Cancer Chemother Pharmacol (2003) 51: 415–421
DOI 10.1007/s00280-003-0606-z

ORIGINAL ARTICLE

Mark K. Polar · Chris Gennings · Misook Park
Mona S. Gupta · David A. Gewirtz

Effect of the vitamin D_3 analog ILX 23-7553 on apoptosis and sensitivity to fractionated radiation in breast tumor cells and normal human fibroblasts

Received: 28 October 2002 / Accepted: 10 February 2003 / Published online: 11 April 2003

Abstract *Purpose*: Previous work from this laboratory has demonstrated that the vitamin D_3 analogs EB 1089 and ILX 23-7553 enhance the response of breast tumor cells to ionizing radiation and promote radiation-induced apoptotic cell death. The current studies were designed to more closely simulate clinical radiotherapy in the treatment of breast cancer by examining the utility of ILX 23-7553 as an adjunct to fractionated ionizing radiation. The potential toxicity to normal tissue of the combination of ILX 23-7553 and fractionated radiation was assessed in a model of BJ human fibroblasts in culture. *Methods*: MCF-7 cells and human fibroblasts were treated with fractionated radiation alone (5×2 Gy over 3 days), ILX 23-7553 alone (50 n*M*) or ILX 23-7553 followed by 5×2 Gy. Viable cell numbers were determined by trypan blue exclusion and apoptosis by the TUNEL assay. A statistical model of additivity was utilized to assess the nature of the interaction between ILX 23-7553 and fractionated radiation. *Results*: Radiation and ILX 23-7553 each alone reduced viable cell numbers by $72 \pm 3.1\%$ and $62 \pm 4.8\%$, respectively. Pretreatment with ILX 23-7553 followed by 5×2 Gy reduced viable cell numbers by $93.2 \pm 0.7\%$. The interaction between ILX 23-7553 and fractionated radiation appeared to be additive despite the fact that the combination of ILX 23-7553 and fractionated radiation also promoted a twofold increase in apoptotic cell death. ILX 23-7553 failed to enhance the response to radiation or to promote apoptosis in BJ human foreskin fibroblasts. *Conclusions*: ILX 23-7553 enhanced the antiproliferative and apoptotic effects of fractionated ionizing radiation in MCF-7 breast cancer cells. These effects appeared to be selective in that similar responses were not observed in a model of normal human fibroblasts. Vitamin D_3 analogs such as ILX 23-7553 may prove to have utility in combination with conventional radiotherapy of breast cancer as well as other malignancies which are sensitive to vitamin D_3.

Keywords ILX 23-7553 · Fractionated radiation · Breast tumor cells · Vitamin D_3

M. K. Polar · M. S. Gupta · D. A. Gewirtz (✉)
Department of Pharmacology/Toxicology, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980230, Richmond, VA, USA
E-mail: gewirtz@hsc.vcu.edu
Tel.: +1-804-8289523
Fax: +1-804-8288079

M. K. Polar · M. S. Gupta · D. A. Gewirtz
Department of Medicine, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980230, Richmond, VA, USA

C. Gennings · M. Park
Department of Biostatistics, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980230, Richmond, VA, USA

Introduction

Each year approximately 200,000 women in the United States are diagnosed with breast cancer, and of these about 40,000 die from the disease [1]. Although decades of research have been focused on developing an understanding of the etiologies and mechanisms by which malignant tumors arise in order to improve treatment, breast cancer remains the leading cause of cancer death for women between the ages of 20 and 59 [2, 3]. Since many of the current chemotherapeutic regimens utilized in the treatment of breast cancer are quite toxic, there has been considerable interest in developing approaches that would serve to enhance drug sensitivity without a concomitant increase in host toxicity. Vitamin D_3 and vitamin D_3 analogs are being considered for such applications as these agents appear to have the capacity to interfere with the growth of breast tumor cells [4, 5, 6] and, under the appropriate experimental conditions, promote apoptotic cell death [7, 8].

Studies from the laboratories of a number of investigators (reviewed in reference 9) as well as our own work [10, 11, 12] have demonstrated that vitamin D_3 and analogs such as EB1089 and ILX 23-7553 can be effectively combined with chemotherapeutic drugs such

as Adriamycin as well as ionizing radiation [10, 11, 12]. In our hands, these combinations have resulted in enhancement of the response of breast tumor cells to both Adriamycin and ionizing radiation as well as the promotion of apoptosis in breast tumor cells which are relatively refractory to this mode of cell death [13]. Previous studies utilizing the vitamin D_3 analogs have involved a single high (supraclinical) dose of radiation [10, 12]. However, since patients are routinely treated with fractionated radiation in doses on the order of 2–2.5 Gy, the current studies were performed utilizing ionizing radiation at doses of 2 Gy administered over a period of 3 days to achieve a total dose of 10 Gy. Lower concentrations of ILX 23-7553 were used compared to those in previous studies [12] in order to assess the potential utility of a drug concentration that might more readily be achieved in the clinic. To address the issue of therapeutic ratio (i.e. to determine whether a normal tissue might also be sensitized to radiation), studies combining the vitamin D_3 analog with radiation were also performed in human fibroblasts.

The current work established that ILX 23-7553 is additive with fractionated radiation in reducing viability of MCF-7 breast tumor cells while having little impact on sensitivity to fractionated radiation in human fibroblasts. The combination of ILX 23-7553 and fractionated radiation was also significantly more effective than either modality alone in preventing proliferative recovery of MCF-7 breast tumor cells and in promoting apoptotic cell death. In dramatic contrast, no apoptosis was evident in the fibroblasts exposed to the combination of ILX 23-7553 and fractionated radiation.

Materials and methods

Cells and reagents

The p53 wild-type MCF-7 human breast tumor cell line was obtained from NCI (Frederick, Md.). ILX 23-7553 was provided by ILEX Products (San Antonio, Tx.). RPMI-1640 and supplements were obtained from GIBCO Life Technologies (Gaithersburg, Md.). Wild-type BJ fibroblasts were graciously provided by the laboratory of Dr. Shawn Holt and Dr. Lynne Elmore (Department of Pathology, Virginia Commonwealth University). Reagents used for the terminal transferase end-labeling (TUNEL) assay (terminal transferase, reaction buffer, and fluorescein-dUTP) were purchased from Boehringer Mannheim (Indianapolis, Ind.). All other reagents used in the study were of analytical grade.

Cell culture

The MCF-7 cell line was grown from frozen stocks in basal RPMI-1640 medium supplemented with 10% fetal calf serum, 2 m*M* L-glutamine and penicillin/streptomycin at 37°C under a humidified atmosphere containing 5% CO_2. BJ fibroblasts were grown in D9C medium at 37°C under a humidified atmosphere containing 5% CO_2.

Cell viability

The effects of fractionated irradiation and ILX 23-7553, alone and in combination, on cell viability were determined by trypan blue exclusion. For the combination studies, cells were treated with 50 n*M* ILX 23-7553 (unless otherwise indicated) for 72 h followed by five doses of 2 Gy administered over a period of 3 days to provide a cumulative dose of 10 Gy. Cells were harvested 24 h after the final irradiation using trypsin and stained with 0.4% trypan blue dye. The percentage of trypan blue staining cells was determined by phase contrast microscopy.

TUNEL assay for DNA fragmentation

The method of Gavrieli et al. [14] was utilized to assess apoptotic cell death. Cells were fixed after cytospins and the fragmented DNA in cells undergoing apoptosis was detected using an In Situ Cell Death Detection Kit (Boehringer-Mannheim). In this assay, the fragmented DNA in individual cells was end-labeled using fluorescein-dUTP at strand breaks by the enzyme terminal transferase. The cells were then washed, mounted in Vectashield and photographed using a Nikon fluorescent microscope.

Synergism vs additivity

The predicted responses for the colony forming assay were determined using the following additivity model: mean $(y) = \exp(B_0 + B_1x_1 + B_2x_2)$, where y is the observed response (percent reduction in cell viability), x_1 is the concentration of ILX 23-7553 (nanomoles), x_2 is the dose of ionizing radiation (Gray), B_0 is an unknown parameter associated with the intercept, B_1 is an unknown parameter associated with the slope of x_1, and B_2 is an unknown parameter associated with the slope of x_2. Parameter estimates were found using a generalized least squares criterion for nonlinear models. A constant variance was assumed across the concentration range of the drug/radiation. The Gauss-Newton iterative algorithm was used in PROC NLIN in SAS (version 8.01) to find parameter estimates. To compare the observed response at each combination point to that predicted under the hypothesis of additivity, a prediction interval was used following the method of Gennings et al. [15]. An overall test for additivity [16] was based on testing the hypothesis that the mean response under the hypothesis of additivity is the true mean response. The estimated responses under the hypothesis of additivity were provided by the additivity model. The estimated responses for the true means were provided by the sample means of each mixing group.

Results

Effect of the combination of ILX 23-7553 and fractionated radiation on viable cell numbers

Previous work from this laboratory has demonstrated that the vitamin D_3 analogs, EB 1089 and ILX 23-7553, enhance the response of MCF-7 breast tumor cells to Adriamycin and ionizing radiation [10, 11, 12]. To determine if the vitamin D_3 analog ILX 23-7553 could effect a similar response of breast tumor cells to fractionated ionizing radiation, viable cell numbers were determined by trypan blue exclusion 24 h after the final irradiation (5×2 Gy) alone or with ILX 23-7553 pretreatment at 50 n*M*. Figure 1 shows that viable cell numbers were decreased by $72 \pm 3.1\%$ and $62 \pm 4.8\%$, respectively, for cells treated with fractionated ionizing radiation or ILX 23-7553 alone when compared to untreated controls. Pretreatment of MCF-7 breast tumor cells with ILX 23-7553 significantly enhanced the effectiveness of the irradiation treatment. Cells treated with ILX 23-7553 in combination with fractionated ionizing radiation showed a $93.2 \pm 0.7\%$ reduction in viable cell numbers. In previous work [10, 12], as well as

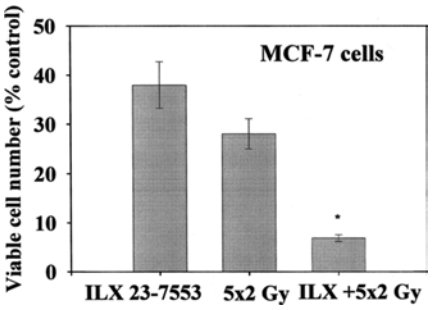

Fig. 1 Effects of ILX 23-7553 treatment followed by fractionated radiation on viable cell numbers. MCF-7 cells were treated with 50 n*M* ILX 23-7553 for 72 h followed by exposure to 5×2 Gy fractionated radiation. Viable cell numbers were determined by trypan blue exclusion 24 h after the final dose of radiation. The viable cell population was decreased by 62% and 72%, respectively, after treatment with ILX 23-7553 or fractionated radiation alone. Pretreatment with ILX 23-7553 followed by fractionated radiation decreased viable cell numbers by 93%. Results are presented as means ± SE for an average of 11 experiments. *$P < 0.001$ vs ILX 23-7553 alone and 5×2 Gy alone

in ongoing studies using another vitamin D_3 analog, EB 1089, in combination with fractionated radiation (manuscript in preparation), we have found that clonogenic survival studies provide results which closely parallel those generated by determining viable cell numbers.

Statistical analysis was also performed to evaluate the nature of the interaction between ILX 23-7553 and fractionated radiation. Cell viability data were compared for cells treated with ILX 23-7553 alone at doses as low as 5 n*M* and 10 n*M*, fractionated radiation alone at doses as low as 5×0.5 Gy and 5×1 Gy, and cells treated with ILX 23-7553 in combination with fractionated radiation in the model of additivity. Table 1 shows that the observed surviving fractions were not significantly different from those predicted by the model of additivity, indicating that the interaction between ILX 23-7553 and fractionated radiation is additive.

Promotion of apoptosis by the vitamin D_3 analog ILX 23-7553 in combination with irradiation

We have previously reported that irradiation alone does not induce a primary apoptotic cell death response in MCF-7 breast tumor cells [17]. To determine if the enhanced ability of fractionated ionizing radiation to decrease cell viability when pretreated with ILX 23-7553 could be due, in part, to the induction of apoptosis, the TUNEL assay was performed to assess DNA fragmentation. Figure 2 shows that DNA fragmentation, which is indicative of apoptotic cell death, was not induced by fractionated ionizing radiation alone; however, as in our previous study [12], a small but significant degree of DNA fragmentation occurred with ILX 23-7553 alone. When cells were treated with ILX 23-7553 followed by fractionated radiation the primary apoptotic cell death response was enhanced when compared to cells treated with ILX 23-7553 alone.

To determine the temporal response of MCF-7 cells to the combination of ILX 23-7553 and fractionated radiation, the extent of apoptosis induction was assessed between 5 and 7 days after irradiation. On days 5 and 6, there was little or no difference evident in the extent of apoptosis in the absence or presence of ILX 23-7553. On day 5, cells treated with ILX 23-7553 alone yielded 0.25% apoptosis while the combination of ILX 23-7553 and radiation yielded 0.29% apoptosis. On day 6, ILX 23-7553 treatment alone resulted in 0.51% apoptosis induction while the combination yielded 0.79% apoptosis. In contrast, while day-7 samples showed 2.60% of the cell population undergoing apoptosis with ILX 23-7553 alone, treatment with the ILX 23-7553 and fractionated radiation in combination resulted in apoptosis in 5.06% of the cell population. The induction of apoptosis by the combination on day 7 was significantly greater than that for ILX 23-7553 treatment alone. As expected, no apoptosis was observed in cells treated with fractionated radiation alone.

Although apoptosis was determined using only a single assay, current studies combining EB 1089 with fractionated radiation indicate that the extent of apoptosis assessed by TUNEL reflects that found using complementary assays such as alkaline unwinding and FACS analysis (manuscript in preparation).

Effect of the combination of ILX 23-7553 and fractionated radiation on recovery of breast tumor cells

Delayed recovery of proliferative function has been reported after irradiation of breast tumor cells in

Table 1 Additive interaction between ILX 23-7553and fractionated radiation. The percent reductions in viable cell numbers for each treatment alone were as follows: 5×0.5 Gy 29%, 5×1 Gy 53%, 5 n*M* ILX 23-7553 33%, 10 n*M* ILX 23-7553 63%. The results of viability assays were compared with values predicted using a statistical model of additivity. The observed values were not significantly different from those predicted under additivity ($P = 0.189$); therefore, the assumption of additivity was not rejected

Treatment combination		Observed response (fractional reduction in viable cell numbers)	Predicted response under assumption of additivity	95% prediction intervals under assumption of additivity
Radiation	ILX 23-7553 (n*M*)			
5×0.5 Gy	5	0.67	0.58	0.48–0.69
5×1.0 Gy	5	0.78	0.72	0.62–0.82
5×0.5 Gy	10	0.66	0.74	0.62–0.87
5×1.0 Gy	10	0.78	0.79	0.66–0.92

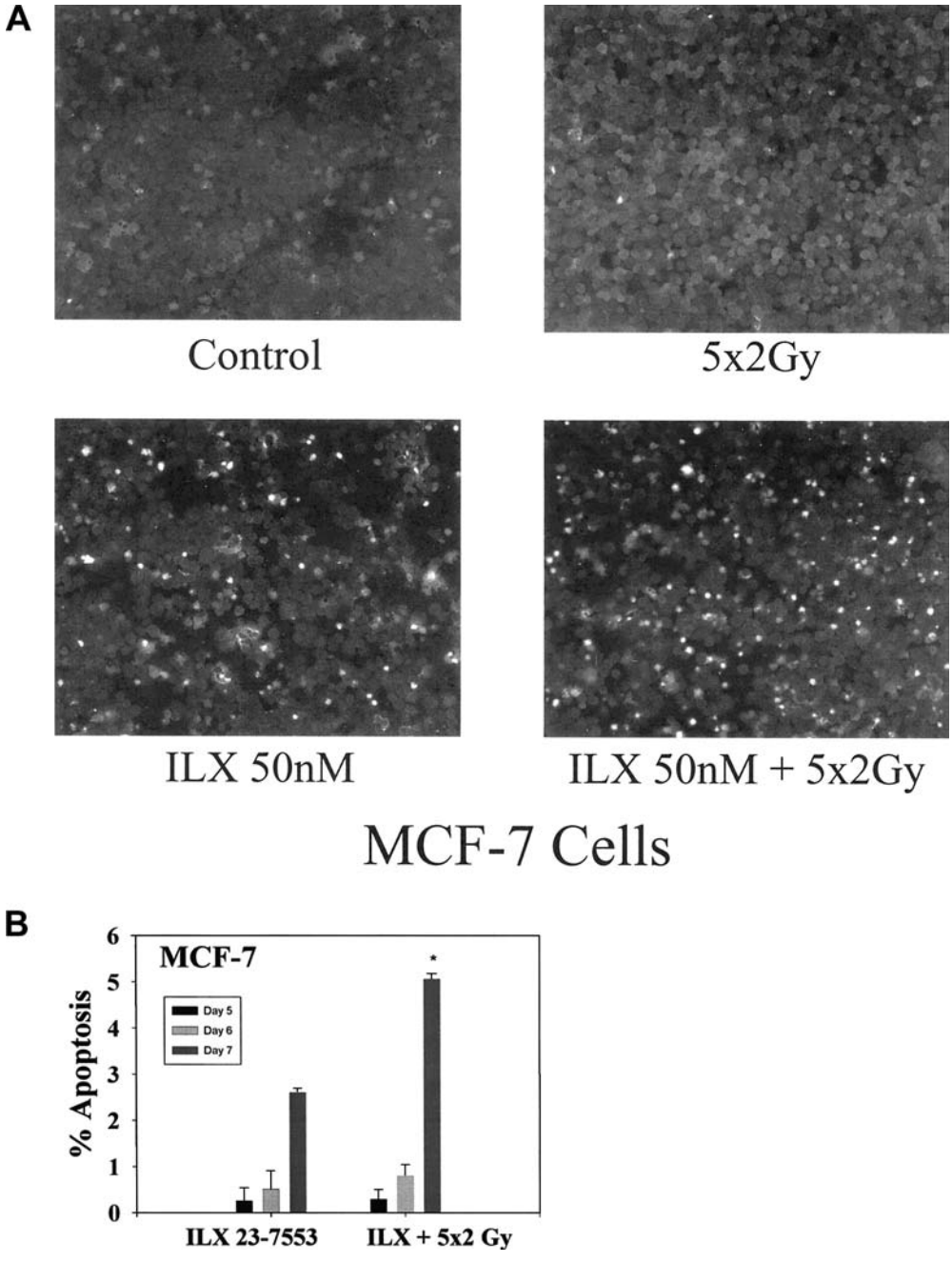

Fig. 2 **A** Induction of apoptosis by ILX 23-7553 in combination with fractionated ionizing radiation. Cells were cytospun onto glass slides after treatment with 50 n*M* ILX 23-7553 followed by exposure to 5×2 Gy radiation. **B** Percent TUNEL-positive cells. DNA fragmentation indicative of apoptosis was evident on day 5 in 0.25% and 0.29% of cells, respectively, following treatment with ILX 23-7553 alone or in combination with radiation. On day 6, ILX 23-7553 resulted in 0.51% apoptosis while pretreatment with ILX 23-7553 followed by fractionated radiation resulted in 0.79% apoptosis. ILX 23-7553 treatment alone and in combination with radiation resulted in 2.60% and 5.06% apoptosis, respectively, on day 7. Results are presented as means ± SE for an average of three experiments. $*P < 0.02$ vs ILX 23-7553 alone

culture [18]. To determine if the enhanced response of breast tumor cells to fractionated radiation would influence the ability of the cells to recover proliferative function, cells were exposed to radiation or ILX 23-7553 alone, or ILX 23-7553 prior to fractionated radiation, and viable cell numbers were assessed 9 days after the last dose of radiation, when recovery of proliferative function is detected (not shown). Figure 3 shows that with ILX 23-7553 alone, the number of viable cells was 61.6 ± 7.71% of controls. Radiation alone was quite effective in interfering with cell growth, resulting in only 6.4 ± 0.30% viable cells compared to controls. The combination of ILX 23-7553 and fractionated radiation reduced the number of viable cells by approximately threefold, to 2.2 ± 0.39% of controls.

Evidence for a selective effect of ILX 23-7553 on the response to radiation

In addition to assessing the effects of ILX 23-7553 on the breast tumor cell response to radiation, we investigated whether normal human BJ skin fibroblast cells would demonstrate increased growth suppression and/or loss of viability when treated with ILX 23-7553 prior to fractionated radiation. Under conditions identical to those used in the studies with MCF-7 breast

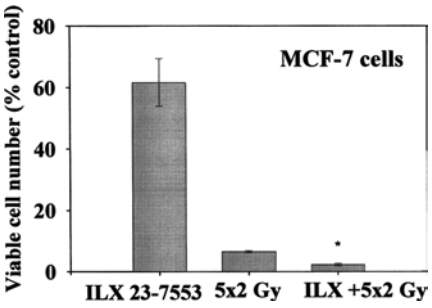

Fig. 3 Effects of ILX 23-7553 on recovery of MCF-7 cells treated with fractionated radiation. Viable cell numbers were determined by trypan blue exclusion 9 days after treatments were completed. The combination of ILX 23-7553 and radiation resulted in a threefold lower viable cell number when compared to cells treated with fractionated radiation alone. Results are presented as means ± SE for an average of six experiments. $^{*}P < 0.001$ vs 5×2 Gy alone

tumor cells, Fig. 4A indicates that viable cell numbers were decreased by 16.5 ± 4.2% and 56.9 ± 1.6%, respectively, in cells treated with 50 n*M* ILX 23-7553 and fractionated radiation alone, compared to growth of untreated controls. Cells treated with ILX 23-7553 in combination with fractionated radiation demonstrated a 57.3 ± 5.4% reduction in viable cell numbers, which did not differ significantly from cells treated with fractionated radiation alone. This assay was also repeated using 200 n*M* ILX 23-7553 and yielded a similar lack of interaction between ILX 23-7553 and radiation (data not shown). TUNEL analysis confirmed that ILX 23-7553 does not induce a primary apoptotic cell death response in wild-type BJ skin fibroblasts when used alone or in combination with fractionated radiation (Fig. 4B).

Discussion

Previous work from this laboratory has shown that the vitamin D_3 analogs EB 1089 and ILX 23-7553 enhance the response to ionizing radiation in breast tumor cells [10, 12]. Since these studies focused on a relatively high single dose of radiation (10 Gy) as well as a relatively high dose of ILX 23-7553 (200 n*M*), we were interested in applying these findings to a more clinically relevant exposure protocol, lower fractionated doses of radiation and a lower concentration of ILX 23-7553. Although there are no data currently available relating to the concentrations of this vitamin D_3 analog that might be achieved in the clinic, it is known that normal vitamin D_3 concentrations in the plasma are in the range 25–85 n*M* [19, 20, 21]. Therefore, 50 n*M* of ILX 23-7553 would not be expected to be associated with patient toxicity. The selectivity of the drug–radiation interaction was examined by assessing the response in a normal cell line, human BJ foreskin fibroblasts.

This work demonstrates that ILX 23-7553 interacted additively with fractionated radiation in terms of reducing viable cell numbers. In addition, prior exposure to ILX 23-7553 appeared to confer sensitivity to apoptosis in breast tumor cells. This observation is noteworthy because these cells lack caspase 3, which has frequently been implicated as a critical executioner caspase in the apoptotic cell death pathway [22]. Nevertheless, the relatively small absolute degree of apoptosis observed may be related to the absence of functional caspase 3 in these cells.

It is particularly noteworthy that a concentration of ILX 23-7553 which promotes apoptosis in combination with fractionated radiation in the breast tumor cell fails to influence the response to radiation or promote apoptosis in a normal human fibroblast cell line. This observation strongly suggests that the use of analogs such as ILX 23-7553 may provide a favorable therapeutic ratio in clinical situations where breast tumor cells are exposed to DNA damage from ionizing radiation or chemotherapeutic agents. A selective enhancement of the radiation response in MCF-7 cells has also been observed using the vitamin D_3 analog, EB 1089 (manuscript in preparation).

Studies were also performed to determine if ILX 23-7553 influences recovery of breast tumor cells after fractionated radiation. Cells were treated with ILX 23-7553 followed by irradiation with 5×2 Gy and cell numbers were monitored for an additional 9 days. This procedure is in contrast to assessment of cell viability shortly after the last dose of radiation in the design of the other experimental protocols presented in this report and permits cells to recover proliferative capacity. In this series of studies, we observed an approximately threefold greater decline in viable cell numbers in cells treated with ILX 23-7553 and radiation as compared to cells treated with radiation alone.

An important question to address is the role that apoptosis plays in radiation sensitivity in this experimental system. Although apoptosis does occur with the combination of ILX 23-7553 and radiation (as well as with ILX 23-7553 alone), the effect of the combination appears to be no greater than additive. In part, this may be related to the small absolute number of apoptotic cells. In addition, ILX 23-7553 may simply be altering the modes of growth inhibition and/or cell death. In this context, it is well established that the tumor cell response to irradiation can include growth arrest and cell death by apoptosis as well as through reproductive cell death or mitotic catastrophe [23, 24, 25].

In contrast to the apparent lack of impact of apoptosis on viability of the breast tumor cells, in the case of the fibroblasts, failure to enhance the response to radiation occurs coincident with the failure of the combination to promote apoptosis. In this case, as well as in the case of fibroblasts or HME31 breast epithelial cells exposed to EB 1089 in combination with 5×2 Gy of radiation, we have observed less than additive effects and no induction of apoptosis (manuscript in preparation). Therefore, the lack of signaling to promote apoptosis may provide some degree of protection to normal cells. Although the basis

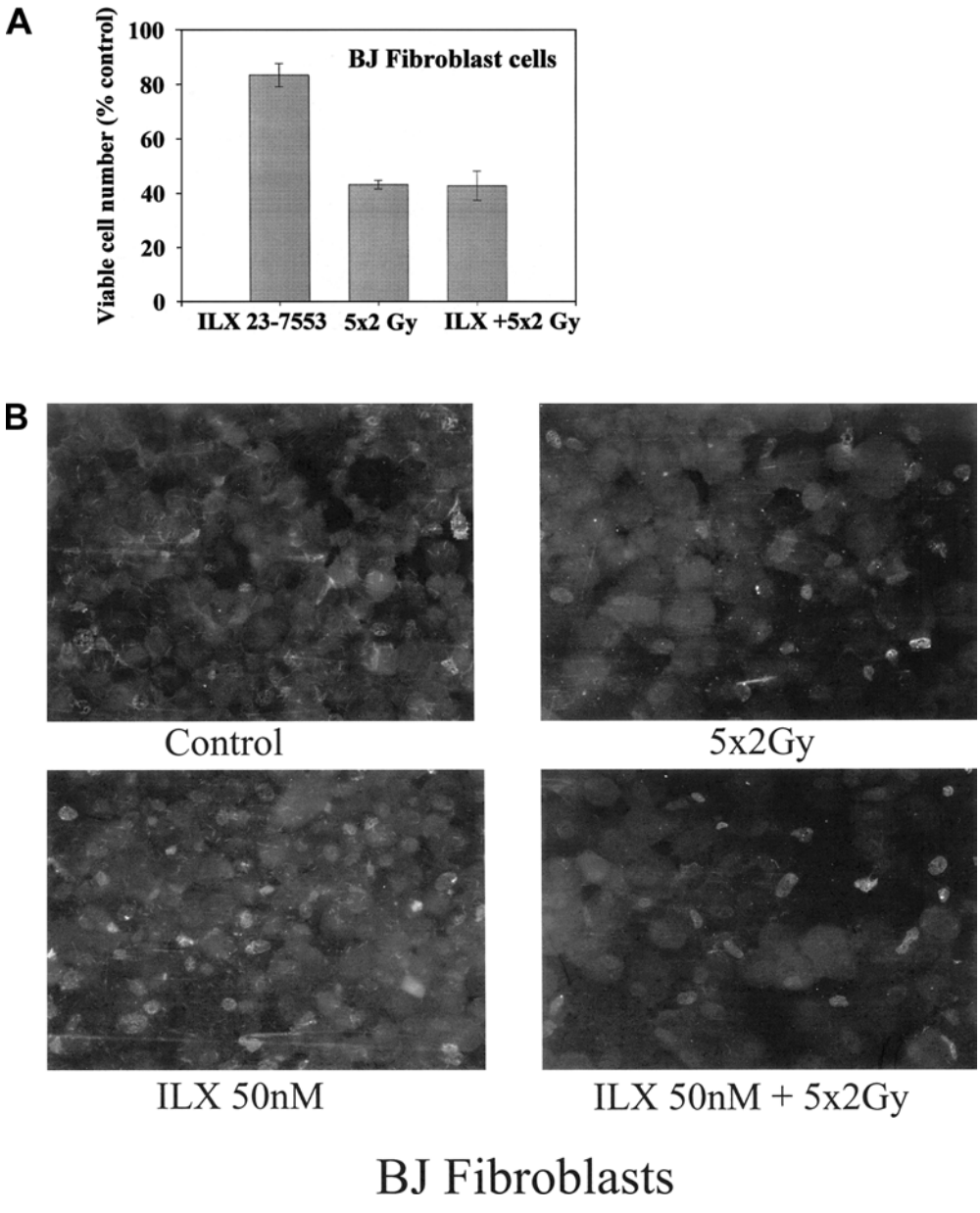

Fig. 4 **A** Effect of ILX 23-7553 treatment followed by fractionated radiation on the number of viable normal BJ fibroblasts. Cells were pretreated with 50 n*M* ILX 23-7553 for 72 h followed by exposure to 5×2 Gy fractionated radiation. Viable cell numbers were determined by trypan blue exclusion 24 h after the final dose of radiation. Cell numbers were decreased by 16.5% and 56.9%, respectively, after treatment with ILX 23-7553 and fractionated radiation alone. Treatment with ILX 23-7553 followed by fractionated radiation decreased viable cell numbers by 57.3%. Results are an average of four experiments. **B** Lack of apoptosis induction in normal BJ fibroblasts following treatment with ILX 23-7553 in combination with fractionated ionizing radiation. Cells were cytospun onto glass slides after pretreatment with 50 n*M* ILX 23-7553 followed by exposure to 5×2 Gy radiation

for these differential responses is not currently understood and is under investigation in our laboratory, a recent report demonstrates that vitamin D_3 protects normal cells while inhibiting growth of tumor cells [26]. Furthermore, our recent studies using tumor cell xenografts have demonstrated that the vitamin D_3 analog EB 1089 enhances the decline in tumor cell volume after irradiation without any evident increase in toxicity to the test animal [27]. Taken together, these observations support the concept of selective enhancement of the breast tumor cell response to conventional radiotherapy by vitamin D_3 and its analogs.

Acknowledgements This work was supported by U.S. Army DAMD 17-96-1-6167, American Institute for Cancer Research Award 99A091 and a grant from ILEX Products Inc. Mona Gupta was supported by American Institute for Cancer Research Award 2A068.

References

1. American Cancer Society (2001) Cancer facts and figures 2001. Atlanta, GA
2. Greenlee RT, Hill-Harmon MB, Murray T, Thun M (2001) Cancer statistics, 2001. CA Cancer J Clin 51:15
3. Pisani P, Parkin DM, Bray F, Ferlay J (1999) Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer 83:18
4. Colston KW, Mackay AG, James SY, Binderup L, Chander S, Coombes RC (1992) EB1089: a new vitamin D analogue that inhibits the growth of breast cancer cells in vivo and in vitro. Biochem Pharmacol 44:2273
5. Mathiasen IS, Colston KW, Binderup L (1993) EB 1089 a novel vitamin D analogue has strong antiproliferative and differentiation inducing effects on cancer cells. J Steroid Biochem Mol Biol 46:365
6. Wu G, Fan RS, Li W, Ko TC, Brattain MG (1997) Modulation of cell cycle control by vitamin D3 and its analogue EB1089 in human breast cancer cells. Oncogene 25:1555

7. Simboli-Campbell M, Narvaez CJ, van Weelden K, Tenniswood M, Welsh J (1997) Comparative effects of 1,25(OH)2D3 and EB1089 on cell cycle kinetics and apoptosis in MCF-7 breast cancer cells. Breast Cancer Res Treat 42:31
8. Narvaez CJ, Welsh J (2001) Role of mitochondria and caspases in vitamin D-mediated apoptosis of MCF-7 breast cancer cells. J Biol Chem 276:9101
9. Gewirtz DA, Gupta MS, Sundaram S (2002) Vitamin D3 and vitamin D3 analogues as an adjunct to cancer chemotherapy and radiotherapy. Curr Med Chem Anticancer Agents 2:683
10. Sundaram S, Gewirtz DA (1999) The vitamin D3 analog EB 1089 enhances the response of human breast tumor cells to radiation. Radiat Res 152:47
11. Sundaram S, Chaudhry M, Reardon D, Gupta M, Gewirtz DA (2000) EB 1089 enhances the antiproliferative and apoptotic effects of adriamycin in MCF-7 breast tumor cells. Breast Cancer Res Treat 63:1
12. Chaudhry M, Sundaram S, Gennings C, Carter H, Gewirtz DA (2001) The vitamin D analog ILX-23-7553 enhances the response to adriamycin and irradiation in MCF-7 breast tumor cells. Cancer Chemother Pharmacol 47:429
13. Gewirtz DA (2000) Growth arrest and cell death in the breast tumor cell in response to ionizing radiation and chemotherapeutic agents which induce DNA damage. Breast Cancer Res Treat 62:223
14. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 19:493
15. Gennings C, Schwartz P, Carter WH Jr, Simmons JE (1997) Detection of departures from additivity in mixtures of many chemicals with a threshold model. J Agric Biol Environ Stat 2:198
16. Bae DS, Gennings C, Carter WH Jr, Yang RS, Campain JA (2001) Statistical analysis of interactive cytotoxicity in human epidermal keratinocytes following exposure to a mixture of four metals. Toxicol Sci 63:132
17. Watson NC, Di YM, Orr MS, Fornari FA Jr, Randolph JK, Magnet JK, Jain PT, Gewirtz DA (1997) Influence of ionizing radiation on proliferation c-myc expression and the induction of apoptotic cell death in two breast tumor cell lines differing in p53 status. Int J Radiat Biol 72:547
18. Schmidt-Ullrich RK, Contessa JN, Dent P, Mikkelsen RB, Valerie K, Reardon DB, Bowers G, Lin PS (1999) Molecular mechanisms of radiation-induced accelerated repopulation. Radiat Oncol Investig 7:321
19. Jacques PF, Felson DT, Tucker KL, Mahnken B, Wilson PW, Rosenberg IH, Rush D (1997) Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. Am J Clin Nutr 66:929
20. Harris SS, Dawson-Hughes B (1998) Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. Am J Clin Nutr 67:1232
21. Kristinsson JO, Valdimarsson O, Sigurdsson G, Franzson L, Olafsson I, Steingrimsdottir L (1998) Serum 25-hydroxyvitamin D levels and bone mineral density in 16–20 year old girls: lack of association. J Intern Med 243:381
22. Janicke RU, Ng P, Sprengart ML, Porter AG (1998) Caspase-3 is required for alpha-fodrin cleavage but dispensable for cleavage of other death substrates in apoptosis. J Biol Chem 273:15540
23. Szumiel I (1994) Ionizing radiation-induced cell death. Int J Radiat Biol 66:329
24. Hendry JH, West CM (1997) Apoptosis and mitotic cell death: their relative contributions to normal-tissue and tumour radiation response. Int J Radiat Biol 71:709
25. Chang WP, Little JB (1992) Delayed reproductive death in X-irradiated Chinese hamster ovary cells. Int J Radiat Biol 60:483
26. Manggau M, Kim DS, Ruwisch L, Vogler R, Korting HC, Schafer-Kortin GM, Kleuser B (2001) 1Alpha, 25-dihydroxyvitamin D3 protects human keratinocytes from apoptosis by the formation of sphingosine-1-phosphate. J Invest Dermatol 117:1241
27. Sundaram S, Sea A, Feldman S, Strawbridge R, Hoopes PJ, Demidenko E, Binderup L, Gewirtz DA (2003) The combination of the a potent vitamin D3 analog, EB 1089 with ionizing radiation reduces tumor growth and induces apoptosis of MCF-7 breast tumor xenografts in nude mice. Clin Cancer Res (in press)