Radiat Environ Biophys
DOI 10.1007/s00411-009-0237-9

ORIGINAL PAPER

Rituximab sensitizes a Burkitt lymphoma cell line to cell killing by X-irradiation

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Received: 9 September 2008 / Accepted: 17 July 2009

Abstract Clinical trials with rituximab in combination with chemotherapeutic regimens have shown promising results. Data on the effects of rituximab treatment in combination with irradiation are, however, limited and inconsistent. This study aims to investigate the effects of rituximab (R) on cell death induced by X-irradiation in Raji lymphoma cells and to evaluate its mechanisms. We found the cell growth inhibition by irradiation was enhanced by additional rituximab exposure both in cells precultured with rituximab followed by irradiation (R + irradiation) or in cells treated in the reverse sequence (irradiation + R). R + irradiation combination treatment induced more apoptotic cells than irradiation and irradiation + R treatment as early as 12 h after treatment. At 24 h, both combination treatments, R + irradiation and irradiation + R, showed apoptotic cells, which were significantly different from irradiation alone. G2/M cell cycle arrest was observed after irradiation alone and the combination treatment. The combination treatment revealed an elevation in reactive oxygen species (ROS) generation in a radiation dose-dependent manner. In addition, rituximab enhanced the cell growth inhibition and apoptotic cell death induced by the oxidative agent, H_2O_2. We propose that rituximab mediates a significant in vitro radiosensitizing effect and induces cell cycle changes and apoptosis in Raji cells. ROS probably play an important role in these events.

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Introduction

Traditional radiotherapy and chemotherapy are still the two major approaches in Non-Hodgkin's lymphoma (NHL) treatment. Although the initial response rates to chemotherapy are high, relapse eventually occurs and subsequent chemotherapy regimens are incapable of yielding long-term remission. Rituximab, a human–mouse chimeric monoclonal antibody (mAb) that targets CD20, is the first therapeutic antibody approved by the Food and Drug Administration for the treatment of NHL patients. In vitro data demonstrate that rituximab sensitizes tumor cells to the effects of conventional chemotherapeutic drugs. Clinical trials with rituximab in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy or other chemotherapeutic regimens, either as first-line therapy or for patients with relapsed or refractory aggressive B-cell NHL, have also shown promising results in terms of clinical response rates (Boehrer et al. 2006; Buske et al. 2006; Dearden 2007).

Extensive in vitro and preclinical studies suggest that antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct effects of transmembrane signaling leading to apoptosis and growth arrest may be responsible for the observed clinical effects of rituximab (Jazirehi 2004; Winter et al. 2006). In addition, Bcl-2 plays an important role in a tumor cell's ability to survive cytotoxic stimuli (Alas et al. 2001). The optimal use of the drug combined with chemotherapy in many clinical settings has yet to be clarified. Data on the

Published online: 04 August 2009

effects of rituximab on radiobiological endpoints in lymphoma cells are sparse and contradictory. In one study, rituximab was shown to radiosensitize lymphoma cells and to realize its radiosensitizing activity by modulating apoptosis- and cell cycle-related proteins (Skvortsova et al. 2005, 2006). Recently, Kapadia et al. (2008) reported that rituximab exerts radiosensitizing and radioprotective effects at different doses on Raji lymphoma cells. Some researchers suggest that these radiobiological effects of rituximab should be carefully considered in the design of radioimmunotherapeutic trials (Kapadia et al. 2008; Skvortsova et al. 2005, 2006). Ionizing radiation is known to induce intracellular oxidative stress. Recent studies suggest that reactive oxygen species (ROS) may play an important role during apoptosis induction. Many stimuli such as tumor necrosis factor, anticancer drugs, and chemopreventive agents stimulate cells to produce ROS and subsequent activation of the cell death machinery. ROS can directly activate the mitochondrial permeability transition and result in mitochondrial membrane potential (MMP) loss (Yi et al. 2002). The induction of apoptosis triggered by irradiation combined with rituximab was found to be stipulated by mitochondrial dissipation and AIF translocation in Raji cells (Skvortsova et al. 2006). ROS were reported to play a role in cell death mediated by combining complement and high levels of rituximab (Bellosillo et al. 2001). These data prompted us to investigate the influence of rituximab on Raji cell death and on cell cycle distribution in response to X-ray irradiation in vitro. We also evaluated the contribution of intracellular ROS production on the effects of combination treatments.

Materials and methods

Cell line and irradiation

The CD20-expressing human Burkitt's lymphoma cell line, Raji, is from Prof. Gao's collection (School of Life Sciences, Lanzhou University, Lanzhou, China). The Raji cells were grown in RPMI-1640 medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Si-Ji-Qing Biotechnology, Hangzhou, China), 2 mM glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, and 100 mg/ml streptomycin (complete medium [CM]) at 37°C and 5% CO_2 in a humidified incubator. On the day of the experiment, cells were counted by means of a hemocytometer following standard Trypan Blue exclusion methodology and were used only if viability exceeded 90%. A stock solution of 10 mg/ml of rituximab, chimeric IgG, was purchased from Roche. 2′,7′-Dichlorofluorescein diacetate (DCFH-DA) was obtained from Beyotime and sulforhodamine B (SRB) was obtained from Sigma. For X-irradiation treatment, a 8MV Varian Clinac 2100 accelerator at a dose rate of 80 cGy/min was used. The doses ranged from 0 to 10 Gy. All procedures were carried out at room temperature.

Determination of cell survival

Cells were seeded at a density of 2×10^4 cells in 1 ml CM per well in 48-well plastic plates. Cell cultures were treated by X-irradiation or H_2O_2 alone, or combined with rituximab. The combination treatment contained two ways of rituximab addition sequence. Rituximab (R) was either added 12 h before X-irradiation (R + irradiation), or within 1 h after X-irradiation (irradiation + R). In the case of H_2O_2 treatment, the combination treatment also contained the two, R + H_2O_2 and H_2O_2 + R treatment. Cell growth was measured in the SRB-staining assay by determination of whole culture protein. Briefly, upon completion of the incubation, the cells were fixed by adding 50 µl of cold 80% (w/v) trichloroacetic acid (TCA) (final concentration, 10% TCA) for 1 h at 4°C. The plates were then washed five times with tap water and dried. One hundred microliters of SRB solution (0.4% in 1% acetic acid, w/v) was added to each well, and the culture was incubated for 10 min at room temperature. Unbound SRB was removed by washing five times with 1% acetic acid, and the plates were air dried. The bond stain was solubilized with Tris buffer, and the absorbance was read on an automated spectrophotometric plate reader at a single wavelength of 515 nm. Viability assays were performed at 7 days of post-irradiation incubation and at 2 days of post-H_2O_2 incubation under standard conditions. All experiments were performed at least thrice. The survivals were calculated by: mean optical density (OD) of treated cells/mean OD of control cells × 100%.

Cell cycle analysis

Cell cycle analysis was performed by flow cytometric measurement of the DNA content of the cells. Raji cells were treated by X-irradiation alone and in combination with rituximab in two ways as described above. The exposed cells were incubated and harvested at appropriate time points. The harvested cells were centrifuged and resuspended in 3 ml 95% ice-cold ethanol for fixation, and stored at −20°C. Before flow cytometry, the cells were stained at 4°C for at least 3 h in phosphate-buffered saline containing 0.1% Triton X-100, 5 µg/ml propidium iodide (Sigma), and 20 µg/ml RNase A. The cells were incubated for at least 15 min at room temperature in the dark, and the DNA content of the preparation was analyzed with flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). At least 10,000 events were acquired using CELL Quest software.

Determination of apoptotic cells

Apoptotic cells were assayed by morphology and the Annexin V-Kit. Following exposure to X-irradiation alone and in combination with rituximab, cells were washed and divided into two parts. One was used for morphologic evaluation with acridine orange–ethidium bromide (AO–EB) double staining. Cells were stained with double staining and assessed by fluorescence microscopy. A viable cell possesses a uniform bright green nucleus and orange cytoplasm. An early apoptotic cell still has a green nucleus, but its chromatin becomes condensed and manifests bright green patches. A late apoptotic cell shows bright orange areas of condensed chromatin in the nucleus, and a necrotic cell manifests uniform bright orange nucleus. At least 300 cells were counted in each slide. The other part was incubated with AV-FITC, PI (2 μg/ml), and binding buffer (140 mM NaCl, 2.5 mM CaCl, and 10 mM HEPES/NaOH, pH 7.4). Flow cytometry was performed on FACS Calibur flow cytometer and analyzed by means of CellQuest software (Becton Dickinson, San Jose, CA). Cells progress from annexin V-negative/propidium iodide-negative (AV^-/PI^-) to AV^+/PI^- to AV^+/PI^+ as they become apoptotic.

Measurement of reactive oxygen species

The intracellular accumulation of ROS was assayed using DCFH-DA, an uncharged, cell permeate fluorescent probe. Inside the cells, DCFH-DA is cleaved by nonspecific esterases forming DCFH, which is the nonfluorescein form and is oxidized to the fluorescent compound DCF in the presence of H_2O_2. DCFH-DA was commonly used to detect H_2O_2, but it is now accepted that it is also sensitive to other peroxides. DCFH-DA was dissolved in DMSO to a working concentration of 20 mM before use. Raji cells were treated with irradiation alone and in combination with rituximab, incubated at 37°C for various periods of time, and then collected by centrifugation. Cells were resuspended in RPMI-1640 medium without red phenol, and loaded with 1 μM DCFH-DA. The intensity of fluorescence was recorded by a fluorescence spectrophotometer, with an excitation filter of 485 nm and an emission filter of 535 nm. The ROS level was calculated as [ROS] = mean intensity of exposed cells/mean intensity of unexposed cells.

Data analysis

The radiation survival curves were fitted according to the linear-quadratic model:surviving fraction = $\exp(-\alpha D-\beta D^2)$. ID50 is the radiation dose causing 50% growth inhibition. Radiosensitization (RS) was expressed by the dose enhancement factor (DEF): ID50(−rituximab)/ID50(+rituximab). Student's *t*-test was used to determine the significance of differences between two group means.

Results

Radiosensitization by rituximab

The radiation dose–survival curves of the Raji cells treated with irradiation alone or in combination with rituximab are shown in Fig. 1. Treatment with irradiation resulted in dose-dependent growth inhibition of Raji cells. A typical shoulder was observed on the curve with irradiation alone, the cell growth inhibition in Raji cells by irradiation was enhanced by addition of rituximab at a concentration of 20 μg/ml. To determine if rituximab administration sequence may affect radiosensitization of tumor cells, growth inhibition assays were performed by pretreating cells with 20 μg/ml of rituximab followed by increasing doses of irradiation (R + irradiation) or the reverse (irradiation + R). ID50 for Raji cells treated with X-irradiation alone, R + irradiation and irradiation + R exposure were 4.1, 2.5, and 2.9 Gy, respectively. While both rituximab treatments lead to radiosensitizing effects on Raji cells, the response to irradiation + R treatment was smaller compared with R + irradiation treatment, with corresponding DEF figures of 1.4 and 1.6, respectively.

Cell cycle analysis

In addition to the cell proliferation assay, cell cycle distributions were analyzed by flow cytometry of propidium

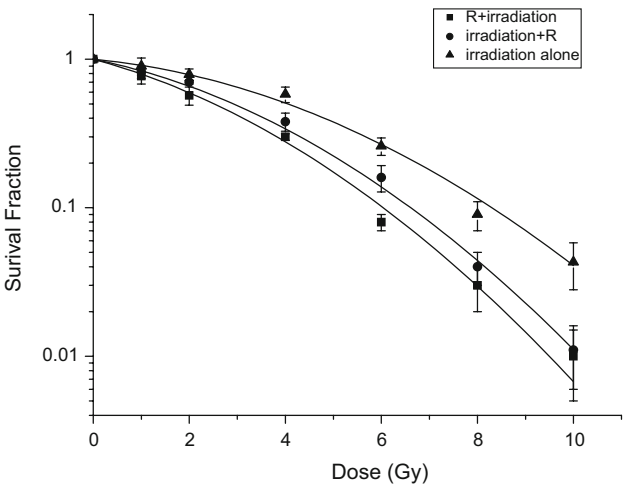

Fig. 1 X-irradiation survival curves, generated by fitting individual data of the SRB-staining assay to a linear-quadratic model, for X-irradiation alone and irradiation combined with rituximab in two ways (R + irradiation and irradiation + R). Calculated values of α and β, repectively, are 0.07 and 0.025 for irradiation alone; 0.2 and 0.03 for R + irradiation and 0.15 and 0.03 for irradiation + R. *Error bars* indicate standard deviations. The experiment was repeated thrice

iodide stained Raji cells. Compared with untreated cells, irradiation alone and in combination with rituximab lead to a reduced proportion of cells in S phase and induced accumulation in the G2/M phase, which peaked after 24 h exposure (Fig. 2). Treatment with irradiation alone and in the combination irradiation + R resulted in comparable cell cycle distributions. Interestingly, at doses lower than 4 Gy, Raji cells treated with R + irradiation showed a significantly lower increase in the proportion of G2/M phase cells than after the two other treatment modes (Fig. 2).

Rituximab enhances apoptosis induction after irradiation

To determine the induction of apoptosis, Raji cells treated with irradiation alone and in combination with rituximab were incubated for 12, 24, and 48 h and subjected to AO/EB double staining and FACS analysis. As shown in Table 1, in time-course experiments R + irradiation combination treatment induced more apoptotic cells than irradiation and irradiation + R treatment as early as 12 h after treatment. At 24 h, compared with irradiation alone, irradiation + R treatment showed obvious apoptotic cells as well, similar to R + irradiation. However, Raji cells revealed accelerated apoptosis in response to irradiation at 48 h, which showed no significant difference with the cells with R + irradiation and irradiation + R treatment. Although early and late apoptotic cell could be distinguished by AO/EB staining under fluorescence microscopy, less apoptotic cells were detected with AO–EB double staining assay than with FACS Calibur flow cytometer. This discrepancy may due to some early apoptotic cells that manifested no obvious morphologic changes but were detected by FACS. As to apoptosis enhancement with rituximab after irradiation, the results obtained with both AO/EB double staining and FACS assay showed the same trend.

ROS contribute to radiosenzitization by rituximab in Raji cells

To investigate the signaling factors involved in the radiosensitizing effects of rituximab on Raji cells, we measured the kinetics of total ROS generation by using DCFH-DA. In Raji cells incubated with rituximab, an increased production of ROS that lasted for 4–6 h and gradually disappeared 8 h later was observed. Ionizing radiation is known to induce intracellular oxidative stress. As seen in Fig. 3, either irradiation alone or combination treatment with rituximab led to maximum increase in ROS generation within 4 h post treatment, followed by a gradual decrease to the basal level at 24 h. Exposure of Raji cells to X-irradiation resulted in an about three-fold enhanced ROS generation compared to the unirradiated control. Further increase in ROS levels was observed after combined treatment with irradiation and rituximab. The levels of ROS at 2 h in Raji cells treated with irradiation alone, R + irradiation, and

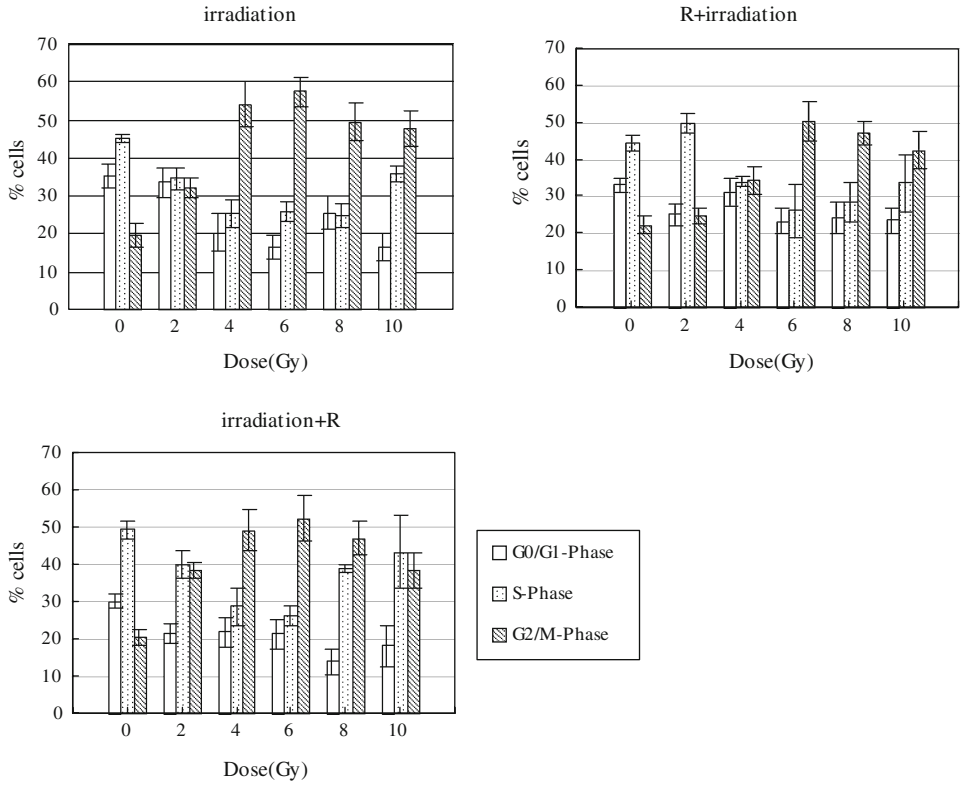

Fig. 2 Cell cycle analysis of Raji cells after X- irradiation and combination treatment with rituximab. Indicated is the percentage of cells in G0/G1, S, and G2/M phase 24 h after irradiation alone, R + irradiation, and irradiation + R treatment. *Error bars* indicate standard deviations. The experiment was repeated thrice

Table 1 Effects of rituximab on irradiation-induced apoptosis

Assay methods	Culture time after treatment (h)	2 Gy			6 Gy		
		Irradiation	R + irradiation (%)	Irradiation + R	Irradiation	R + irradiation	Irradiation + R
AO/EB	12	2.6 ± 0.5	5.6 ± 0.5**	2.5 ± 0.4	4.1 ± 0.4	8.2 ± 0.5**	5.1 ± 0.4
	24	4.1 ± 0.6	11.7 ± 1.1**	9.4 ± 0.5**	8.4 ± 0.8	14.8 ± 1.3**	15.4 ± 0.7**
	48	13.4 ± 1.1	15.7 ± 1.7	16.6 ± 1.1*	21.6 ± 2.8	22.6 ± 2.8	19.9 ± 2.2
AV/PI	12	6.75 ± 0.71	19.91 ± 0.79**	10.21 ± 0.89*	5.85 ± 2.36	29.24 ± 1.84**	21.97 ± 1.12*
	24	14.15 ± 2.42	27.84 ± 1.95*	23.03 ± 1.91*	27.73 ± 1.30	38.49 ± 1.70*	35.84 ± 0.88*
	48	29.20 ± 1.80	34.97 ± 1.60*	33.8 ± 1.90	38.78 ± 1.88	42.88 ± 3.31	46.34 ± 5.32

Raji cells were treated with ionizing radiation and in combination with rituximab. The kinetics of apoptosis was measured by AO/EB double staining and AV/PI co-staining after treatment with irradiation, rituximab, and combination thereof for 12, 24, and 48 h. Data are given as mean and standard deviation. The experiments were repeated two to three times

* Denotes marked difference when compared with irradiation alone. * $P < 0.05$, ** $P < 0.01$

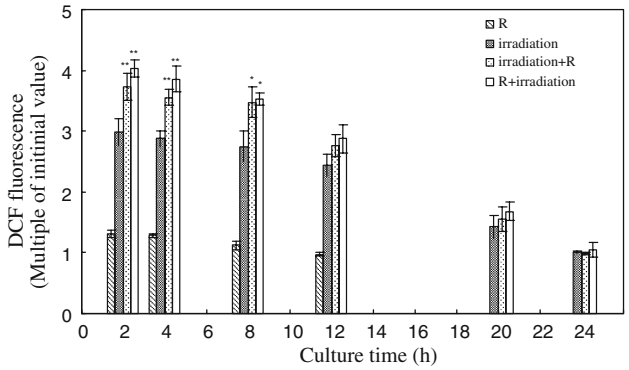

Fig. 3 Enhancement of ROS production by combination treatment of irradiation with rituximab Raji cells treated with 4 Gy of X-irradiation alone, or in combination of with rituximab. At given time intervals, cells were incubated with 1 μM of DCFH-DA for 30 min and analyzed by fluorescence spectrophotometry. Data are given as mean and standard deviation. The experiment was repeated thrice. *$P < 0.05$; **$P < 0.01$ versus irradiation alone

irradiation + R increased 2.98-fold, 4.02-fold, and 3.73-fold related to unirradiated controls, respectively. To determine the role of intracellular redox changes or oxidative stress on the cell death induced by rituximab combined with irradiation, cells were treated with an oxidative agent, H_2O_2, alone or combined with rituximab. Application sequences tested were similar to those used in combinations of irradiation and rituximab. Application of H_2O_2 induced a concentration dependent growth inhibition and apoptosis in Raji cells (Fig. 4). Both combination treatments, R + H_2O_2 and H_2O_2 + R, showed similar significant reduced cell survival and enhanced apoptosis. The viability of cells incubated with H_2O_2 at a concentration of 100 μM for 48 h was 65.6% of the control value. The viabilities of cells treated with rituximab in the presence of 100 μM H_2O_2 were decreased in a statistically significant manner to 50.2% in R + H_2O_2 ($P < 0.05$) and 48.9% in H_2O_2 + R ($P < 0.05$), respectively (Fig. 4a). Quantitation of apoptotic cells showed a significant increase in the apoptotic rate (from 4.2 to 16.4%) after Raji cells were treated with 100 μM H_2O_2 for 48 h. When cells were treated with rituximab (20 μg/ml) in the presence of 100 μM H_2O_2 for 48 h, the percentage of apoptotic cells increased to 30.8% in R + H_2O_2 ($P < 0.01$) and 29.0% in H_2O_2 + R ($P < 0.01$), respectively (Fig. 4b). We conclude that rituximab sensitizes Raji cells to intracellular oxidative stress caused by H_2O_2, which implies that ROS contribute to the radiosensitizing effect of rituximab in combination treatments.

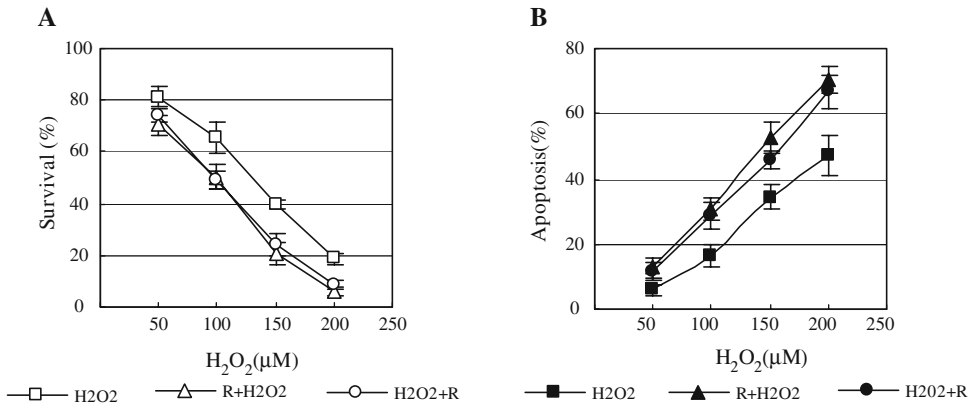

Fig. 4 Rituximab enhances H_2O_2-induced growth inhibition in Raji cells. **a** Cells were treated with different concentrations of H_2O_2, alone or in combination with rituximab. After treatment, cell viability was estimated by the SRB method. Data are given as mean and standard deviation. The experiment was repeated thrice. **b** Effects of rituximab on apoptosis induced by H_2O_2. Data are given as mean and standard deviation. The experiment was repeated thrice

Discussion

Berinstein et al. (1998) showed that 3 months after rituximab treatment, rituximab serum levels remained at 20.3 μg/ml. Thus, the concentration of 20 μg/ml rituximab was selected for our study.

The colony-forming assay is traditionally used to measure cellular radiation susceptibility and is the accepted standard cell survival assay. However, this assay, which requires long-term culture (usually 10–21 days), is time-consuming and laborious and is open to subjective interpretation due to manual counting. Moreover, the assay is less reliable for cells with low colony-forming capacity. Several researchers have reported the use of cell proliferation assays, such as tetrazolium (MTT)- and SRB-base assays, as an alternative to the colony-forming assay for measurement of radiation susceptibility in cultured cells (Ristić-Fira et al. 2008). After radiation treatment, cells destined to die can still undergo one or more cell divisions. Therefore, it may take a considerable period of time before these cells express their radiation-induced damage. Previous studies have shown that the results of the SRB assay are highly comparable to those obtained with clonogenic assays when exponential growth is guaranteed during the assay and when sufficient time is allowed for delayed radiation-induced cell death to occur (Pauwels et al. 2003, 2006; Simoens et al. 2006). To allow sufficient time for the delayed radiation-induced cell death, the duration of the assay should be equivalent to at least six cell doubling times. Raji cells are moderately tumorigenic and do not grow in soft agar, nor in dilution limit. In the present study, we investigated the radiosensitizing effect of rituximab by SRB. In addition, to overcome a decrease in reliability due to very low cell concentrations at high doses of irradiation, the cells were seeded in 48-well plates instead of 96-well plates. This created a broader margin for longer incubation times of higher cell concentrations. In the present study, the cell growth inhibition in Raji cells by irradiation, from low doses to high doses, was enhanced by 20 μg/ml concentration of rituximab, which was in accordance to Skvortsova et al.'s report (2005, 2006).

Previous studies have revealed that signaling through CD20 by rituximab can induce certain lymphoma cell lines to undergo apoptosis (Alas et al. 2001; Jazirehi 2004; Winter et al. 2006). Raji cells treated with rituximab at concentrations up to 200 μg/ml for 48 h did not exhibit a specific cell cycle arrest and programmed cell death, but only a slight growth inhibition in vitro (data not shown). Therefore, a major role of cell cycle redistribution in rituximab-induced radiosensitization is unlikely. Raji cells are mutated in p53 and thus we did not observed G1 arrest after treatment with rituximab, X-rays, and combination thereof. It is noteworthy that the extent of G2/M arrest induced by R + irradiation treatment at 24 h was significantly lower than that induced by irradiation alone or irradiation + R at doses below 4 Gy. As the delay in G2/M phase is proposed to be a period for DNA repair (Wilson 2004), we postulated that at low dose of X-irradiation, rituximab interfere with the repair of X-irradiation-induced DNA damage somehow by regulating the cell cycle check point, whereas at higher dosage, the cells with serious damaged DNA were forced to arrest at G2/M phase for repairing. When the repair process fails, cells undergo mitotic death, or alternatively, apoptosis. We here showed that rituximab enhances apoptosis induced by irradiation at 24 h. However, the cells irradiated alone also manifested profound apoptosis at 48 h, which had no significant difference with combination treatment. The death process of irradiated cells has been shown to involve two principal mechanisms: mitotic and apoptotic cell death. As apoptosis after combination treatment does not appear to be the only way of cell death, we suggest that also another death mechanism (mitotic) may occur after combination treatment.

Ionizing radiation is known to induce intracellular oxidative stress (Hosokawa et al. 2002). ROS generated by irradiation have extremely high rate constants for various biomolecules, rapidly react with base and sugar moieties to cause DNA damage, and with lipids to induce membrane lipid peroxidation. They are possible triggers of radiation-induced apoptosis (Cui et al. 2004; Woo et al. 2003). In the present study, an obvious increase in intracellular ROS levels was observed after combination treatment with irradiation and rituximab. These may be correlated with higher apoptosis induction and cell growth inhibition in Raji cells undergoing combination treatment. Moreover, direct exposure of cells to rituximab was shown to sensitize Raji cells to H_2O_2 as seen by reduced survival and enhanced apoptosis, which lends further support to the notion that ROS generation might be an important step for radiation-induced apoptosis in Raji cells.

Although ROS were once regarded simply as toxic agents implicated in antimicrobial defense, there is now considerable evidence that they act as intra- and extracellular messengers (Fruehauf and Meyskens 2007). Recent developments suggest that a number of diverse apoptotic stimuli share a mechanistic pathway characterized by the generation of ROS (Burkitt et al. 2004; Kagan et al. 2004). On stimulation with anti-CD20, the CD20 antigen recruits Src family members (specifically, Lyn, Fyn, and Lck) and an undetermined adaptor molecule to its cytoplasmic regions (Deans et al. 1995; Popoff et al. 1998). ROS have been shown to inhibit the activity of certain tyrosine phosphatases, and to induce the phosphorylation and activation of Src and Syk family kinases (Esposito et al. 2003; Yan and Berton 1996). Thus, apoptosis after rituximab and irradiation combination treatment may be associated to Src

family PTK activation induced by anti-CD20, which is essential and required for subsequent PLCγ2 tyrosine phosphorylation, mobilization of intracellular calcium, and caspase 3 activation (Unruh et al. 2005). In addition, the increased expression of CD20 surface antigen that correlated with radiation-induced changes in the generation of ROS may be responsible for enhanced responsiveness to CD20-specific antibody therapies (Gupta et al. 2008). In our study, radiosensitivity of cells was slightly higher in R + irradiation than in irradiation + R treatment. We suggest that a certain level of intracellular ROS production induced by rituximab pretreatment, though non-cytotoxic by itself, may facilitate the induction of cell killing by subsequent irradiation. Similar phenomena were manifested in cytotoxic effects of As_2O_3 in leukemia cells (Ramos et al. 2005). EBV-infected Raji cells, as well as another EBV-positive cell line, have been shown to possess efficient DNA damage repair systems. Up to 59% of DNA double-strand breaks induced by 50 Gy γ-rays were repaired within 1 h after irradiation in Raji cells (Mustonen et al. 1999). Because of the quick and efficient DNA repair, intracellular molecular events caused by addition of rituximab within 1 h postirradiation may have reduced effects on inducing cell death.

In conclusion, our data show that rituximab increases radiosensitivity of Raji cells in vitro. Raji cell response after combined treatment is mediated in part by induction of apoptosis. Little difference was seen with regard to cell survival, apoptosis induction, and cell cycle delay with two ways of rituximab adding sequence. ROS, which act as an important intra- and extracellular messengers besides causing DNA damage and inducing membrane lipid peroxidation, play a role in cell death induced by combination treatment with rituximab and irradiation. To optimize therapy regimen with rituximab, it is necessary to further explore the mechanisms of radiosensitization by rituximab.

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