Radiosensitization by Gemcitabine

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Gemcitabine is a potent radiosensitizer in both laboratory studies and in the clinic. Initial laboratory studies showed that gemcitabine radiosensitizes a wide variety of rodent and human tumor cells in culture. Maximum

Introduction

Gemcitabine (2’β,2’-difluorodeoxycytidine) is an analog of cytarabine (cytosine arabinoside) with clinical activity against a variety of solid tumors, particularly pancreatic[1,2] and non–small-cell lung cancer.[3,4] Gemcitabine is distinguished from other chemotherapeutic agents by its relatively low toxicity (typically mild fatigue and modest bone-marrow suppression), its broad spectrum of activity against a variety of cancers, and its ability to perturb deoxynucleotide metabolism at clinically achievable concentrations.[5] Gemcitabine must be phosphorylated by deoxycytidine kinase to produce cytotoxicity.[6] The key phosphorylated metabolites are: (1) difluorodeoxycytidine diphosphate (dFdCDP), which can inhibit ribonucleotide reductase, resulting in perturbation of deoxyribonucleoside 5¢-triphosphate (dNTP) pools,[7] particularly d-adenosine triphosphate (dATP) pool depletion,[8] and (2) difluorodeoxycytidine triphosphate (dFdCTP), which blocks the DNA polymerases necessary for replication by competing with dCTP.[9,10] These and other studies suggest that dFdCTP (which can become incorporated into DNA in the form of difluorodeoxycytidine monophosphate [dFdCMP]) is the metabolite responsible for cytotoxicity.

In addition to its cytotoxic effects, gemcitabine is a potent radiation sensitizer of EMT6 rodent tumor cells[11] and a variety of human tumor cell lines[8,12,13] by a continuous exposure to noncytotoxic concentrations of gemcitabine (10 nM) for up to 16 to 24 hours.[8] Because gemcitabine is typically administered once weekly as an infusion, we determined whether radiosensitization could be obtained by exposing cells for 2 hours to clinically relevant concentrations of the drug. In view of the prolonged retention of the toxic metabolites,[8,14] we hypothesized that this brief treatment with gemcitabine could produce significant delayed radiosensitization. We found that radiosensitization equivalent to or greater than that resulting from a 24-hour continuous incubation with a low concentration of gemcitabine occurred 24 to 48 hours after a 2-hour exposure to 100 nM or 3 µM gemcitabine.[15] Because plasma levels greater than 10 µM are routinely obtained in clinical infusions, these findings suggested that gemcitabine would be a clinical radiosensitizer.

Mechanism of Radiosensitization

Role of dATP Pool Depletion and Cell-Cycle Redistribution in Radiosensitization

Our initial efforts focused on determining which metabolite was chiefly responsible for the mechanism of sensitization: dFdCTP (the metabolite responsible for cytotoxicity) or dFdCDP (which produces ribonucleotide reductase inhibition and dNTP pool depletion). Substantial correlative evidence suggests that dFdCTP is not responsible for increased radiation sensitivity. For instance, we found that after exposure of colon cancer cells to gemcitabine, dFdCTP accumulated rapidly, reaching a plateau level within 6 hours of exposure. This rapid rise contrasted with the fact that a minimum of 4 hours was required to develop detectable sensitization, and 16 to 24 hours were needed to produce the maximum effect. This disparity of time courses suggests that dFdCTP accumulation is not responsible for sensitization.[16] Similarly, we found that the same radiosensitization was produced in two different pancreatic cancer cell lines despite a tenfold difference in intracellular dFdCTP concentration.[12] In addition, direct measurement of dCMP incorporation (which results from intracellular dFdCTP) shows a poor
correlation with radiosensitization. [Shewach DS, unpublished data, May 1998] These and other data show that dFdC levels tend to correlate well with cytotoxicity, but are not closely associated with radiosensitization.

In contrast, our data tend to support the hypothesis that the critical event in gemcitabine-mediated radiosensitization is inhibition of ribonucleotide reductase by dFdCDP, leading to perturbation of dNTP pools (in particular, dATP). We have found that dATP-pool depletion after gemcitabine treatment occurs with a time course that correlates with radiosensitization for both colon [8] and pancreatic cancer cells. [12] These changes were associated with a substantial change in the cell-cycle distribution, with most cells progressing into early to mid S phase.

Although these findings demonstrate that dATP pools and ribonucleotide reductase activity are important factors in radiosensitization, we have also found that sensitization appears to be affected by cell-cycle phase. For instance, the maximum sensitization we could achieve using a 4-hour exposure in HT29 human colon cancer cells was an enhancement ratio of 1.4, whereas during a 24-hour exposure to gemcitabine 10 nM, an enhancement ratio of 1.8 was obtained. This longer exposure was accompanied by redistribution of cells into S phase. [8]

Additional evidence supporting the role of cell-cycle redistribution comes from our study of radiosensitization after gemcitabine removal. We found that maximum radiosensitization was obtained 24 hours after drug exposure, when there was concurrent dATP-pool depletion and redistribution of cells into S phase. There was detectable dATP depletion 72 hours after drug exposure, but cell cycle had normalized and no significant radiosensitization was detected. [15] In vivo studies of mouse intestine demonstrate similar kinetics. [17] These findings are also in agreement with a recent study that directly assessed the role of cell-cycle phase in gemcitabine-mediated radiosensitization using synchronized V79 cells. Although gemcitabine increased the radiation sensitivity of all cell-cycle phases, the effect was greatest in S-phase cells. [18]

**Role of Apoptosis in Radiosensitization**

The critical lesion produced by ionizing radiation appears to be the DNA double-strand break. Hence, we used pulsed-field gel electrophoresis to assess the effect of gemcitabine on the induction and repair of radiation-induced DNA damage in HT29 human colon cancer cells under two conditions that produced substantial radiosensitization (immediately after a 24-hour exposure to gemcitabine 10 nM or 24 hours after a 2-hour exposure to 100 nM). Both of these conditions produced a radiation enhancement ratio of 1.8. In contrast to our findings with other antimetabolites, such as bromodeoxyuridine [19] and fluorodeoxyuridine [20], gemcitabine treatment did not increase radiation-induced damage nor did it decrease damage repair during the first 4 hours after radiation. [15] This result has recently been confirmed. [21] These findings suggest that gemcitabine does not affect the primary DNA lesion, but the results of this lesion.

Given the lack of effect of gemcitabine on the induction and immediate repair of radiation damage, we hypothesized that gemcitabine could act as a radiation sensitizer by lowering the threshold for radiation-induced apoptosis. There is mounting evidence that several chemotherapeutic drugs, including gemcitabine, [22] activate the cellular apoptotic machinery, and that alterations in the expression of p53, bcl-2, and bcl-x directly affect the sensitivity of cancer cells to chemotherapy. [23-25]

To begin to assess the role of apoptosis in gemcitabine-mediated radiosensitization, we investigated the effect of gemcitabine on radiation-induced apoptosis in HT29 and SW620 human colon cancer cells, UMSCC-6 human head and neck squamous cancer cells (which are sensitized by gemcitabine), and A549 human lung cancer cells (which are not sensitized by gemcitabine). We have found that gemcitabine significantly enhances apoptosis in cell lines that are radiosensitized (Figure 1). We also found that although apoptosis played a relatively minor role in the clonogenic death produced by radiation alone, it accounted for a substantial fraction of the loss of clonogenicity produced by the combination of gemcitabine and radiation (data not shown). These findings suggest that gemcitabine shifts the pattern of radiation-induced cell death from a nonapoptotic to an apoptotic mechanism.

We have performed similar experiments with A549 lung cancer cells (Figure 2). Although HT29 and A549 cells show similar sensitivity to the cytotoxic effects of gemcitabine, A549 cells are not radiosensitized by noncytotoxic concentrations of gemcitabine. Importantly, exposure of A549 cells to gemcitabine prior to radiation does not result in an important increase in radiation-induced apoptosis (Figure 3). We believe this represents strong correlative evidence that apoptosis plays a key role in gemcitabine-mediated radiosensitization.

**Clinical Trials Combining Gemcitabine and Radiation**
Head and Neck Cancer

Based on these preclinical data, we have developed clinical trials combining radiation and gemcitabine. Our first study focuses on patients with unresectable head and neck cancers. (All patients are evaluated by our otolaryngology group for unresectability. Eligible patients for all of our trials have total granulocytes > 1,500/µL; platelets > 100,000/µL; blood urea nitrogen < 40 mg/dL; and creatinine < 2.0 mg/dL.) The treatment plan consists of radiation therapy (70 Gy in 35 2-Gy fractions) combined with weekly doses of gemcitabine (administered Monday morning) at a starting dose of 300 mg/m²/week. As described above, this dose was chosen to be well below the chemotherapeutic dose of gemcitabine (1,000-1,200 mg/m²/week) based on our preclinical data that gemcitabine is a potent radiosensitizer under nontoxic conditions.

The primary objective of this clinical protocol is to determine the maximum dose of gemcitabine that can be administered concurrently with standard external-beam irradiation. Dose-limiting toxicity was defined according to Radiation Therapy Oncology Group (RTOG) criteria as any grade 3 toxicity or grade 4 mucositis (as grade 3 mucositis commonly occurs with standard therapy) in more than two of six patients at a common dose level. The secondary objectives of this protocol are to determine response (although the study is not powered for this end point), and to determine if phosphorylated metabolites of gemcitabine can be detected in tumor specimens.

This protocol is now approaching completion, and initial results have been presented in abstract form.[26] Sixteen patients have been entered into this trial, eight of whom received gemcitabine 300 mg/m²/week. All patients developed confluent mucositis and required gastrostomy tube feedings. Although the toxicity was substantial, it was scored as grade 3 (by RTOG criteria), which did not preclude dose escalation. No other grade 3 toxicity was noted in more than one patient. Because of the severity of these reactions, we did not dose escalate but observed patients for late toxicity prior to further dose escalation. One patient developed a deep ulceration and another patient developed an esophageal stricture that could not be successfully dilated 6 months after treatment; both were grade 4 toxicities. This demonstrated that the starting dose level of 300 mg/m²/week was above the maximum tolerated dose.

Although we were distressed by these toxicities, we felt this was a remarkable confirmation of the value of our preclinical studies in guiding our starting dose level to be well below the level used when gemcitabine is administered as a cytotoxic agent. Indeed, our findings are in marked contrast to those of other investigators who used full-dose gemcitabine with standard radiation with truly disastrous results.[27] Eight additional patients have been accrued at gemcitabine 150 mg/m²/week. Additional toxicity at this dose level has suggested that the eventual phase II dose for this schedule will be 10-50 mg/m²/week.

Although a formal phase II trial will be required to determine the actual response rate, seven of eight patients who received gemcitabine 300 mg/m²/week achieved a pathologic complete response, based on endoscopic biopsies obtained 3 months after treatment completion. No patient who achieved a complete response has relapsed locally. Of the four evaluable patients with squamous cell cancer of the head and neck who received gemcitabine 150 mg/m²/week, the three patients who underwent biopsy have achieved a pathologic complete remission. The remaining patient, who has not yet been biopsied, has had a radiographic complete response. These results compare well to the best results reported using multiagent chemotherapy with radiation, which produces significant mucositis and hematologic toxicity, and encourages us to try to improve treatment through rational clinical design.

In addition to these results, a number of patients have undergone biopsies 2 hours after receiving the first dose of gemcitabine (but earlier in the day than the first dose of radiation, which begins about 6 hours after gemcitabine administration). Patient biopsies ranged in size from 22 mg to 125 mg. Analysis of the soluble nucleotides from the tumor extracts demonstrated that there were detectable levels of dFdCTP in all biopsies. There was no significant difference in the concentration of dFdCTP in tumor tissue among patients who achieved a complete response. A mean level of 300 mg/m²/week, although the mean level of dFdCTP was lower at the reduced dose.

Because of the significant toxicity associated with a once-weekly treatment regimen, we thought it was worthwhile to use an animal model to assess alternate schedules. We chose to use C3H mice bearing a syngeneic tumor (SCCVII cells) to measure tumor responses, and the mucosal toxicity model of Xu and colleagues.[28] We evaluated two issues that were relevant to our clinical observations. First, we wanted to determine whether once-weekly or twice-weekly gemcitabine (matched to produce equivalent systemic toxicity) caused more mucosal toxicity when used in combination with radiation. (We chose the twice-weekly schedule based on our hypothesis from our cell-culture studies that twice-weekly administration might radiosensitize all five radiation fractions,
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whereas once-weekly administration would only be anticipated to sensitize two to three fractions.) Radiation was delivered in five daily fractions (Monday through Friday), with gemcitabine administered either on Monday, or on both Monday and Wednesday. We wished to know whether once-weekly or twice-weekly gemcitabine with radiation (matched to produce the same toxicity) had a greater antitumor effect. We found that twice-weekly gemcitabine with radiation was more effective than once-weekly administration (Fields MT, unpublished data, October 1998). These data suggest that it may be worthwhile to pursue clinical trials using twice-weekly gemcitabine with radiation.

**Pancreatic Cancer**

We have initiated several clinical trials for patients with either resected or unresectable pancreatic cancer. These clinical trials have taken two forms. The first, and perhaps more traditional, approach has been to use a standard radiation dose (50.4 Gy in 1.8-Gy fractions), with dose-escalating gemcitabine, starting at 300 mg/m², given every Monday. Separate trials are under way, both as preoperative therapy[29] (with the intention of performing curative resection) and for patients with unresectable disease.[30] Early results show that in contrast to the experience with head and neck cancer, the dose could be escalated, and patients in both studies have tolerated gemcitabine 500 mg/m² given once weekly. Another clinical trial under way has used twice-weekly gemcitabine.[31] Dose-limiting toxicity in these trials has not yet been reached. A significant fraction of patients have required decreased gemcitabine doses due to hematologic toxicity; although it is not clear that this toxicity is greater than would be anticipated from gemcitabine alone.

More recently, we have initiated a second type of protocol for patients with either resected or unresectable disease. This approach gives full-dose gemcitabine (1,000 mg/m² administered once a week) with dose-escalating radiation. The logic underlying this approach assumes that systemic spread is a crucial component of the morbidity and mortality of pancreatic cancer, and that improved therapy will result from attempting to achieve simultaneous radiosensitization of local disease and systemic treatment. Our initial radiation dose was 1.6 Gy given 5 days/week for 15 fractions (starting dose, 24 Gy), with gemcitabine administered each Monday. After a 1-week break, patients receive a second cycle of gemcitabine, consisting of weekly gemcitabine for 3 additional weeks. This approach appears to be well tolerated and has produced some promising, objective responses. We plan to escalate the radiation dose by increasing the dose/fraction, with a goal of maintaining a short radiation treatment time for this group of patients with a guarded prognosis.

**Conclusions**

Gemcitabine is a potent radiation sensitizer. Although the mechanism of action is not yet defined, the key proximal event appears to be inhibition of ribonucleotide reductase producing dATP pool depletion, followed by cell-cycle redistribution into S phase. These events lower the threshold for radiation-induced apoptosis. Promising preliminary clinical results have been produced by combining gemcitabine and radiation for patients with unresectable head and neck and pancreatic cancers. These studies have been based, in general, on pharmacologic principles defined in the laboratory. The optimal dose and schedule are not yet known, but given the number of investigators working in this area, phase II trials are expected to be under way in the next year.

**References:**


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