Korean Journal of Oral and Maxillofacial Radiology 2006; 36 : 189-98

Effects of 2-deoxy-D-glucose and quercetin on osteoblastic differentiation and mineralization in irradiated MC3T3-E1 cells

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ABSTRACT

Purpose : To investigate the in vitro response of MC3T3-E1 osteoblastic cells to X-ray in the presence and absence of 2 deoxy-D-glucose (2-DG) and quercetin (QCT).

Materials and Methods : The MC3T3-E1 cells were cultured in an α-MEM supplemented with 5 mM 2-DG or 10 μM QCT and then the cells were incubated for 12 h prior to irradiation with 2, 4, 6, and 8 Gy using a linear accelerator (Mevaprimus, Germany) delivered at a rate of 1.5 Gy/min. At various times after the irradiation, the cells were processed for the analyses of proliferation, viability, cytotoxicity, and mineralization.

Results : Exposure of the cells to X-ray inhibited the tritium incorporation, 3-(4, 5-dimethylthiazol-2yl-)-2, 5-diphenyl tetrazolium bromide (MTT)-reducing activity, and alkaline phosphatase (ALP) activity, and caused cytotoxicity and apoptosis in a dose-dependent manner of the X-ray. This effect was further apparent on day 3 and 7 after the irradiation. RA+2-DG showed the decrease of DNA content, cell viability, and increase of cytotoxicity rather than RA. ALP activity increased on day 7 and subsequently its activity dropped to a lower level. 2-DG suppressed the calcium concentration, but visual difference of number of calcified nodules between RA and RA+2-DG was not noticed. RA+QCT showed the increase of DNA content, cell viability, but decrease of cytotoxicity and subG1 stage cells in the cell cycle, and increased calcified nodules in von Kossa staining rather than the RA. ALP activity showed significant increases on day 7 and subsequently its activity dropped to a lower level.

Conclusion : The results showed that the 2-DG acted as a radiosensitizing agent and QCT acted as a radioprotective agent respectively in the irradiated MC3T3-E1 osteoblast-like cells. (***Korean J Oral Maxillofac Radiol 2006; 36 : 189-98***)

KEY WORDS : 2-deoxy-D-glucose; Quercetin; Radiation; MC3T3-E1 Cells

Introduction

External irradiation is an important modality in the treatment of head and neck malignancies. In spite of this valuable effect, irradiation delays fracture union, increases the incidence of fibrous union, causes osteoradionecrosis, and adversely affects the quality of bone grafts.[1-3] Histologically, these irradiated bones consistently produce the decreased osteocyte number, suppressed osteoblastic activity, and diminished vascularity observed.[4] Furthermore, the histologic changes in irradiated bone correlate with impaired mechanical properties.[5] The main aim of successful radiation therapy is to eliminate completely the neoplastic cells without causing significant damage to normal tissues. A number of biophysical and radiobiological approaches are being studied to achieve this aim. Theoretically, an interesting possibility could be the use of a radiosensitizer for tumors. 2-deoxy-D-glucose (2-DG), a glucose antimetabolite and an inhibitor of the glycolytic pathway, has been suggested as a suitable candidate to achieve this objective.

The radio-modifying effects of 2-DG could be mediated by changes in the metabolic flux and reduction of energy flow by the inhibition of glycolysis. The cellular processes leading to the repair and fixation of radiation-induced DNA damage require a continuous supply of metabolic energy in the form of adenosine triphosphate (ATP) produced by the respiratory and/or the glycolytic pathway. If the energy supply is reduced below a certain critical threshold, repair of DNA damage is completely inhibited. 2-DG inhibits DNA repair in cells by causing reduction of glycolysis, thereby enhancing the radiation damage at high 2-DG concentrations.[6] Investigations to test this suggestion in tumor bearing animal models showed that 2-DG could indeed increase the radiation-induced tumor cell loss, tumor regression and survival of mice with tumors.[7]

Received September 18, 2006; accepted November 8, 2006
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The presence of 2-DG after irradiation increased radiation-induced micronuclei formation.[8,9] Examination of the effects of 2-DG in normal laboratory mice under similar experimental conditions revealed that the presence of 2-DG actually reduced the radiation-induced chromosomal damage in normal bone marrow cells. The differential effects of 2-DG on the radiation response of normal and malignant cells could result from energy-linked modifications of repair processes as suggested earlier.[10-13]

Flavonoids are a group of about 4000 naturally occurring compounds that are ubiquitously present in fruits, vegetables, other plants. They have many biological effects and it is believed that many of these effects are due to their antioxidant properties. Flavonoids may exert antioxidant effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers or metal ion chelators. One of the most abundant natural flavonoids, present in a large number of fruits and vegetables, is quercetin (QCT). Its antioxidant effect was documented in many experimental studies.[14-16] An animal study demonstrated the protective effect of QCT against ultraviolet A-induced oxidative stress.[17] Rats given QCT before irradiation had a high level of antioxidant enzymes, such as glutathion reductase and catalase, than those not given QCT.[15] It may be postulated that QCT exerts a protective effect on irradiation damage with its positive antioxidant effect.

Many works[16-18] suggested that the effects of radiation on irradiated MC3T3-E1 osteoblasts include decreased proliferation, decreased collagen production, increased alkaline phosphatase (ALP), and induces terminal differentiation. The effects of 2-DG or QCT on the radiation response in MC3T3-E1 cells are poorly understood. The present work was undertaken to study the in vitro response of MC3T3-E1 osteoblastic cells to X-ray irradiation and to investigate further the effect of 2-DG and QCT on the radiation-induced damage.

Materials and Methods

1. Cell culture

Murine osteoblastic MC3T3-E1 cells (ATCC, CRL-2593) were cultured in alpha-minimum essential medium (a-MEM) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) and antibiotics. The cultures were maintained at 37°C with a gas mixture of 5% CO_2/95% air and subcultures were performed with 0.05% trypsin-0.02% EDTA in Ca^{2+}, Mg^{2+}- free phosphate buffered saline (DPBS; Gibco BRL Co. USA). One million cells per millimeter were resuspended in either 2 mL or 100 μL media for spreading onto either 6-well or 96-well flat-bottomed plates (Falcon, Becton Dickinson, NJ, USA), respectively. Cultures were switched to a fresh batch of the same medium twice weekly.

2. Cellular irradiation and differentiation induction

MC3T3-E1 cells were replated in 6- or 96- well flat-bottomed plates at doses of 5×10^5 cells/ mL, and the final content of media in each plate were recommended as follows : 6-well plate, 2 mL, 24-well plate, 500 μL, 96-well plate, 200 μL. After the cell numbers reached the level of 70-80% confluence, culture media were changed with α-MEM supplemented 5 mM 2-DG or 10 μM QCT, and then the cells were incubated for 12 hours before irradiation with 2, 4, 6, and 8 Gy using an X-ray delivered at a rate of 1.5 Gy/min using a linear accelerator (Mevaprimus, Germany).

The cells were then incubated in α-MEM supplemented with 10% FBS, 5 mM β-glycerol phosphate, and 50 μg/mL ascorbic acid. Fresh medium was supplied to cells on 3-day intervals depending on experimental purpose. At various times after irradiation, the cells were processed for analyses of proliferation, viability, cytotoxicity, and mineralization.

1) Measurement of DNA synthesis

The level of DNA synthesis by MC3T3-E1 cells after irradiation was measured by adding 1 μCi of [methyl-^{3}H] thymidine deoxyribose (TdR) into each well for the last 24 h of the culture period. The cells were then collected with a cell harvester (Inotech Inc., Switzerland) and the TdR content were measured using a liquid scintillation counter (Packard Instrument Co., Downers Grove, IL, USA).

2) Measurement of cell viability

3-(4, 5-dimethylthiazol-2yl-)-2, 5-diphenyl tetrazolium bromide (MTT) was used for the evaluation of cell viability. MC3T3-E1 cells were cultured in α-MEM supplemented with 10% FBS and antibiotics and irradiated at various levels. At various times, 10 μL of MTT solution (5 mg/μL in PBS as stock solution) was added into each well and then the cells were incubated for 4 h more hours at 37°C. To measure the absorbance, 70 μL of acidic isopropanol was added to each well and the plates were read at 560 nm using a SpectraCount™ (Packard, Instrument Co.) ELISA reader.

3) Determination of cytotoxicity

Cellular cytotoxicity induced by the radiation was measured using a trypan blue exclusion assay. The cells were cultured in α-MEM supplemented with 10% FBS and irradiated (2, 4, 6,

8 Gy). At various times, the cells were stained with 0.4% trypan blue and 100 cells were counted for each treatment. Cytotoxicity was calculated as follows: % cytotoxicity= [(total cells-viable cells)/ total cell] × 100.

4) Determination of cell cycle (Propidium Iodide staining)

Following irradiation, cells were fixed with 80% ethanol at 4°C for 24 h, and then incubated overnight at 4°C with 1 mL of propidium Iodide (PI) staining mixture (250 μL of PBS, 250 μL of 1 mg/mL RNase in 1.12% sodium citrate, and 500 μL of 50 μg/mL PI in 1.12% sodium citrate). After staining, 1×10^4 cells were analysed using the FACS Calibur® system (Becton Dickinson, San Jose, California, U.S.A.)

5) Measurement of alkaline phosphatase activity

At various times during the irradiation, cells were collected and vigorously resuspended in a lysis buffer (50 mM Tris-HCl, pH 7.2, 0.1% Triton X-100, and 2 mM $MgCl_2$). ALP activity of each sample was determined by an established technique with *p*-nitrophenyl phosphate as the substrate.

Two hundred milliliter of ALP yellow (pNPP) liquid substrate for ELISA (Sigma Chemical Co., A3469) was transferred to each well of 96-multiwell ELISA plates, and then each sample (50 μL) was added to the wells. After incubation for adequate time, the reaction was stopped by the addition of 50 μL of 3N NaOH and optical density was measured at 405 nm using a Spectra Count™ (Packard, Instrument Co.) ELISA reader. ALP activity was expressed as mM pNPP/mg protein.

6) Determination of Ca deposition

Control and irradiated MC3T3-E1 cells were cultured in α-MEM supplemented 10% FBS, 5 mM β-glycerol phosphate, and 50 μg/mL ascorbic acid. At various times (0-3 weeks) during the irradiation, the deposition of calcium was measured by inductively coupled argon plasma-atomic emission spectrometry (ICP-AES) (Spectroframe-EOP; Spectro Analytical Instruments GmbH, Kleve, Germany).

7) Determination of crystalline formation (von Kossa staining)

After various times of the irradiation, MC3T3-E1 cells were fixed in 10% formaldehyde for 30 min at room temperature and then washed several times with 10 mM Tris-HCl, pH 7.3.

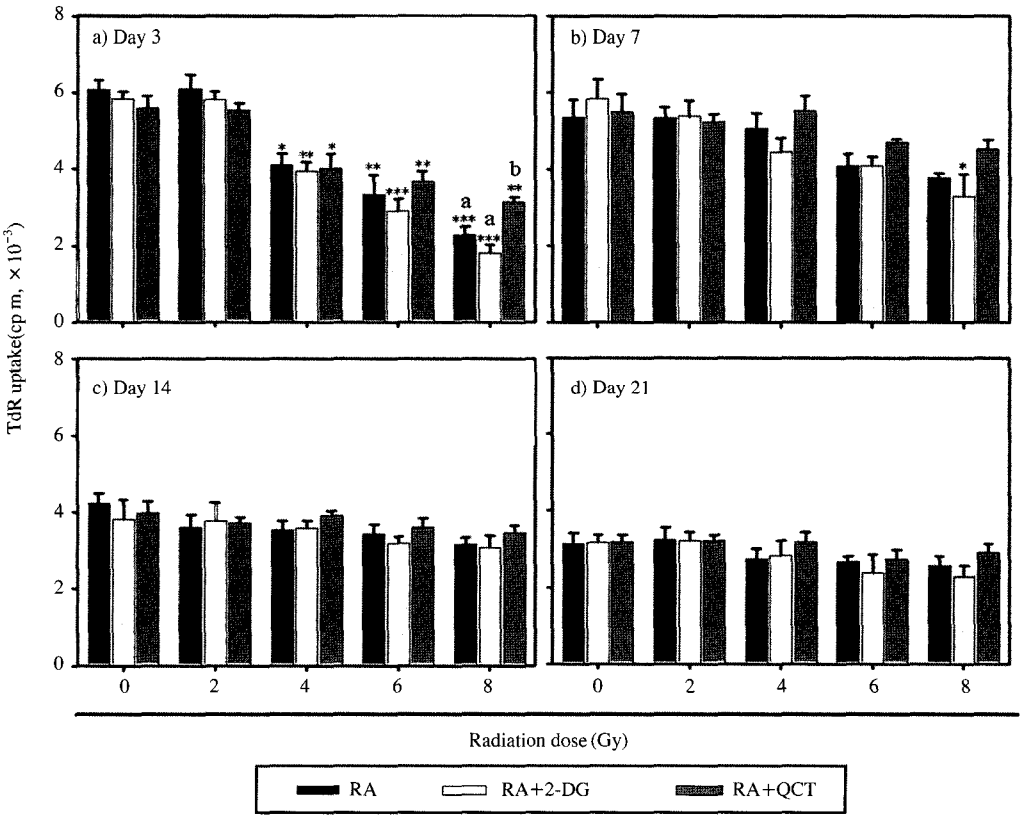

Fig. 1. Effects of irradiation on DNA synthesis in MC3T3-E1 cells. The cells were exposed to 2, 4, 6 and 8 Gy of X-ray in the presence and absence of 2-DG or QCT. After the exposure, TdR uptake was measured on day 3(a), 7(b), 14(c), and 21(d), respectively. Each bar shows the mean±S.E. of three separate experiments. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ represent significant differences between the experimental and control values. $^{a\text{-}b}$ indicate significant differences among the groups irradiated with the same dose.

The fixed cells were incubated with 5% silver nitrate for 5 min in sunlight, washed twice with distilled water, and then observed under microscope.

3. Statistical analyses

The results are expressed as mean ± standard error (SE). One-way ANOVA using SPSS ver 10.0 software was used for multiple comparisons. In addition, statistical analysis was performed using ANOVA followed by Duncan's test and a value of $p < 0.05$ was considered significant.

Results

1. DNA synthesis

To determine the level of DNA synthesis by MC3T3-E1 cells after irradiation, the TdR uptake level of the DNA was determined. This is because the rate of DNA synthesis is a reflection of proliferation under many conditions.

Fig. 1a shows the rate of DNA synthesis on the 3rd day after irradiation; there was a dose-dependent decrease in TdR uptake level in the irradiation group (RA) and the irradiation with 2-DG group (RA+2-DG) and irradiation with QCT group (RA+QCT). Especially there was a significant decrease at 4, 6, and 8 Gy. RA+2-DG showed a decreased TdR uptake compared with RA, but a higher tendency than RA+QCT. Therefore, RA+QCT showed the highest level of TdR uptake at 8 Gy. On day 7, 14, and 21 after irradiation, there was no significant difference of the TdR uptake between groups (Fig. 1).

On day 3 after irradiation, MC3T3-E1 cells showed proliferating stages and various DNA content levels. Doses of 4, 6, 8 Gy suppressed the proliferation of MC3T3-E1 cells and RA+2-DG suppressed TdR levels more effectively than RA, but not significantly. RA+QCT showed increasing of the TdR level rather than RA, especially at 8 Gy. On day 14 and 21 after irradiation, most of the cells ceased proliferation, the DNA content were decreased, and there was no marked differences between the various radiation doses among the groups.

2. Cell viability and morphological changes

MC3T3-E1 cells were treated with 2, 4, 6, 8 Gy and cell viability was determined by the MTT assay. There was a dose-dependent decreased in cell viability on day 3, 7, and 14. RA+2-DG and RA+QCT showed a similar tendency compared with RA, but RA+2-DG showed more reduced cell viability compared with RA or RA+QCT. RA+QCT showed less reduced cell viability than RA (Fig. 2).

Despite the reduced cell viability, when observed visually, the cells did not appear to be dead or dying. On day 3 there was an apparent decrease in cell volume at 8 Gy (Fig. 3-a). On day 14 at 8 Gy, the amount of calcification was decreased compared with the control or RA+2-DG (Fig. 3-b). On day 21 at 8 Gy, there was a decreased quality of calcification because of undifferentiated cells, especially in RA+2-DG. But the

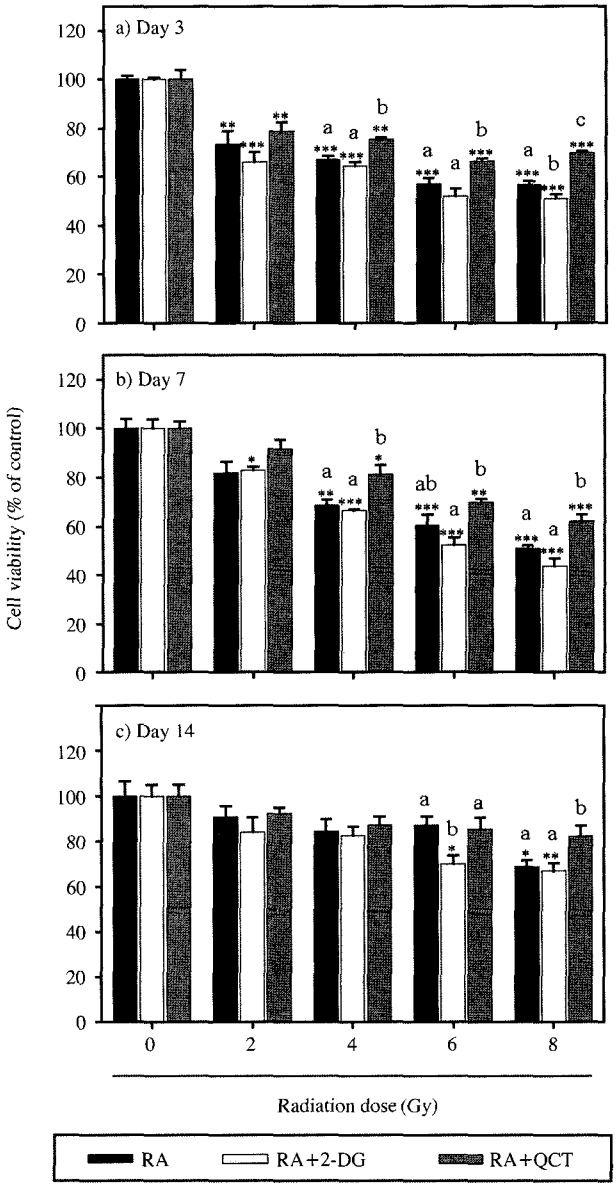

Fig. 2. Effects of irradiation on cell viability in MC3T3-E1 cells. The cells were exposed to 2, 4, 6 and 8 Gy of X-ray and cell viability was measured by MTT assay on day 3 (a), 7 (b), and 14 (c), respectively. [a-c] indicate significant differences among the groups irradiated with the same dose.

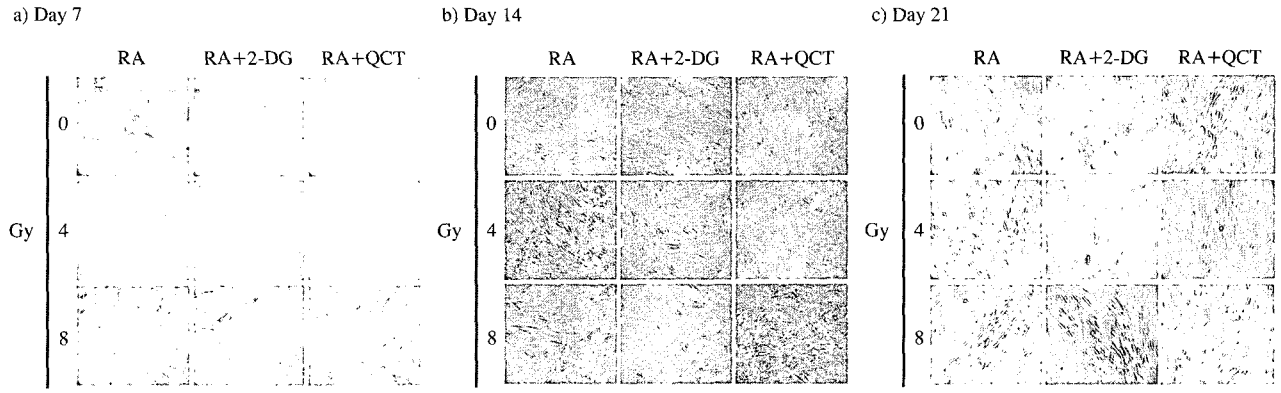

Fig. 3. Effects of irradiation on morphological changes in MC3T3-E1 cells. Micrographic features were taken on day 3 (a), 14 (b), and 21 (c) after the irradiation. A representative result from three independent experiments is shown.

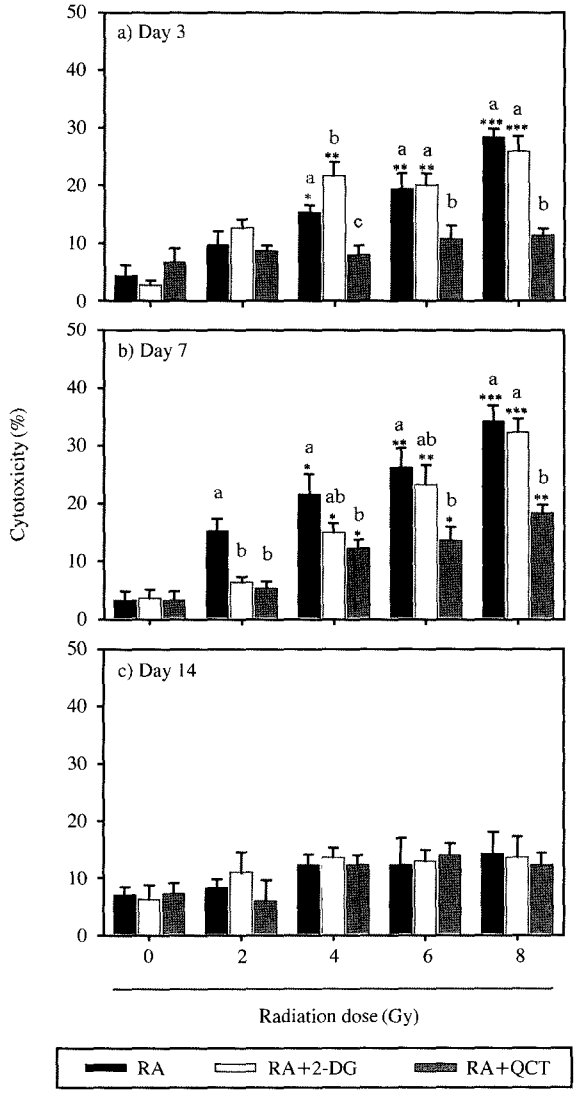

Fig. 4. Effects of irradiation on cytotoxicity in MC3T3-E1 cells. The cells were irradiated with the indicated X-ray and processed for the trypan blue exclusion assay after 3(a), 7(b), and 14(c) days of the treatment.

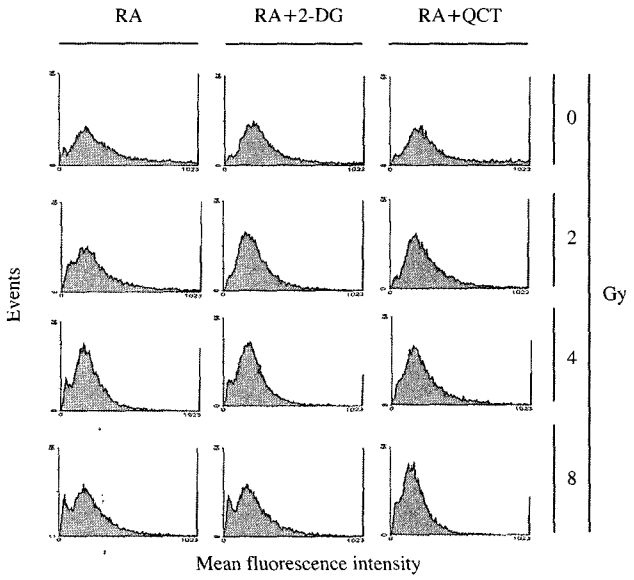

Fig. 5. The effects of irradiation on the cell cycle progression in MC3T3-E1 cells. Cells were irradiated with the indicated dose of X-ray, stained with propidium iodide, and analysed by flow cytometry on day 7 after irradiation. A representative result from triplicate experiments is shown.

morphological differences could not be differentiated between RA and RA+QCT (Fig. 3).

3. Cytotoxicity

On day 3 there was a dose dependent increase in cytotoxicity in RA. RA+2-DG showed an increasing of cytotoxicity in accordance with the radiation dose, especially at 4, 6, 8 Gy. But RA+QCT did not show an increasing of cytotoxicity. RA and RA+2-DG showed higher levels of cytotoxicity than RA +QCT (Fig. 4-a). On day 7 there was a similar tendency as day 3 (Fig. 4-b). On day 14 there was no difference correlating

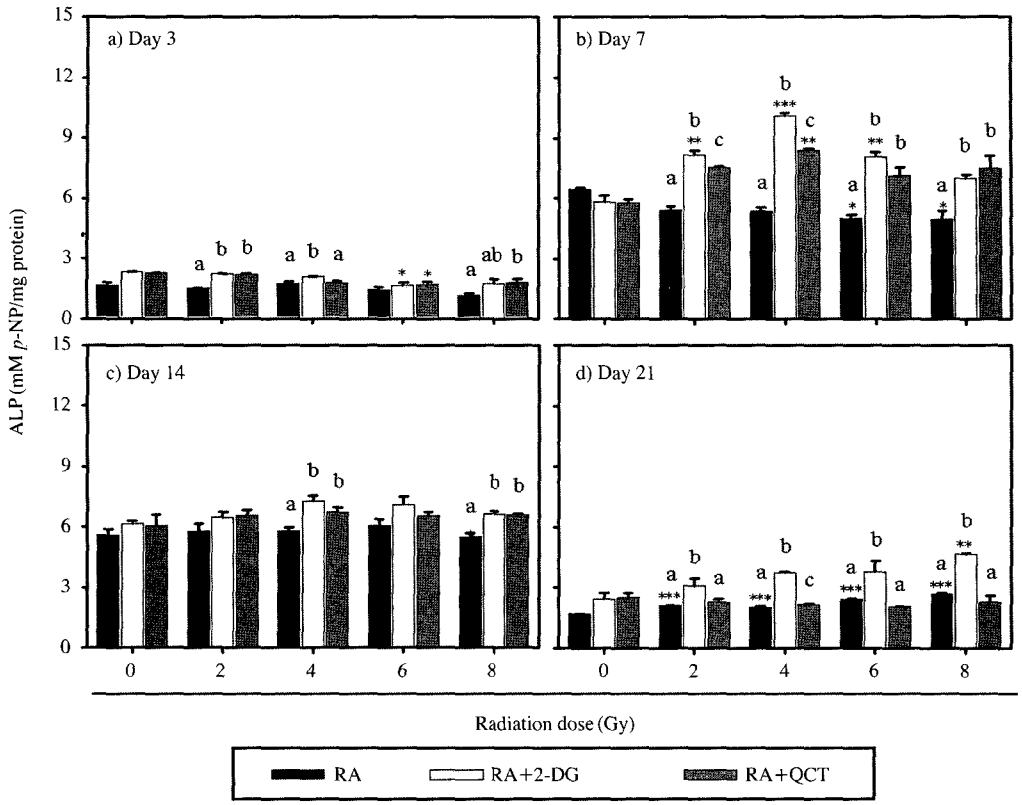

Fig. 6. Effects of irradiation on ALP activity in MC3T3-E1 cells. The cells were exposed to 2, 4, 6 and 8 Gy of X-ray and ALP activity was measured on day 3 (a), 7(b), 14(c), and 21(d), respectively.

with the radiation doses or between groups (Fig. 4-c).

4. Propidium Iodide (PI) staining

To ascertain if the effects of radiation and 2-DG or QCT was linked to cell cycle, PI staining was performed by flow cytometry. On day 7 the fraction of cells in the sub-G1 phase was increased in accordance with the radiation dose. The flow cytometric data indicates that 8 Gy caused a significant increasing of cells in sub-G1 phase. With the presence of QCT during liquid holding at 8 Gy, the radiation-induced sub-G1 phase cells were decreased; however, 2-DG did not markedly influence the fraction of sub-G1 phase cells (Fig. 5).

5. ALP activity

The activity of ALP increased significantly and reached the maximal level on day 7 and dropped to a level on day 3 in RA. RA+2-DG increased ALP activity at 2, 4, 6, and 8 Gy on days 7, and 21 compared with RA. RA+QCT increased ALP activity on days 3, 7, and 14. On day 21 RA+QCT showed similar ALP activity compared with RA (Fig. 6).

6. Calcium deposition

On days 3 and 7 at 2 and 4 Gy, RA+QCT showed an increase in calcium deposition. RA and RA+2-DG showed similar calcium levels at 6 and 8 Gy. On day 14, RA+2-DG showed lower calcium levels at 6 and 8 Gy compared with RA, and RA+QCT showed increasing calcium levels at 8 Gy (Fig. 7).

7. von Kossa staining

On day 21 bone-like nodules had formed in RA (Fig. 8-b). At 4 and 8 Gy the number of bone-like nodules were decreased. RA+2-DG showed a similar number of bone-like nodules with RA, but RA+QCT exhibited an increased number of bone-like nodules (Fig. 8).

Discussion

In vivo bone formation progresses through distinctive developmental stages that include commitment of mesenchymally-derived cells to the osteoblast lineage, mitogenetic growth of osteoblast precursors, expression of the differentiat-

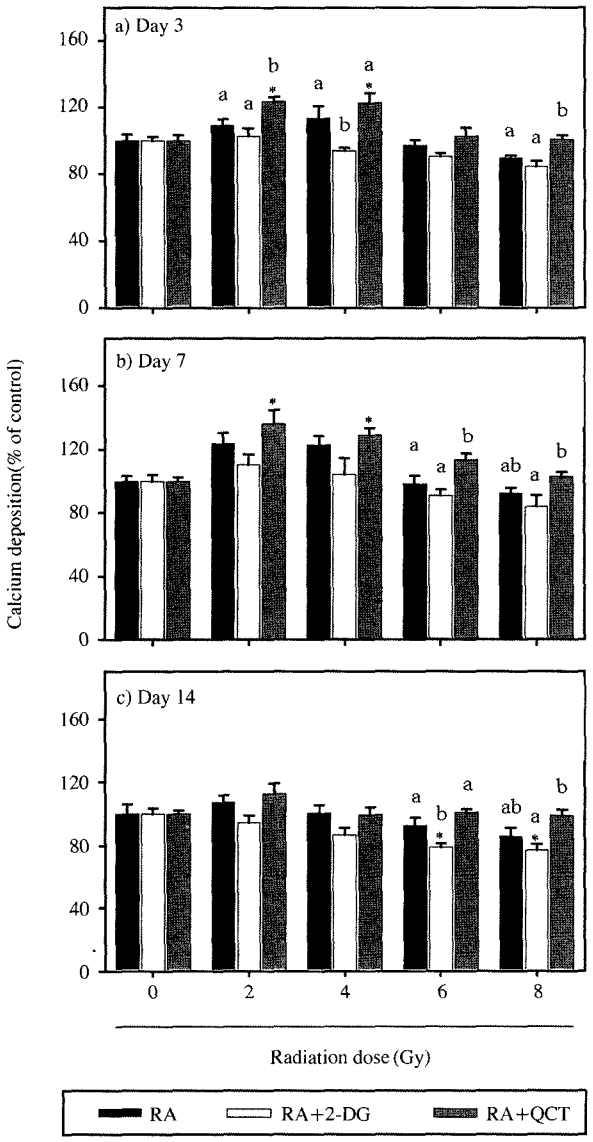

Fig. 7. Effects of irradiation on calcium deposition in MC3T3-E1 cells. The cells were exposed to 2, 4, 6 and 8 Gy of X-ray and calcium deposition was measured on day 3(a), 7(b), and 14(c), respectively.

ed osteoblast phenotype, and ultimately, formation of mineralized extracellular matrix by postmitotic, terminally differentiated osteoblasts.

MC3T3-E1 osteoblast-like cells in culture demonstrate a temporal sequence of development characterized by distinct proliferative and differentiated stages. The initial stage of development is characterized by cell proliferation and high levels of type I collagen gene expression, biosynthesis, and secretion, but the cells remain undifferentiated as evidenced by low levels of ALP activity, inability to effectively assimilate newly synthesized collagen into extracellular matrix, and the absence of mineralization. Down-regulation of replication and the expression of differentiated osteoblast functions characterize the next developmental stage, which occurs approximately 10 days after plating. At this time, an increase in alkaline phosphatase activity -a marker of the osteoblast phenotype- and the acquisition of specialized bone function, consisting of collagen deposition into an extracellular matrix, occur. A final phase of MC3T3-E1 maturation begins at about day 20 and is defined by matrix calcification associated with a progressive increase in extracellular matrix accumulation and alkaline phosphatase activity.[18,19]

Irradiation induces terminal differentiation in some other culture systems, such as human neuroblastoma[20] and fibroblasts.[21] In these systems, induction of differentiation is accompanied by cessation of proliferation. In the osteoblast, it seems that inhibition of the cell proliferation by irradiation may induce osteoblast differentiation.[2,22] The altered rate of proliferation appeared to be a function of an attenuated growth rate in irradiated cells and not an initial radiation-induced cell death, as remained evident with repeated subcultivation of viable cells.

In this study, the culture of MC3T3-E1 osteoblast cells, irradiation induced dose-dependent decreases in total DNA

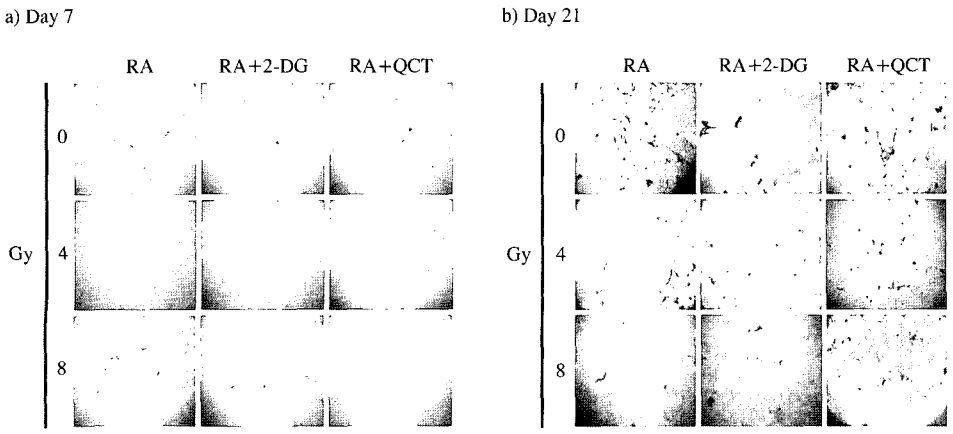

Fig. 8. Effects of irradiation on mineralization of MC3T3-E1 cells. The microphotographs show the calcific nodules according to the duration of culture after irradiation in the presence and absence of 2-DG or QCT in MC3T3-E1 cells (× 100). A representative result from triplicate experiments is shown.

content, decreases in cell viability and calcium deposition, but increases in cytotoxicity. DNA content and total calcium content in whole cell cultures were decreased by irradiation, indicating that the decreased number of osteoblasts by irradiation consequentially decreased the calcified matrix of bone. The activity of ALP increased significantly and reached the maximum level around day 7 and its activity dropped to a lower level on day 3. This pattern of ALP activity is similar to that of the rat calvarial osteoblasts.[23] Rissanen et al.[24] showed a dose-dependent decrease in bone mineral content after irradiation of the humerus and femur of growing and adult dogs. Matsumura et al.[22] showed that the calcium content of irradiated cells was greater than that of non-irradiated. One reason that the increase of ALP activity showed no direct relation to the effect of irradiation in promoting calcification.

2-DG is a structural analogue of glucose differing at the second carbon atom by the substitution of hydrogen for a hydroxyl group. 2-DG, like glucose, enters cells and is phosphorylated to the DG phosphate but, due to the unsuitability of the phosphate as a substrate for glucose phosphate isomerase, undergoes little further metabolism. As a consequence of enhanced transport and hexokinase activity and low glucose phosphatase, DG phosphate accumulates to higher levels in cells.

2-DG as a modifier of radiosensitivity has been based on the assumption that 2-DG probably interferes with the energy-linked processes of repair and fixation of DNA lesions. The exact mechanisms underlying the modulation of radiation responses by 2-DG are not yet known. An understanding of the underlying mechanism necessitates the elucidation of the molecular nature of the radiation-induced critical lesions and the processes associated with its repair/fixation and effect of 2-DG thereupon.[6]

2-DG treatment causes death in human breast cancer cell lines by the activation of the apoptotic pathway. The breast cancer cells treated with 2-DG accelerated their own demise by initially expressing high levels of glucose transporter protein, which allowed increased uptake of 2-DG and subsequent induction of cell death.[25]

In mouse bone marrow, the administration of 2-DG within a few minutes after irradiation reduces the radiation-induced cytogenetic damage. On the other hand, in transformed cells, 2-DG induced an enhancement of radiosensitization by halopyrimidines. The differential effects of 2-DG on the radiation response of normal and malignant cells could result from energy linked modifications of repair processes as suggested earlier.[10,11,26] However, repair processes, and consequently the expression of radiation damage, could also be influenced by changes in the duration of cell cycles, since 2-DG has been shown to inhibit cell proliferation. DNA repair is more strongly dependent upon glycolysis than the rate of cell proliferation. The reason for a stronger dependence of repair processes on the rate of glycolysis is not presently clear and need further study.

Evidence indicates that DNA double strand break (dsb) constitutes one of the critical molecular lesions leading to various cellular responses, including chromosome aberrations and lethality.[27,28] Efficient and accurate rejoining of DNA breaks is crucial for the maintenance of genetic integrity and for cell survival. Because of the importance of dsb in the manifestation of radiation damage and the differential modification of damage by 2-DG, Jha and Pohlit[29] had investigated the effect of 2-DG on the repair of dsb and on cell survival. In exponentially growing euoxic EAT cells, they showed that 2-DG inhibited the repair of those dsb which were repaired with slow kinetics, probably by reducing the cellular ATP level. As a consequence, cells were left with more dsb, leading to reduced cell survival. In hypoxic and euoxic tumor cells, 2-DG was known to enhance radiation damage. However, the magnitude of enhancement was less in euoxic cells when compared with hypoxic tumor cells.[27]

To achieve an increase of radiation damage in tumors, it would be necessary to maintain relatively high concentrations of 2-DG (~5 mM) in the tumor for a few hours after irradiation. If the concentration of 2-DG in the tumor is low, a reduction of the radiation damage in cancer cells may result.[6]

After irradiation below 2 Gy, cells successfully repair from sublethal DNA damage. Dwarakanath and Jain[30] reported that the radiation effects were pronounced at 4 Gy when compared with controls, while at doses above 6 Gy there was significant radiation toxicity and death. In this study, 2-DG could enhance the radiation damage in proliferating MC3T3-E1 cells. 2-DG suppressed the proliferation of cells, and showed decreased calcification. RA+2-DG showed the decrease in DNA content and cell viability, and increased cytotoxicity. The amount of ALP was not similar to RA, but increased on day 7. Therefore, the 2-DG modified the effects of radiation to decrease cell proliferation and to induce cell differentiation. And at 6 and 8 Gy, RA+2-DG showed decreased calcium content compared to RA, but it was difficult to differentiate calcification nodules. The radiomodifying mechanism of 2-DG is not clearly known; further studies concerning the effect at DNA levels and the comparison between the normal cells and cancer cells are needed.

QCT is a common bioflavonol composed of two benzene rings linked with a heterocyclicpyrone ring and a water-soluble pigment. QCT is a potent antioxidant, providing cardiovascular protection by reducing oxidative damage to LDL-cholesterol, the underlying cause of heart disease. Its antioxidant effect has been documented in many in vitro and in vivo experimental studys. QCT has an ability to scavenge reactive oxygen species produced by the photo-oxidative stress induced by ultraviolet light.[15] Also QCT, an inhibitor of heat-shock response, dose dependently suppresses the p53 protein accumulation induced by X-rays at more than 100 μM[31]. In conjunction with X-rays, flavonoid compounds were tested for their effects on Reuber H35 hepatoma cells.[32] In this combination, QCT caused an enhancement of radiation-induced cell death. This enhanced cytotoxicity was only observed when the flavonoids were applied following irradiation and is attributed to a decrease repairing of DNA radiation damage with a concomitant reduction in the rate of cell repopulation. QCT not only seem to act as radiation enhancers but, it also seems to inhibit potential antitumor activity. A study[16] on the antioxidative flavonoids in gamma-ray irradiated mice suggested that the radioprotective effect may be attributed to the hydroxyl radical scavenging potency in a direct or an endogenous enzyme mediated manner.[16] In spite of extensive investigation, the mechanism of QCT has not yet been completely elucidated.

In this study, RA+QCT showed increases in DNA content and cell viability, but decreases in cytotoxicity and subG1 stage cells during the cell cycle. The amount of ALP increased on day 7 and its activity dropped to a control level on day 21. QCT suppressed the decrease of cell proliferation by irradiation and induced cell differentiation at the early differentiation stage, and did not affect at the maturation stage, thereupon QCT did not affect the cell differentiation.

The effect of radiation in MC3T3-E1 cells was modified by 2-DG and QCT. 2-DG suppressed the proliferation of cells, induced the differentiation, and decreased calcification. QCT induced cell proliferation and differentiation, and increased calcification. Consequently, 2-DG acted as a radiosensitizing agent and QCT acted as a radioprotective agent in the irradiated MC3T3-E1 osteoblast-like cells.

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