Radiotherapy and Oncology 83 (2007) 97–101
www.thegreenjournal.com

Experimental radiobiology

Gemcitabine radiosensitizes multiple myeloma cells to low let, but not high let, irradiation☆

Stéphane Supiot[a,b], Francois Thillays[a,b,c], Emmanuel Rio[b], Sébastien Gouard[a], Alfred Morgenstern[d], Frank Bruchertseifer[d], Marc-André Mahé[b,c], Jean-François Chatal[a,b,c], François Davodeau[a], Michel Chérel[a,b,c,*]

[a]*Département de recherche en cancérologie, Université de Nantes, Nantes, France,* [b]*CRLCC Nantes Atlantique — Centre René Gauducheau, Nantes-St-Herblain, France,* [c]*Faculté de Médecine, Université de Nantes, France,* [d]*Institute for Transuranium Elements, Karlsruhe, Germany*

Abstract

The radiosensitizing properties of gemcitabine in relation to low Linear Energy Transfer (LET) particles (Cobalt 60) and high-LET particles (alpha-RIT ^{213}Bi-radiolabeled CHX-DTPA-B-B4) were analyzed. Three multiple myeloma cell lines (LP1, RPMI 8226, U266) were irradiated with or without 10 nM gemcitabine 24 h prior to radiation. Gemcitabine led to radiosensitization of LP1 and U266 cells with low-LET (Radiation Enhancement Ratio: 1.55 and 1.49, respectively) but did not radiosensitize any cell line when combined with high-LET.

Keywords: Radioimmunotherapy; Alpha particles; Homologous recombination; Antimetabolites; Multiple myeloma

Gemcitabine (2′,2′-difluoro-2′deoxycytidine) is an effective treatment for many solid tumors, mainly non-small-cell lung cancers, cancers of the bladder and of the pancreas. It is a potent ionizing radiation sensitizer *in vitro* and in preclinical studies. Encouraging tumor responses have been observed in clinical studies though associated with high toxicity in some cases (for review [19]).

The mechanisms of gemcitabine action are relatively well documented but those specifically related to its radiosensitizing action are unclear and still under study. Its cytotoxic and radiosensitizing properties depend on its intracellular phosphorylation into di- and triphosphate metabolites. Competition between the triphosphate and 2′-deoxycytidine 5′-triphosphate (dCTP) incorporation into DNA would be the main cause of its cytotoxicity [9,22]. The diphosphate is a potent inhibitor of ribonucleotide reductase, depleting the 2′-deoxyadenosine 5′-triphosphate (dATP) pools in tumor cells and thus inhibiting DNA synthesis [1,7,17,23]. There is a strong correlation between the radiosensitizing effects of gemcitabine *in vitro* and dATP depletion, but no relation with the triphosphate level or incorporation into DNA [13,14,23].

Gemcitabine's radiosensitizing effects also appear to involve changes in cell cycle distribution and induction of apoptosis (for review [12]). They occur preferentially when cells accumulate in the S phase [11,16,18,21]. Since high linear energy transfer (LET) particles can overcome the radioresistance of cells in the S phase [2], Latz et al. hypothesized that combining gemcitabine with high-LET particles might prove to have a highly supra-additive lethal action on tumor cells [11]. However, since gemcitabine increases S phase cells killing by targeting homologous repair (HR) pathways [27], a more logical hypothesis would be that high-LET irradiation effects which are not sensitive to cell cycle distribution should not be enhanced by gemcitabine.

To test this hypothesis, we compared the effects of combining gemcitabine with radiation from low-TEL particles (^{60}Cobalt gamma rays) and high-TEL particles (alpha particles emitted during alpha-radioimmunotherapy (RIT)) on clonogenic survival in three human multiple myeloma cell lines.

Methods and materials

Gemcitabine was purchased from Lilly France (St. Cloud, France) and diluted in culture medium to the desired final concentration.

In order to reflect the biological heterogeneity of multiple myeloma, we selected 3 HMCL presenting different characteristics, that may cause them to respond differently

☆ Supported by: Association pour la Recherche sur le Cancer (ARC) S.S., E.R.

0167-8140/$ - see front matter © 2007 Elsevier Ireland Ltd. All rights reserved. doi:10.1016/j.radonc.2007.02.005

to ionizing radiation. The human myeloma cell lines, RPMI 8226 and U266, were obtained from the American Type Culture Collection (Rockville, MD, USA), and LP1 was obtained from DSMZ (Braunschweig, Germany). These cell lines were cultured in RPMI 1640 (Biowhittaker Europe, Verviers, Belgium), supplemented with 10% heat-inactivated fetal calf serum (Biomedia, Boussens, France) and 2 mM L-glutamine, at 37 °C, 5% CO_2 and 100% humidity. Cells were transferred into fresh culture medium, and cell concentration was adjusted to 5×10^5 cells/ml, 48 h before each assay.

Cells were irradiated using a ^{60}Co source (Theratron, Atomic Energy of Canada Limited), at a 1 Gy/min dose rate.

B-B4 monoclonal antibody, a murine IgG that recognizes syndecan-1 (CD 138) antigen, was kindly provided by Dr. John Wijdenes (Diaclone Research, Besançon, France) [28]. A sample of this antibody (50 mg/g) was allowed to react with twice-crystallized pepsin (Sigma) for 2 h at 37 °C. The mixture was fractionated by exclusion-diffusion on a Superdex G200 column (Pharmacia) equilibrated with 100 nM phosphate buffer to obtain F(ab′)2 fragments for use in all experiments at a 2 nM concentration. The ^{213}Bi generator was kindly provided by the TransUranium Institute (Karlsruhe, Germany). ^{213}Bi was selectively eluted by 2 ml of a solution containing 0.1 M HCl and 0.1 M NaI. B-B4 antibody was conjugated to the bifunctional chelating agent CHX-A″-DTPA (cyclohexyldiethylenetriaminepenta-acetic acid), synthesized in our laboratory, as described by Brechbiel et al. [3]. The antibody was incubated with 50 eq (mol/mol) CHX-A″-DTPA in Hepes buffer (0.1 M, pH 8) and, after overnight incubation at room temperature, purified by HPLC on a Sephadex G200 gel-filtration column (Amersham Biosciences, Saclay, France). Mean chelate number per antibody was 2, as assessed with 4 eq of a buffered citrate-acetate (0.02—0.15 M, pH 5.5) ^{111}In solution. B-B4 immunoreactivity was modified only slightly by addition of chelate (>90%). A 10—100 μg sample of B-B4-CHX-A″-DTPA was incubated in a solution of freshly eluted ^{213}Bi for 15 min at 37 °C. The specific activity of the radiolabeled antibody was 150 MBq/mg. Radiochemical purity, checked by ITLC-SG using 10% TCA as solvent, was >90%.

Cells (1×10^6) were incubated during 24 h with varying concentration of gemcitabine. They were pelleted, resuspended in 0.2 ml PBS, and fixed by addition of 2 ml of ice-cold 70% ethanol/30% PBS. Fixed cells were pelleted, vigorously suspended in PBS, and incubated for 30 min at 37 °C with 100 μg/ml RNAse A (Sigma—Aldrich) and 40 μg propidium iodide (Sigma—Aldrich). The fluorescence of the stained cells was analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Data were analyzed with ModFit LT2 (Becton Dickinson).

After a 24-h incubation with gemcitabine, cells were resuspended in RPMI 1640 with 10% FCS at 10^4 cells/ml, and plated in 96-well microtiter plates (Nunclon) at 50 μl/well. They were incubated for 12 or 36 h at 37 °C before addition of ^{3}H-thymidine (Amersham Biosciences, 37 kBq/well), harvested 4 h later and washed. The amount of incorporated radioactivity was counted using a liquid scintillation counter (1450 Microbeta Plus, Wallac, Finland).

Survival, i.e., the ability of cells to maintain their clonogenic potential and form colonies, was assessed using the limiting dilution method [25]. After gemcitabine and radia-

Table 1
Effects of gemcitabine (GMZ) on proliferation, clonogenic survival and cell distribution in three multiple myeloma cell lines

	LP1				RPMI 8226				U266			
	GMZ (nM)				GMZ (nM)				GMZ (nM)			
Proliferation (%)	10		62.0 ± 8.8		10		60.5 ± 4.9		10		54.2 ± 5.9	
Clonogenic survival (%)	10		71.9 ± 19.5		10		67.3 ± 4.8		10		56.7 ± 22	
Cell distribution pattern (%)		G1	S	G2M		G1	S	G2M		G1	S	G2M
	0	48.5 ± 3.5	38 ± 1.4	13.5 ± 2.1	0	32.5 ± 0.7	49 ± 2.8	18.5 ± 2.1	0	48 ± 5.7	39.5 ± 6.4	12.5 ± 0.7
	7	24.5 ± 6.4	70.5 ± 4.9	5 ± 1.4	10	7 ± 1.4	84.5 ± 4.9	8.5 ± 3.5	8	9.5 ± 4.9	86.5 ± 4.9	4 ± 0.0