or at least comparable overall response rate compared with literature data on patients with MF treated with other nucleoside analogs, such as fludarabine and pentostatin.

Two other studies have shown good activity when using gemcitabine for the treatment of patients with refractory T-cell lymphoma.

Sallah *et al*.[3] reported their experience in 10 patients with refractory and relapsed T-cell malignancies treated with gemcitabine. Two patients had CTCL, 2 prolymphocytic leukemia (PLL), 2 nodal PTCL, 2 small lymphocytic lymphoma (SLL), 1 anaplastic and 1 angiocentric lymphoma. The drug dose was the conventional 1,200 mg/m^2 on days 1, 8 and 15 of each 28-day cycle. Of the 10 patients, two achieved a complete response (1 PLL and 1 anaplastic) and four a partial response (2 CTCL, 1 angiocentric, 1 PTCL) for an overall response rate of 60%. The median and mean duration of response was 13 and 16 months, respectively.

The second trial was conducted at the M.D. Anderson Cancer Center.[4] Thirty-three pretreated CTCL patients received gemcitabine at a lower dose of 1000 mg/m^2 for six or more cycle. Thirty-one patients had mycosis fungoides; the overall response rate was 68% including[3] complete response.

These findings show that gemcitabine has substantial activity and accetable toxicity in previously treated patients with mycosis fungoides and peripheral T-cell lymphoma. For these reasons we runned a phase IIb multicenter study[5] with gemcitabine as primary chemotherapy of patients with advanced CTCL (or pretreated only with PUVA or radiotherapy). The patients were recruited from the Italian Cutaneous Lymphoma Study Group.

Between June 2002 and February 2004, 32 patients with untreated MF, PTCLU and SS were treated with gemcitabine in 7 Italian institutions. Twenty-six of 32 patients had a diagnosis of MF, 5 were diagnosed with PTCLU, and only 1 patient had SS. All patients with MF were classified with T3 or

T4, N0, M0 disease using the TNM classification for T-cell lymphoma and patients with PTCLU were classified with stage IV disease according to the Ann Arbor staging system. The median age of the patients was 58 years (range 25-77 yrs); 22 patients were male and 10 were female. Of the 32 patients studied, 4 had been previously treated with local radiotherapy, 10 had received previous PUVA therapy, and 8 had been treated previously with PUVA and radiotherapy, whereas 10 patients had not received any previuos treatment.

Gemcitabine was given to all patients on days 1, 8, 15 of a 28-day schedule at a dose of 1200 mg/m^2 per day for a total of 6 cycles.

The overall response rate (CR+PR) was 75% (24 of 32 patients). The CR were 22% (7 of 32 patients) and 53% (17 of 32 patients), respectively. Patients with MF had a CR rate of 23% (6 of 26 patients) and a PR rate of 50% (13 of 26 patients). Conversely, patients with PTCLU had a CR rate of 20% (1 of 5 patients) and a PR rate of 80% (4 of 5 patients). The one patient with a diagnosis of SS had no response after therapy.

Of the 7 patients who achieved a CR, 3 were still in disease remission after a median follow-up of 10 months (range 4-22). The median PFS and OS were, respectively, 10 and 19 months.

Toxicity

Hematologic toxicities: anemia of WHO grade III was observed in 5-10% of patients, neutropenia of WHO grades III and IV in 20% and 10% of patients, respectively, WHO grade III and IV thrombocytopenia in 20% and 10% of patients, respectively.

Non-hematologic toxicity: transient elevations in liver transaminases were observed in 5-10% of patients. Renal and pulmonary toxicity was very rare; WHO grade III-IV less than 1%. Flu-like symptoms with headache, fever, myalgias and fatigue occurred in up to 10% of patients. No alopecia usually occurs during gemcitabine therapy. Neurotoxicity in connection with gemcitabine is rare. Peripheral edema occurs in 10% of patients. These toxicities were usually mild and reversible after the end of therapy.

In our phase IIb study Gemcitabine was well tolerated. With regard to hematologic toxicity, 37,5% had WHO grade 1-2 anemia and 3% had grade 3 anemia. Grade 3-4 thrombocytopenia and neutropenia were observed in12% and 16% respectively. With regard to non-hematological toxicity, the most significant was hepatic toxicity. Grade 1-2-3 hepatic toxicity was observed in 37% of the patients and grade 4 hepatic toxicity was reported in 3% of the patient. However, the hepatic injury was reversible in all cases.

Conclusions

These findings led us to conclude that gemcitabine has an high activity in untreated patients with CTCL.

Its modest toxicity profile and the easy schedule of administration make gemcitabine an ideal agent for consideration in the development of chemotherapy regimens. In particular, it would be interesting to evaluate the use of two different nucleoside analogs (fludarabine or pentostatin plus gemcitabine) in modulating the entry route into DNA and their action in terms of direct cytoxicity and apoptosis, respectively. Earlier investigations demonstrated the possibility of potentiating fludarabine with low doses of gemcitabine. In addition, combinations of gemcitabine with other compounds are under investigation. For example pralatrexate, a 10-deazaaminopterin derivative, is a novel antifolate designed to have high affinity for the reduced folate carrier type

1 (RFC-1).[6]

This high affinity of pralatrexate for RFC-1 significantly improves its internalization into cells. Preclinical and clinical studies have demonstrated that pralatrexate has significant activity against T-cell lymphomas, in particular preclinical studies in models of B-cell lymphomas and T-cell lymphomas have demonstrated that pralatrexate has more activity than traditional antifolates.

Toner *et al*.,[6] demonstrated a synergism between pralatrexate and gemcitabine in cell lines *in vitro* and in lymphoma xenografts and the strongest synergistic effect was noted when pralatrexate was administered prior to gemcitabine.

Combinations of pralatrexate and gemcitabine were further compared with the combination of methotrexate and cytosine arabinoside, pralatrexate and cytosine arabinoside, and methotrexate and gemcitabine. Pralatrexate and subsequent gemcitabine were determined to be the most cytotoxic combination. The combination of pralatrexate with gemcitabine is currently being explored in a phase I/II clinical trial.

At the time of publication, a phase I/IIa nonrandomized, open-label, multicenter clinical trial (sponsored by Allos Therapeutics Inc) is recruiting patients with relapsed or refractory NHL (expected 84 patients), to assess the combination of pralatrexate and gemcitabine.

During the phase I portion of the trial, 54 patients would receive weekly, intravenous doses of pralatrexate beginning at 15 mg/m^2, followed by gemcitabine the next day beginning at a dose of 400 mg/m^2 for 3 or 4 weeks, as well as vitamin B12 and folic acid supplementation. The primary endpoints would be determinations of the MTD, the recommended phase II clinical dose, safety and pharmacokinetics. In the phase II portion of the trial, an additional 30 patients would be treated, and the primary endpoints would be evaluated as tolerability and preliminary efficacy.

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