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ORIGINAL ARTICLE

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In vitro assessment of nucleoside analogs in multiple myeloma

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Abstract *Purpose*: To identify nucleoside analogs that may be effective for multiple myeloma (MM), we tested fludarabine, clofarabine, arabinosylguanine, cytarabine, troxacitabine, and gemcitabine in MM cell lines. *Methods*: We employed biologic and biochemical assays in MM cell lines to evaluate the clinical potential of these nucleoside analogs. *Results*: Among these purine and pyrimidine nucleoside analogs, fludarabine, clofarabine and gemcitabine were the most potent. MM cell lines, resistant to commonly used chemotherapeutic agents for this disease, were more sensitive to gemcitabine with an IC_{50} in the nanomolar range. The greater cytotoxicity of gemcitabine in MM cells was consistent with greater accumulation of gemcitabine triphosphate, the major cytotoxic metabolite of this drug. MM.1S cells accumulated >100 μ*M* gemcitabine triphosphate but accumulated <20 μ*M* of the other analogs as the respective triphosphates. In addition incubation with gemcitabine resulted in inhibition of DNA synthesis. Incubation with 25, 50 or 100 n *M* gemcitabine resulted in a dose- and time-dependent increase in the cell population with a $subG_1$ DNA content indicative of apoptosis. *Conclusions*: These results suggest that gemcitabine is a potent nucleoside analog in MM cell lines including cell types resistant to other chemotherapeutic agents. The greater activity of gemcitabine compared to other analogs seems to be due to favorable metabolism of this agent.

Keywords Multiple myeloma · Gemcitabine · Nucleoside analogs

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Introduction

Multiple myeloma (MM) is a malignancy of clonal B cells and accounts for 10% of all hematologic malignancies with 14,600 new cases diagnosed in the US in 2003 [20]. The standard therapies for this fatal disease are associated with a median survival of 4 to 5 years from diagnosis, which may be moderately enhanced by the use of high-dose chemotherapy and autologous stem cell transplantation [1, 2]. The survival rate of MM patients has only modestly changed over the past three decades and consequently new treatment approaches need to be developed.

A number of nucleoside analogs are currently used for the treatment of acute and indolent hematologic malignancies that include tumors of myeloid and lymphoid lineage. Based on the extensive experience with hematologic tumors, one would expect that nucleoside analogs that are effective in leukemias and lymphomas could play a role in the treatment of MM [18, 36]. Unfortunately, limited activity has been seen in MM. Because of the success of cytarabine in leukemias and lymphomas, the first attempt was made with this drug [21]; the response rate was very poor with one partial remission among 14 treated patients. This could be partially explained by the cellular pharmacokinetics of

cytarabine triphosphate (ara-CTP). Compared with the cells obtained from patients with lymphomas treated with the same dose of cytarabine, plasma cells from MM patients accumulated significantly less ara-CTP. Additionally, cells obtained from MM patients with >25% bone marrow involvement accumulated significantly less ara-CTP than cells obtained from MM patients with fewer MM cells in the bone marrow. This profile in normal versus tumor cells partly explains the profound hematologic toxicity in these patients and, taken together, these findings indicate that MM cells do not phosphorylate cytarabine effectively nor retain ara-CTP with great efficiency.

An identification of the malignancies which respond to nucleoside analog therapy suggests that effective cellular uptake of the analog and a modest growth fraction of the malignant population each can predict efficacy [44]. Because MM is an indolent disease, analogs active in indolent hematologic malignancies have been examined. The two nucleoside analogs that play a major role in the treatment of B-cell chronic lymphocytic leukemia, hairy-cell leukemia, and indolent lymphomas, are fludarabine and cladribine [7, 39]. Unfortunately, neither of these deoxyadenosine analogs has exhibited sufficient activity in vitro in myeloma cell lines or in clinical trials to justify continued clinical evaluation in this disease [34]. Results of phase II clinical trials in MM with fludarabine [23, 28] and with cladribine [18, 36] were completely negative for patients with MM.

The ability of gemcitabine, a pyrimidine nucleoside analog, to induce apoptosis has been investigated in MM cell lines [32]. In glucocorticoid-resistant MM cell lines, it has been demonstrated that 10 μ*M* gemcitabine is able to induce a significant degree of apoptosis while cladribine fails to induce apoptosis even at higher concentrations [13]. In studies in which the combination of gemcitabine and paclitaxel was used to induce apoptosis in MM cell lines, it has been found that the degree of apoptosis with paclitaxel is dependent on Bcl-2 expression while the gemcitabine-induced apoptosis is independent of Bcl-2 status [10]. In short, these results suggest a potential role of gemcitabine in MM.

Because nucleoside analogs with closely related structures exhibit a diverse spectrum of anticancer activity in human tumor types in the clinic, we undertook a survey of nucleoside analogs used in treatment and in clinical trials to determine their cytotoxicity in MM cell lines. To identify mechanisms of cytotoxicity of these agents, we determined their ability to be converted into their respective triphosphates, their ability to inhibit DNA synthesis, and induction of cell death at doses achievable in plasma during therapy. An appreciation of nucleoside analog metabolism in myeloma cell lines provides a basis for understanding why these drugs may or may not be effective and also provides clues as to the mechanism of resistance to this class of drugs. The objective of this study was to identify the most effective analog that could be subsequently moved into the clinic for treatment of MM.

Materials and methods

Cell lines

Experiments were conducted using MM cell lines developed previously by our group [12, 31]. The original cell line (MM.1S) was established from the peripheral blood of a patient with MM who had been treated with steroid-based therapy. By chronic exposure to glucocorticoid, a steroid-resistant variant was developed. MM.1S cells are sensitive to the killing effects of glucocorticoids while MM.1R cells are resistant to glucocorticoids. U266 MM cells were obtained from the ATCC. RPMI 8226 cells and the melphalan-resistant derivative LR5 MM cells were obtained from Dr. William Dalton [4]. All cells were grown in RPMI-1640 medium (Invitrogen, Baltimore, Md.) supplemented with 10% fetal bovine serum, 2 m *M* glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml fungizone. Cells were maintained at 37°C in an atmosphere containing 5% CO_2.

Drugs and chemicals

Gemcitabine was purchased from Eli Lilly and Company (Indianapolis, Ind.) while cytarabine was obtained from Sigma (St. Louis, Mo.). Nelarabine was purchased from R.I. Chemicals (Orange, Calif.). [^{3}H]Ara-G, cytarabine, and clofarabine were purchased from Moravek Biochemicals (Brea, Calif.). [^{3}H]Troxacitabine and nonradioactive troxacitabine were obtained from Biochem Pharma (Laval, Quebec, Canada). Berlex Laboratories (Alameda, Calif.) provided fludarabine as a sterile, lyophilized powder that was free of antibacterial preservatives. For in vitro investigations fludarabine was dephosphorylated to its nucleoside, F-ara-A. Clofarabine was obtained from Southern Research Institute (Birmingham, Ala.). For HPLC standards, triphosphates of these analogs were custom-synthesized by Sierra Biochemicals (Tucson, Ariz.). All other chemicals were reagent grade.

Cell proliferation assay

This assay was performed as described previously [25]. Briefly, MM cells were cultured into 96-well dishes at a concentration of 25,000 cells per well and incubated with the indicated drugs for 72 h. Cell proliferation was determined using the MTS Cell Titer Aq_{ueous} assay (Promega, Madison, Wis.), which measures the conversion of a tetrazolium compound into formazan by a mitochondrial dehydrogenase enzyme in live cells. The quantity of formazan product as measured by the amount of absorbance at 490 nm is directly proportional to the number of living cells in culture. Each data point is the average of four independent determinations,

and the error bars represent the SD. The results are expressed as the percentage of formazan produced in relation to the cells treated with the control medium in the same assay.

Measurement of intracellular nucleoside triphosphates by HPLC

Nucleotides were extracted using perchloric acid, and the extracts were neutralized with KOH as described previously [48] and stored at −20°C until analyzed. The neutralized extracts were applied to an anion-exchange Partisil-10 SAX column, and eluted at a flow rate of 1.5 ml/min with a 50-min concave gradient (curve no. 7, Waters 600E System Controller) from 60% 0.005 *M* $NH_4H_2PO_4$ (pH 2.8) and 40% 0.75 *M* $NH_4H_2PO_4$ (pH 3.6) to 100% 0.75 *M* $NH_4H_2PO_4$ (pH 3.6). The column eluate was monitored by UV absorption at 256 to 280 nm, and the nucleoside triphosphates were quantitated by electronic integration with reference to external standards [8]. The analog triphosphates were identified by comparing their retention profiles and absorption spectra with those of authentic standards. The intracellular concentration of nucleotides contained in the extract was calculated from a given number of cells of a determined mean volume. The cell number was determined using a Coulter counter (Coulter Electronics, Hialeah, Fl.). This equipment was attached to a channelizer which was used to estimate the mean volume of cells in a given cell population. This volume was used to quantitate the concentration of nucleotides assuming that they were uniformly distributed in the total cell volume. The lower limit of sensitivity of this assay was 10 pmol in an extract of 5×10^6 cells corresponding to a cellular concentration of 1 μ*M*.

Inhibition of DNA synthesis by nucleoside analogs

Exponentially growing cells were incubated with the indicated concentrations of nucleoside analogs for 4 h. After 3 h, 2 μCi/ml [^{3}H]thymidine was added to these cultures, and incubation was continued for an additional hour in a multiscreen assay system (Millipore Corporation, Bedford, Mass.). The cells were then collected on multiscreen-GV filters under vacuum and washed four times each with ice-cold 8% trichloroacetic acid, water, and 100% ethanol. The radioactivity in the acid-insoluble material retained on the filters was measured by scintillation counting and expressed as the percent of control (untreated) value of cells.

Flow cytometry

To determine the distribution of cells within the cell cycle, aliquots of cells (1×10^6 each) were pelleted (500 *g* for 5 min at 4°C), and washed twice in ice-cold phosphate-buffered saline (PBS; 8.1 g NaCl, 1.14 g Na_2HPO_4, 0.22 g KCl, and 0.25 g/l KH_2PO_4), fixed in ice-cold 70% ethanol, and stored at 4°C until analyzed. Before analysis by flow cytometry, the fixed cells were pelleted, washed in PBS, and resuspended in ice-cold flow buffer (PBS containing 0.5% Tween 20, 15 μg/ml propidium iodide, and 5 μg/ml DNase-free RNase). The stained cells were analyzed using an Epics Profile II flow cytometer (Coulter Electronics, Hialeah, Fl.).

Results and discussion

Nucleoside analogs

Nucleosides with closely related structures exhibit cellular metabolic characteristics and action mechanisms that vary widely. Most impressively, it is clear that nucleoside analogs with closely related structures that share metabolic pathways and inhibit similar target enzymes still exhibit a diverse spectrum of anticancer activity in human tumor types in the clinic. Hence, understanding the metabolism and actions of any drug is a prerequisite to optimizing its clinical utility. Nucleoside analogs and adenosine deaminase inhibitor have been effective in the treatment of chronic lymphocytic leukemia, hairy cell leukemia and indolent non-Hodgkin's lymphomas [38]. Since MM is a low-grade neoplasm it was expected to respond to nucleoside analog therapy. However, efficacy has not been established for the use of nucleoside analogs in the treatment of MM. The ability of individual nucleoside analogs, or pairs of analogs to induce cytotoxicity in a variety of MM cell lines has been investigated, but a systematic comparison of nucleoside analogs currently available in clinical settings and clinical trials has not been done. Consequently, in this study we tested, in parallel, whether cytarabine, gemcitabine, troxacitabine, fludarabine, clofarabine or nelarabine are cytotoxic to MM cell lines that are sensitive or resistant to the current therapeutics.

The chemical structures of the compounds tested are shown in Fig. 1. Purine analogs include a guanosine-based compound (prodrug nelarabine) and deoxyadenosine analogs namely fludarabine and clofarabine (Fig. 1). Cytosine-based compounds (cytarabine, troxacitabine and gemcitabine) were among the pyrimidine nucleoside analogs (Fig. 1). Because both nelarabine and fludarabine are prodrugs and metabolized in plasma to free nucleosides, F-ara-A and nelarabine, respectively, we used these free nucleosides for the present investigation.

Nucleoside analog-induced cytotoxicity

The cytotoxicity of each compound was compared in the MM.1S cell line that is sensitive to the synthetic glucocorticoid dexamethasone. To determine the growth-inhibitory effect of analogs, MM.1S cells were incubated

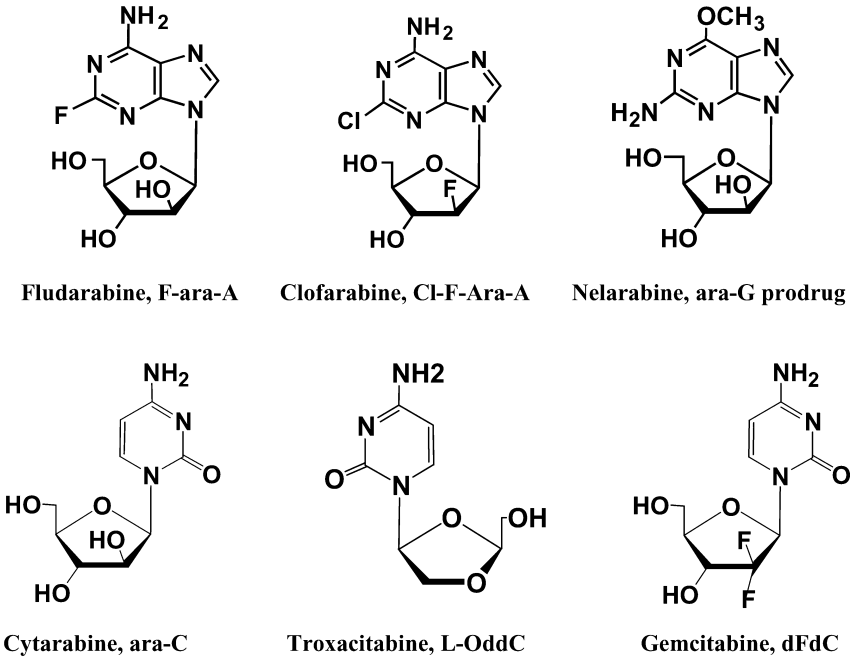

Fig. 1 Chemical structures of purine and pyrimidine nucleoside analogs

for 72 h with the indicated concentration of nucleoside analogs. After incubation, formazan absorbance was determined by the MTS assay. A decrease in absorbance indicates a decrease in cell number. The results are shown in Fig. 2. For the purine compounds, deoxyadenosine analogs clofarabine and F-ara-A were very effective while cells were resistant to nelarabine (Fig. 2A). Among the deoxycytidine analogs, gemcitabine was the most potent with an IC_{50} of approximately 20 n *M* (Fig. 2B). Troxacitabine had a marginal effect while cytarabine was not effective and required concentrations at least 100- to 1000-fold greater than the level of gemcitabine required for a cytotoxic effect.

Because gemcitabine, F-ara-A and clofarabine were the most potent analogs, these were further tested against a number of established MM cell lines that are resistant to the established chemotherapeutic treatments for MM. These cell lines include MM.1R, U266 and RPMI 8226-S which are all resistant to the synthetic glucocorticoid, dexamethasone. LR5 cells were derived from RPMI 8226 cells by stepwise treatment with melphalan and are resistant to this DNA-damaging drug. These cell lines were exposed to the indicated concentrations of gemcitabine, F-ara-A or clofarabine, and the concentration of formazan determined by the MTS assay after 72 h of incubation. The results are shown in Fig. 3.

Of the five MM cell lines, MM.1S and MM.1R were most sensitive to fludarabine (Fig. 3A), clofarabine (Fig. 3B) and gemcitabine (Fig. 3C) with IC_{50} values ranging from 10 to 100 n *M*. Similar to the data in Fig. 2, the most effective compound in MM.1S and MM.1R cells was gemcitabine, followed by clofarabine and F-ara-A. In addition, gemcitabine was the most active compound in RPMI 8226, U266 and LR5 cell lines with tenfold higher concentrations of clofarabine and F-ara-A required to achieve the same level of response. Taken together, these results suggest that among the analogs tested, gemcitabine is the most potent

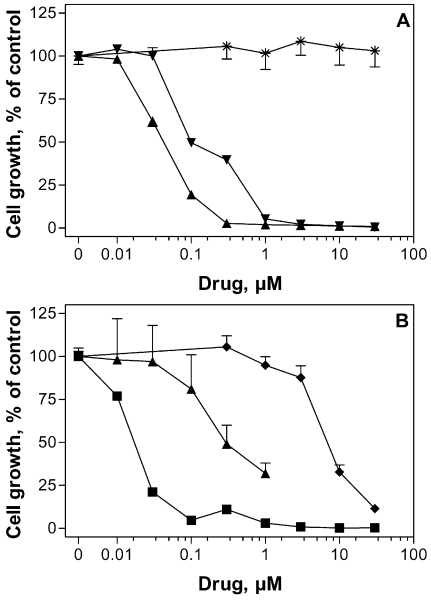

Fig. 2A, B Cytotoxicity of nucleoside analogs in MM.1S cells. MM.1S cells were incubated for 72 h with the indicated concentrations of purine analogs (**A**) or pyrimidine analogs (**B**). Cells were incubated with the purine analogs nelarabine prodrug (✳), F-ara-A (▼) or clofarabine (▲). Cells were incubated with the pyrimidine analogs cytarabine (◆), troxacitabine (▲) or gemcitabine (■). Cell numbers were determined with the MTS assay as described in Materials and methods. The results are presented as percentages in relation to untreated control cells and are the averages of three separate experiments with four determinations per data point in each experiment. The *error bars* represent standard deviation

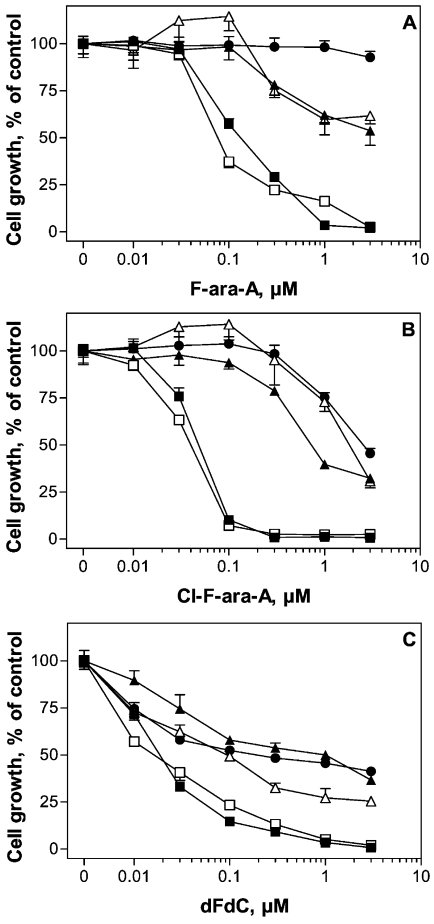

Fig. 3A–C Cytotoxicity of nucleoside analogs in drug-resistant MM cells. Glucocorticoid-sensitive MM.1S cells (■), glucocorticoid-resistant MM.1R (□), U266 (●) and 8226-S (▲) MM cells, and melphalan-resistant 8226/LR5 MM cells (△) were treated with increasing concentrations of F-ara-A (**A**), clofarabine (**B**) or gemcitabine (**C**) for 72 h, and the cell numbers determined by the MTS assay. The results are presented as percentages in relation to untreated control cells and are the averages of a minimum of two separate experiments with four determinations per data point in each experiment. The *error bars* represent standard deviation

Table 1 Accumulation of analog triphosphate in MM.1S cells. MM.1S cells were treated with the indicated concentrations of nucleoside analog for 3 h. Cell extracts were prepared and analyzed by HPLC as described in Materials and methods. The values presented are the means ± SD from three separate experiments

Analog		Analog triphosphate ($\mu M \pm$ SD)	
Commercial name	Chemical name	30 μM	100 μM
Gemcitabine	dFdC	112 ± 04	114 ± 10
Cytarabine	ara-C	20 ± 04	23 ± 02
Troxacitabine	L-OddC	7 ± 01	Not determined
Fludarabine	F-ara-A	3 ± 01	13 ± 03
Clofarabine	Cl-F-ara-A	6 ± 01	18 ± 02
Nelarabine	ara-G	1 ± 0.3	1 ± 0.2

nucleoside analog in MM cell lines that are sensitive and resistant to standard chemotherapeutic agents used for MM.

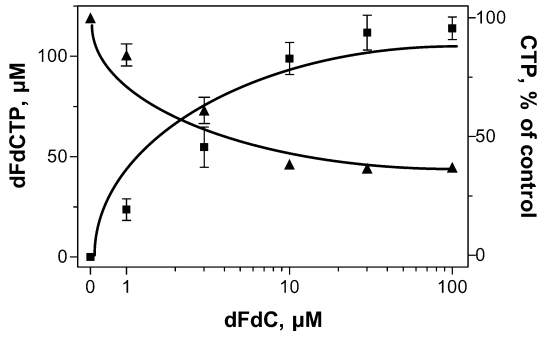

Fig. 4 Accumulation of gemcitabine triphosphate and effect on the CTP pool. MM.1S cells were incubated with various concentrations of gemcitabine for 3 h. Nucleotides were extracted and separated by HPLC, and quantitated as described. Accumulation of gemcitabine triphosphate (■) and decrease in CTP concentration (▲) are shown. Data points are the mean with SD of three separate experiments

Accumulation of analog triphosphates

The cytotoxic capacity of a nucleoside analog is directly linked to its ability to be taken up by cells and phosphorylated to its triphosphate metabolite. We therefore measured the ability of MM.1S cells to convert the nucleoside analogs into their respective triphosphates. MM.1S cells were incubated with either 30 or 100 μM of the nucleoside analog for 3 h and perchloric acid extraction performed. The neutralized acid extract was analyzed by HPLC to determine the concentration of the triphosphate derivative. With gemcitabine at 30 and 100 μM, MM.1S cells accumulated 112 and 114 μM triphosphate, respectively. This was 5- to 100-fold higher than the intracellular triphosphate levels of other nucleoside analogs (Table 1). The relatively higher accumulation of gemcitabine triphosphate may account for the increased sensitivity of MM cells to gemcitabine over the other compounds.

The accumulation of gemcitabine triphosphate was further characterized by incubating MM1.S cells with different concentrations of gemcitabine for 3 h (Fig. 4). The intracellular accumulation of the analog triphosphate was dose dependent but reached a plateau by 30 μM. In association with the formation of gemcitabine triphosphate, there was a corresponding decrease in the endogenous CTP pool, which reached about 40% of the control value at 30 and 100 μM of gemcitabine.

The accumulation of gemcitabine triphosphate (Fig. 5A) and depletion of the CTP pool (Fig. 5B) was also investigated in other MM cell lines. The concentrations of gemcitabine triphosphate were similar in MM1.S and MM1.R cell lines, and were higher than the levels in the U266, 8226, and 8226/LR5 cell lines. The latter three accumulated between 30 and 40 μM of gemcitabine triphosphate. This lower level of triphosphate accumulation may account for a slightly decreased sensitivity of these cells lines to gemcitabine (Fig. 3C), perhaps indicating that patients with melphalan-resistant MM may not be as responsive to this drug. How-

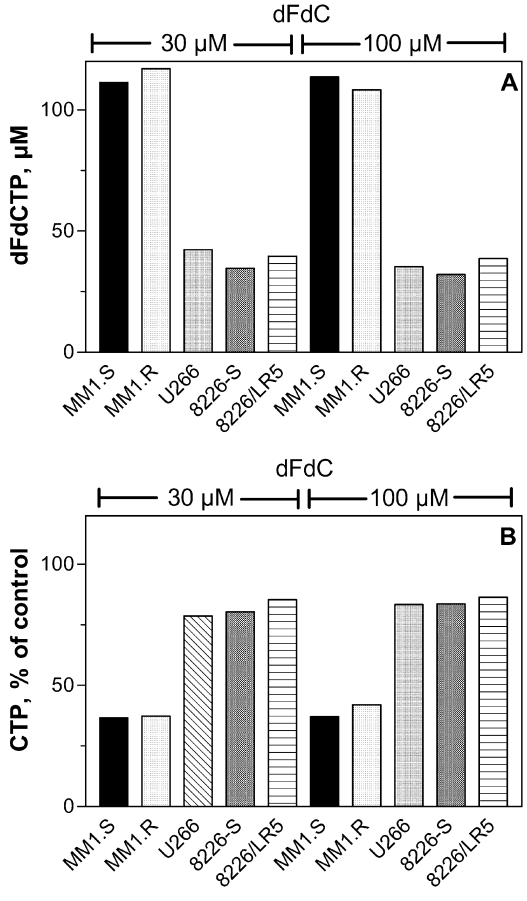

Fig. 5A, B Accumulation of gemcitabine triphosphate and effect on the CTP pool. MM cell lines were incubated with 30 or 100 μ*M* gemcitabine for 3 h. Nucleotides were extracted and separated by HPLC, and quantitated as described. Levels of gemcitabine triphosphate (**A**) and changes in the CTP pool (**B**) are plotted for each cell line. Bars are the means of three separate experiments

ever, the 30 to 40 μ*M* concentration of gemcitabine triphosphate that does accumulate in these cells is significantly higher than that of other nucleoside analogs (Table 1). The depletion of the CTP pool was in accordance with the amount of gemcitabine triphosphate in each cell type.

Previous studies have demonstrated the effect of gemcitabine diphosphate on inactivation of ribonucleotide reductase. This action leads to a decrease in deoxynucleotide pools. Although we believe that gemcitabine has a similar effect in MM cells, unfortunately this could not be tested because of extremely low levels of dNTP pool in these cells. For example, the dATP and dCTP concentration in MM1.S cells is between 1 and 2 μ*M*. The endogenous level of dGTP is in the range 2–4 μ*M* while dTTP is in the range 6–7 μ*M*.

The initial and important step in the formation of triphosphate is the conversion of nucleoside analog to its monophosphate. For all the analogs tested in the present investigation, dCyd kinase has been shown to be responsible for this first step [6, 24, 37, 43]. Although a role of dGuo kinase has been shown for fludarabine [46], and nelarabine [27, 42], the high specific activity of dCyd kinase suggest the importance of this enzyme [6, 43]. Previous studies using purified dCyd kinase have demonstrated that gemcitabine is a preferred substrate for this enzyme. The kinetic value of dCyd kinase for gemcitabine is 1–10 μ*M* compared to 10–20 μ*M* for cytarabine and Cl-F-ara-A [37], and >100 μ*M* for F-ara-A [37] and nelarabine [26]. Troxacitabine has not yet been tested for phosphorylating kinases. In short, the affinity of the kinase for gemcitabine may provide a metabolic benefit for a higher rate of phosphorylation and greater accumulation of gemcitabine triphosphate.

Effect of gemcitabine on DNA synthesis

The mechanism of action for gemcitabine has been described previously and is multitargeted [3, 9, 19]. The triphosphate analog of gemcitabine incorporates into DNA and causes chain termination following the incorporation of one additional deoxynucleotide. In addition, the action of the diphosphate form of gemcitabine on ribonucleotide reductase activity lowers the endogenous deoxynucleoside triphosphate pools, thereby enhancing the incorporation of gemcitabine triphosphate [16]. Furthermore, and consistent with the solid tumor [15] but in contrast to the human leukemia cell lines [6], gemcitabine triphosphate also inhibits CTP synthase in MM cells (Fig. 4). This will block the synthesis of CTP and by mass action that of dCTP as well. The lowered dCTP pool will enhance incorporation of gemcitabine triphosphate into DNA and will release feedback inhibition of dCyd kinase resulting in enhanced phosphorylation of gemcitabine triphosphate [40, 41]. Taken together these multifaceted actions result in inhibition of DNA synthesis. Such properties are missing in other analogs such as cytarabine or troxacitabine and may reflect the greater inhibition of DNA synthesis by gemcitabine compared to other dCyd analogs [3, 9, 19]. Therefore, we measured the ability of gemcitabine to inhibit DNA synthesis in MM cells and compared that to inhibition of DNA synthesis by other deoxycytidine analogs, cytarabine and troxacitabine.

MM.1S cells were incubated with the indicated concentrations of these nucleoside analogs for 3 h, and 1 h prior to harvest [^{3}H]thymidine was added. The radioactivity in the acid-insoluble material was measured by scintillation counting and expressed as the percent of untreated control cells. At a concentration of 0.1 μ*M*, gemcitabine caused a 78% decrease in DNA synthesis in relation to that in control (untreated) cells. In contrast, the same concentration of cytarabine and troxacitabine caused, respectively, a 10% and a 22% decrease in DNA synthesis (Fig. 6). Similar results were obtained when these agents were used for a longer period (24 h, data not shown). Consistent with its effect on cell growth, gemcitabine was much more efficient in causing a decrease in DNA synthesis. The enhanced inhibition of DNA synthesis may contribute to the enhanced efficacy

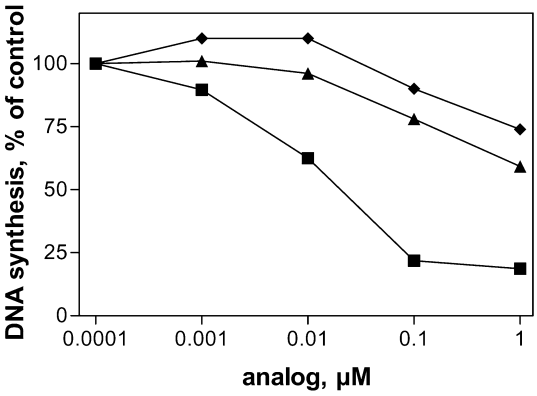

Fig. 6 Inhibition of DNA synthesis in MM.1S cells. MM.1S cells were incubated with the indicated concentrations of cytarabine (◆), troxacitabine (▲) or gemcitabine (■), and 1 h prior to harvest [^{3}H]thymidine was added. The radioactivity in the acid-insoluble material was measured by scintillation counting and is expressed as percent in relation to untreated control cells

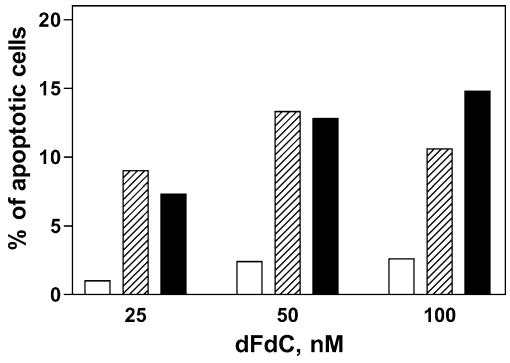

Fig. 7 Gemcitabine-induced apoptosis in MM.1S cells. MM.1S cells were incubated with the indicated concentrations of gemcitabine for 24 h (*open bars*), 48 h (*hatched bars*) or 72 h (*solid bars*) and the percent of cells with a sub-G_1 content of DNA determined by propidium iodide staining followed by flow cytometry

of gemcitabine in inducing apoptosis in the MM cell lines. Consequently, we next measured the ability of gemcitabine to induce apoptosis in MM cell lines.

Gemcitabine induces apoptosis in MM cell line

To determine whether the growth inhibition caused by gemcitabine was due to induction of apoptosis in MM cell lines, MM.1S cells were incubated with either 25, 50 or 100 n *M* gemcitabine for 24, 48 or 72 h. The cells were harvested, fixed and stained with propidium iodide to determine DNA content in a flow cytometric assay. Cells with a less than 2N complement of DNA (sub-G_1 fraction) were scored as apoptotic. The background level of apoptosis in control cells was subtracted. The data in Fig. 7 demonstrate that there was a dose- and time-dependent increase in apoptosis caused by gemcitabine. Similar results were obtained when these cells were incubated with higher concentrations of gemcitabine (10–30 μ*M*) for shorter times (data not shown). Additional features of apoptosis such as annexin V staining, loss of mitochondrial membrane potential and activation of caspase activity have recently been reported by our group [32]. Taken together, these results demonstrate that the cytotoxicity of gemcitabine is due to induction of apoptosis in MM cells.

These results suggest that in comparison with other nucleoside analogs gemcitabine is the most potent nucleoside analog in MM cell lines that are either sensitive or resistant to the standard chemotherapies for this malignancy. Gruber et al. [13] have reported that gemcitabine induces apoptosis in MM and plasma cell leukemia cell lines at a concentration (10 μ*M*) that is clinically achievable. By examining additional nucleoside analogs and determining the mechanism of action in MM cell lines, our results provide a mechanism for this cytotoxicity. Potent induction of apoptosis has also been observed in other MM cell lines and plasmacytoma cells with gemcitabine [30]. The effect of gemcitabine in combination with paclitaxel in MM cell lines [10], and also in a limited phase II clinical trial [11], suggests efficacy with this combination approach, but with a combination of drugs it is difficult to assess the role of each drug individually.

While gemcitabine has been used successfully for the treatment of some solid tumors [5, 17, 22, 29], it is just now being examined for the treatment of hematologic malignancies [33]. A recently published clinical trial with gemcitabine as a single agent for the treatment of MM [47] has shown that because of toxicity gemcitabine is not favorable. Briefly, 29 heavily pretreated, elderly patients with MM received gemcitabine at 1000 mg/m^2 as a 30-min infusion. Of the 23 evaluable patients, 16 had stable disease. This 70% of heavily pretreated elderly patients achieving stable disease demonstrates that gemcitabine is active in this disease. Furthermore, because of hematologic toxicity reported in this study, many patients did not receive all the courses, suggesting that growth factors or other supports to control the hematologic toxicity may improve the quality or quantity of the response rates.

In a recently published clinical investigation [35], 16 heavily pretreated patients with MM received gemcitabine at 1250 mg/m^2 over 30 min. After three courses of gemcitabine as a single agent, the response rate was 31% (one complete remission, one partial remission and three minimal responses). In addition to these responses, an additional eight patients (50%) had stable disease. Overall in this clinical trial, 81% of heavily pretreated patients with MM responded to single-agent gemcitabine. Taken together, these two clinical studies provide sufficient evidence for the efficacy of gemcitabine in MM patients to warrant further investigation into the actions of this drug.

Unfortunately, in both these clinical trials, gemcitabine was infused over 30 min. However, previously it has been demonstrated that the rate of gemcitabine triphosphate accumulation is saturated at a dose-rate of 10 mg/m^2 per minute [14]. Pharmacologic [14, 15, 41]

and clinical evidence [45] strongly suggests that the maximal biochemical effects and clinical benefits are observed when gemcitabine is infused over a fixed-dose rate of 10 mg/m^2 per minute. Hence consideration should be given to administering gemcitabine using this pharmacologically guided fixed dose rate schedule. The toxicity profile may be very different if gemcitabine is infused at a different dose-rate to more favorable patients with MM to fully evaluate the efficacy of this drug.

In summary, we examined several purine and pyrimidine nucleoside analogs in MM cell lines that are sensitive and resistant to standard chemotherapeutic agents used for MM. We found gemcitabine to be the most effective due to the favorable metabolism of this agent. Although several new agents have shown efficacy in MM, a pharmacologically optimal dose-rate of gemcitabine needs to be considered for patients with MM. In addition, further investigation of gemcitabine in a clinical setting as a single agent or in combination is warranted.

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