Induction of autophagic cell death and radiosensitization by the pharmacological inhibition of nuclear factor–kappa B activation in human glioma cell lines

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Object. The intrinsic radioresistance of certain cancer cells may be closely associated with the constitutive activation of nuclear factor–kappa B (NF-κB) activity, which may lead to protection from apoptosis. Recently, non-apoptotic cell death, or autophagy, has been revealed as a novel response of cancer cells to ionizing radiation. In the present study, the authors analyzed the effect of pitavastatin as a potential inhibitor of NF-κB activation on the radiosensitivity of A172, U87, and U251 human glioma cell lines.

Methods. The pharmacological inhibition of NF-κB activation was achieved using pitavastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Growth and radiosensitivity assays were performed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Hoechst 33258 staining, supravisual acridine orange staining, and electron microscopy were performed utilizing 3 glioma cell lines with or without pitavastatin pretreatment to identify apoptosis or autophagy after irradiation.

Results. The growth of these 3 glioma cell lines was not significantly inhibited by pitavastatin at a concentration of up to 1 µM. Treatment with 0.1 µM of pitavastatin enhanced radiation-induced cell death in all glioma cell lines, with different sensitivity. Apoptosis did not occur in any pretreated or untreated (no pitavastatin) cell line following irradiation. Instead, autophagic cell changes were observed regardless of the radiosensitivity of the cell line. An inhibitor of autophagy, 3-methyladenine suppressed the cytotoxic effect of irradiation with pitavastatin, indicating that autophagy is a result of an antitumor mechanism. Using the most radiosensitive A172 cell line, the intracellular localization of p50, a representative subunit of NF-κB, was evaluated through immunoblotting and immunofluorescence studies. The NF-κB of A172 cells was immediately activated and translocated from the cytosol to the nucleus in response to irradiation. Pitavastatin inhibited this activation and translocation of NF-κB.

Conclusions. Autophagic cell death rather than apoptosis is a possible mechanism of radiation-induced and pitavastatin-enhanced cell damage, and radiosensitization by the pharmacological inhibition of NF-κB activation may be a novel therapeutic strategy for malignant gliomas. (DOI: 10.3171/2008.8.JNS17648)

Abbreviations used in this paper: ANOVA = analysis of variance; AVO = acidic vesicular organelle; DMSO = dimethylsulfoxide; DTT = dithiothreitol; EGTA = ethylene glycol tetraacetic acid; FBS = fetal bovine serum; HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A; IkB = inhibitory kappa B; MEM = minimum essential medium; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF-κB = nuclear factor–kappa B; NLS = nuclear localization sequence; PBS = phosphate-buffered saline; PLSD = protected least-significant difference; PMSF = phenylmethysulfonyl fluoride; 3-MA = 3-methyladenine.
cumulation of AVOs after high levels of damage leads to eventual autophagocytosis and cell death.\textsuperscript{11,50}

Ionizing radiation immediately activates the transcription factor NF-κB, which is an important regulator of the immune response\textsuperscript{3,5,36} and a key mediator of the genes controlling cellular proliferation and apoptosis.\textsuperscript{15,20,42,48} The immediate activation of NF-κB after radiation exposure is thought to protect cells from apoptosis.\textsuperscript{13,36} Nuclear factor-κB is usually inactive in the cytoplasm because of its endogenous inhibitor protein, IκB. The phosphorylation of IκB provokes the activation of NF-κB as well as its translocation from the cytosol into the nucleus, where NF-κB activates the expression of specific cellular genes.\textsuperscript{27,42,49} The active form of NF-κB is a dimer most frequently composed of 2 DNA binding subunits, p50 and p65 (RelA). We have reported that NF-κB activity is constitutively activated in malignant astrocytomas, although such activity has not been observed in a human fetal astrocyte strain or in normal cerebral tissue in adult humans.\textsuperscript{33} This aberrant NF-κB activation was assumed not to be due to the overexpression of p50 or p65. Moreover, the proliferation of human glioma cells was significantly restricted through the inhibition of NF-κB activity by using p65 antisense oligodeoxynucleotide or curcumin.\textsuperscript{52,33} These findings indicate that malignant astrocytoma cells exhibit aberrant NF-κB activity, which promotes their proliferation. This NF-κB activity may also play an important role in the antiapoptotic effect in patients with malignant glioma subjected to radiotherapy and chemotherapy. Therefore, we assumed that NF-κB is a potential molecular target in the treatment of malignant gliomas.

Statins are widely prescribed for the treatment of hypercholesterolemia, and recent studies have revealed that this group of drugs may play a beneficial role in cancer therapy.\textsuperscript{4,27,18,39,40} Statins have been reported to inhibit NF-κB activation in breast cancer cells and hepatocellular carcinoma cells.\textsuperscript{12,46} The inhibition of NF-κB activity by statins is known to be due to a blockage of IκB kinase.\textsuperscript{33} We have adopted pitavastatin as a potential inhibitor of NF-κB activation, because this statin is a novel and highly potent inhibitor of HMG-CoA reductase. Pitavastatin is now clinically available and its lipophilic property seems to be advantageous for passing through the blood-brain barrier.\textsuperscript{24,45} Thus, in the present study, we investigated the effect of pitavastatin on NF-κB activity and the radiosensitivity of a human glioma cell line.

Methods

Reagents Used

Pitavastatin (Livalo, or NK-104) was kindly provided by Kowa Co., Ltd., and Nissan Chemical Industries. It was dissolved in DMSO for stock solution, and the final concentration of DMSO was set at 0.05%. Control solutions contained equivalent amounts of DMSO. A rabbit antibody against NF-κB p50 (NLS) was purchased from Santa Cruz Biotechnology, Inc. Nuclear factor-κB p50 (NLS) antibody recognizes an epitope overlapping the nuclear location signal of the p50 subunit of the NF-κB heterodimer. This antibody binds both activated and non-activated forms of NF-κB p50. Acridine orange and MTT were purchased from Wako Junyaku. Hoechst 33258 and 3-MA were purchased from Sigma Chemical Co.

Cell Lines and Cell Culture

The A172, U87, and U251 malignant glioma cell lines were used in the present study. Cells were cultured in flasks containing the Eagle MEM supplemented with 10% FBS at 37°C in a humidified atmosphere of air containing 5% CO₂. When this culture reached confluence, the cells were trypsinized and passaged at a split ratio of 1:3.

Growth and Radiosensitivity Assays

Each growth assay and each radiosensitivity assay was conducted at least 3 times. To evaluate both growth and radiosensitivity, an MTT assay was used as described previously.\textsuperscript{51} For the growth assay, cells were seeded in 96-well microplates at an initial density of 2500 cells/well in 100 μl of medium. After incubation for 24 hours, various concentrations of pitavastatin were added. After further incubation for 4 days, an MTT assay was performed to determine the inhibitory effect of pitavastatin on glioma cell growth. For the radiosensitivity assay, cells were seeded in 96-well microplates at an initial density of 2500 cells/well in 100 μl of medium. After incubation for 24 hours, various concentrations of pitavastatin were added. After further incubation for 24 hours, the medium was replaced with fresh medium and the cells were irradiated using an MBR-1520R-3 system (Hitachi Medico Technology). The radiation dose ranged from 0 to 30 Gy administered in a single fraction. After incubation for 4 days, an MTT assay was performed as follows: 4 hours before the termination of each experiment, 50 μl of PBS containing 5 mg/ml of MTT was added to the culture medium. The medium was then gently removed, and 50 μl of DMSO was added to each well to solubilize a formazan precipitate. Following agitation, the absorbance of each well was measured at a test wavelength of 550 nm with a reference wavelength of 630 nm.

Detection of Apoptosis and Autophagy

To determine whether glioma cells subjected to the various treatments displayed apoptotic morphology, cells on glass coverslips were fixed with methanol and stained with Hoechst 33258 at a concentration of 5 μM prior to analysis using fluorescence microscopy.

Autophagy was assessed using supravalv cell staining with acridine orange as described previously.\textsuperscript{35} Twenty minutes before the termination of each experiment, a final concentration of 1 μg/ml of acridine orange was added to the cultured glioma cells on glass coverslips. When the incubation was completed, cells were gently washed with PBS. The glass coverslips were then removed from the wells and inverted onto a glass slide. Cells were then examined using a fluorescence microscope (BX50, Olympus) equipped with a mercury 100-W lamp, 450- to 480-nm bandpass blue excitation filters, a 500-nm dichroic mirror, and a 515-nm long-pass filter.
Electron Microscopy

After each treatment, glioma cells were harvested by trypsinization, washed twice with PBS, and fixed with ice-cold 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 1 hour. After fixation, the cells were post-fixed in osmium tetroxide in the same buffer for 1 hour and then subjected to electron microscopy analysis. Representative areas were chosen for ultrathin sectioning and viewed with an H-300 transmission electron microscope (Hitachi).

Immunoblotting of NF-κB p50

The A172 cells were seeded in MEM supplemented with 10% FBS in 10-cm tissue culture dishes and were cultured until subconfluence. The medium was then replaced with fresh medium and various concentrations of pitavastatin. After incubation for 24 hours, the cells were irradiated using an MBR-1520R-3 system at 15 Gy. We then obtained nuclear extracts 1.5 and 8 hours after irradiation. Briefly, the cells were harvested with 1 ml of ice-cold PBS and processed in a centrifuge for 1 minute at 5000 rpm at 4°C. The cell pellets were lysed with 0.4 ml of buffer A containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, and 1 mM PMSF for 15 minutes on ice. Twenty-five microliters of 10% Nonidet P-40 solution was added, and the samples were vortexed for 15 seconds before processing in a centrifuge at 15,000 rpm for 5 minutes at 4°C. The pellets were washed once with 0.5 ml of buffer B, which was composed of 20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and 1 mM PMSF. The lysed nuclei were left on ice for 30 minutes and processed in a centrifuge at 15,000 rpm for 5 minutes at 4°C. The nuclear protein concentration was determined using the DC Protein Assay (Bio-Rad Laboratories, Inc.). Nuclear extracts were stored at −80°C until use.

Immunoblot analysis was performed using 10 μg of nuclear extracts of NF-κB p50. Proteins were elec-trotransferred from sodium dodecyl sulfate–polyacrylamide gels onto a polyvinylidene difluoride membrane and were blocked with 5% nonfat milk in Tris-buffered saline–Tween20 buffer (20 mM Tris-HCl, pH 7.4, 135 mM NaCl, and 0.1% Tween20) for 1 hour at room temperature, incubated with the rabbit polyclonal anti–NF-κB p50 (NLS) antibody for 1 hour, and incubated with horse-radish peroxidase–conjugated secondary antibody for 1 hour at room temperature. After extensive washing, immunoreactive proteins were detected using an enhanced chemiluminescence detection system (ECL, Amersham Biosciences Corp.).

Cellular Localization of NF-κB p50 by Immunofluorescence

The A172 cells were seeded on the cover glass in 35-mm tissue culture dishes at 10,000 cells/well in MEM supplemented with 10% FBS and were incubated at 37°C in a humidified atmosphere of air containing 5% CO₂. After incubation for 24 hours, various concentrations of pitavastatin were added. After further incubation for 24 hours, the medium was replaced with fresh medium and the cells were irradiated using an MBR-1520R-3 system at 15 Gy. Cells on the cover glass were washed with cold PBS and fixed with methanol at 1.5 and 8 hours after irradiation. Rabbit polyclonal anti–NF-κB p50 (NLS) antibody was used as the first antibody, and fluorescein-conjugated anti–rabbit antibody was used as the second. Cells were then analyzed under a fluorescence microscope.

Statistical Analysis

All experiments were performed at least 3 times in quadruplicate wells. Data are expressed as the means ± SDs. For simple statistical comparisons (for example, treatment vs control), 1-way ANOVA was first performed to determine statistical significance, followed by the Dunnett t-test. For dose-dependent effects, 1-way ANOVA was applied, followed by the Fisher PLSD test.

Results

Growth Assay and Determination of the Adequate Pitavastatin Concentration

An MTT assay of the 3 glioma cell lines showed that a 4-day treatment with pitavastatin at a concentration between 0.01 and 1 μM had little inhibitory effect on cell proliferation, whereas a concentration of 10 μM had a cytostatic effect (Fig. 1). Various concentrations of pitavastatin were used to determine the cytotoxicity assay in this study.

Radiosensitivity Assay

For the radiosensitivity assay, pitavastatin at a concentration between 0.01 and 1 μM was used because this range had little inhibitory effect on glioma cell growth during a period of 4 days. The MTT assay was performed 4 days after radiation exposure with or without pitavastatin pretreatment. The original absorbance data were plotted, and survival curves were generated (Fig. 2A, C, and E). These survival curves were then corrected for the relative survival of the respective control glioma cells (Fig. 2B, D, and F). Pitavastatin dose-dependently enhanced radiosensitivity, and relative survival following exposure to 30 Gy of radiation and pitavastatin pretreatment at concentrations between 0.1 and 1 μM was significantly reduced in all 3 glioma cell lines. The A172 cells were the most sensitive to irradiation and pitavastatin pretreatment.

Undetectable Apoptosis in Irradiated Glioma Cells

Hoechst 33258 nuclear staining of the 3 cell lines was performed. Control cells as well as the irradiated cells with or without pitavastatin pretreatment of 0.1 μM did not show apoptotic figures such as chromatin condensation or nuclear fragmentation. Representative A172 cells are shown in Fig. 3A–C.

Induction of Autophagy in Irradiated Glioma Cells

Supravital cell staining with acridine orange was performed to detect A VOs, which are a hallmark of autophagic vacuoles in the cytoplasm. As shown in Fig. 4A,
nonirradiated A172 cells exhibited no AVO formation in the cytoplasm, and irradiated A172 cells 1 day after exposure to 15 Gy of radiation demonstrated a small amount of AVO formation (Fig. 4B). Irradiated A172 cells that had been pretreated with 0.1 µM of pitavastatin showed abundant cytoplasmic AVO formation (Fig. 4C). Once 3-MA, an inhibitor of phosphatidylinositol-3 kinase (PI3K) known to inhibit autophagic sequestration, was added just after irradiation, AVO formation was inhibited regardless of pitavastatin pretreatment (Fig. 4D). Both U87 and U251 cells also demonstrated the formation of numerous AVOs following irradiation and pitavastatin pretreatment, which was inhibited by 3-MA (Fig. 4E–H). Furthermore, to quantify the incidence of irradiation-induced and pitavastatin-enhanced autophagy, in each experiment we counted 1000 cells from 4 randomly selected areas under high magnification, and the cells with typical AVO formation were scored. This method is very similar to that used in the determination of an MIB-1 staining index. The 3-MA decreased the percentage of AVO-positive cells in the 3 glioma cell lines obtained after irradiation at 15 Gy alone or with pitavastatin pretreatment (Table 1).

To assess whether radiation-induced and pitavastatin-enhanced autophagy is a protective response or an anticancer effect, an MTT assay was performed using the A172 cells to evaluate the cytotoxicity of the radiation (15 Gy) and the pitavastatin pretreatment (0.1 µM) with or without 3-MA (1 mM). A preliminary experiment was performed to confirm the adequate 3-MA concentration, and 1 mM was selected because this dose had no growth inhibitory effect. Figure 5 shows the percent cell number obtained from the MTT assays in which the control experiment was deemed to be 100%. With 3-MA treatment, the percent of irradiated, pitavastatin-treated A172 cells increased from 65 to 90% and the percent of irradiated cells without pitavastatin increased from 88 to 96%.

The standard method of detecting autophagy is to examine the ultrastructure of treated cells by transmission electron microscopy; thus, the ultrastructure was examined in the 3 glioma cell lines. Figure 6 shows that irradiation with or without pitavastatin pretreatment induced autophagy. Whereas untreated control cells showed normal nuclear and cytoplasmic morphology, irradiated cells developed autophagic vacuoles that contained lamellar structures or digested materials. Irradiated cells pretreated with pitavastatin showed an increased formation of large and small autophagic vesicles. The U87 and U251 cells also showed similar ultrastructural results (data not shown).

**Nuclear Factor-κB Expression of A172 Cells After Irradiation**

Immunoblot analysis of the nuclear extract of control A172 cells demonstrated that NF-κB p50 protein levels had increased by 1.5 hours after irradiation and were restored to normal levels by 8 hours after irradiation (Fig. 7). Pretreatment of the A172 cells with pitavastatin led to a dose-dependent suppression of the immediate radiation-induced increase in the NF-κB protein in the nuclear extract, implying that pitavastatin pretreatment suppresses the radiation-induced early activation of NF-κB.

**Subcellular Localization of NF-κB**

Immunofluorescence staining revealed a dynamic change in the intracellular localization of the NF-κB p50 of A172 cells following irradiation. In control cells that were not irradiated, the localization of NF-κB p50 was almost perinuclear (Fig. 8A), whereas NF-κB increased in the nucleus by 1.5 hours after irradiation (Fig. 8B) and was restored by 8 hours after irradiation (Fig. 8C).
Fig. 2. Line graphs demonstrating the effect of pitavastatin on the survival of irradiated A172 (A and B), U87 (C and D), and U251 (E and F) cells. Survival curves were normalized to nonirradiated controls at each pitavastatin concentration. Pitavastatin dose-dependently enhanced the radiosensitivity of all 3 glioma cell lines. The most sensitive cell line was A172. Statistical analysis was performed using 1-way ANOVA followed by the Fisher PLSD. *p < 0.05. O.D. = optical density.
A172 cells pretreated with 0.1 µM pitavastatin, NF-κB p50 remained in the cytoplasm 1.5 (Fig. 8D) and 8 hours (Fig. 8E) after irradiation. Together with the immunoblotting data, these results showed that pitavastatin inhibits the early translocation of activated NF-κB from the cytoplasm into the nucleus after irradiation.

Discussion

Nuclear factor-κB is now known as one of the most common molecular biomarkers for cancer for several reasons: NF-κB activity is elevated in most cancer cells including malignant gliomas,\(^{10,33}\) radiation and some cytotoxic drugs immediately activate NF-κB,\(^{3,5,10}\) and NF-κB is involved in the transcriptional regulation of genes important in antiapoptosis.\(^{5,10,15,36,42,49}\) In the present study, we demonstrated that pitavastatin dose-dependently increases the radiosensitivity of A172, U87, and U251 cells, but that the radiosensitivities of these cell lines are different. We found that irradiation induces autophagic cell death rather than apoptosis and that pitavastatin enhances autophagic cell death following irradiation. Furthermore, the NF-κB of A172 cells was immediately activated and then translocated from the cytosol to the nucleus in response to irradiation, and pitavastatin inhibited this activation and translocation of NF-κB. These results indicate that the activation of transcription factor NF-κB, an immediate early response following exposure to ionizing radiations, may protect cells from autophagic cell death after irradiation. Pitavastatin may inhibit intrinsic radioresistance by preventing immediate NF-κB activation.

Autophagy, as an alternative form of programmed cell death, recently has been shown to contribute significantly to the antineoplastic effects of radiotherapy. It is known that irradiation barely induces apoptosis in glioma cells.\(^{31,30,50,51}\) However, some authors have reported that autophagy occurs in irradiated glioma cells.\(^{22,50}\) Autophagy was originally described as a process of protein degradation and recycling in response to starvation or stress. In some cancer cells, autophagy may be a protective response to survive anticancer treatments by blocking the apoptotic pathway. In contrast, other cancer cells undergo autophagic cell death after cancer therapies.\(^{29}\) In the present study, to determine whether the autophagic response of glioma cells to radiation together with pitavastatin treatment was a protective response or an antitumor ef-

### TABLE 1: Incidence of irradiation-induced and pitavastatin-enhanced autophagy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% AVO-Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A172</td>
</tr>
<tr>
<td>control</td>
<td>3.2</td>
</tr>
<tr>
<td>radiation</td>
<td>42.4</td>
</tr>
<tr>
<td>radiation + 3-MA</td>
<td>11.7</td>
</tr>
<tr>
<td>radiation + pitavastatin</td>
<td>76.3</td>
</tr>
<tr>
<td>radiation + pitavastatin + 3-MA</td>
<td>16.7</td>
</tr>
</tbody>
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* Radiation was equal to 15 Gy, 3-MA to 1 mM, and pitavastatin to 0.1 µM. One thousand cells were counted and AVO-positive cells were scored.
Fig. 4. Immunofluorescence photomicrographs obtained after supravital staining to detect AVOs. Control A172 cells showing no cytoplasmic AVO formation (A), irradiated A172 cells demonstrating AVO formation (B), irradiated A172 cells with 0.1-µM pitavastatin pretreatment demonstrating abundant cytoplasmic AVO formation (C). The addition of the autophagy inhibitor 3-MA (1 mM) prevented AVO formation in irradiated A172 cells pretreated with pitavastatin (D). Irradiated U87 (E) and U251 cells (G) with 0.1-µM pitavastatin pretreatment demonstrating abundant cytoplasmic AVO formation. The 3-MA (1 mM) prevented AVO formation of U87 (F) and U251 cells (H). Acridine orange, original magnification ×1000.

Fig. 5. Bar graph revealing the effect of 3-MA on the cytotoxicity of irradiated A172 cells. The A172 cells were seeded at 2500 cells per well in 96-well plates and were incubated for 24 hours. After exposure to 0.1 µM of pitavastatin for 24 hours, the medium was changed and the cells were exposed to 15 Gy of radiation. Immediately after irradiation, 1 mM of 3-MA was added and the cells were cultured for an additional 96 hours. Cell numbers were assessed by performing an MTT assay. Results are shown as the means ± SD. Pit = pitavastatin pretreatment; Rad = radiation treatment. Statistical analysis was performed using 1-way ANOVA followed by the Dunnett t-test. *p < 0.05.
fect, we used 3-MA to inhibit autophagy. Once autophagy was prevented by 3-MA, the cytotoxic effect of irradiation and pitavastatin was suppressed, suggesting that radiation-induced and pitavastatin-enhanced autophagy is an antitumor effect, not a protective response.26,29

In this study, we attempted the pharmacological inhibition of NF-κB to sensitize glioma cells to irradiation. Similarly, several NF-κB inhibitors such as indomethacin, curcumin, and proteasome inhibitor PS-341 have been shown to act as radiosensitizers against cancer cells.6,9,41 The present study provided important in vitro findings that pitavastatin at a low dose (0.01–1 µM) radiosensitizes human glioma cells. This dose is very similar to the expected plasma levels of pitavastatin in patients with hypercholesterolemia.24 Although pitavastatin at a concentration of 10 µM had a cytostatic effect on the 3 glioma cell lines we studied, no growth inhibition was observed in any of the cell lines at a concentration of 0.1 µM. We recently reported similar experimental cytotoxicity data by using normal endothelial cells, which were not growth-inhibited at a pitavastatin concentration up to 1 µM.47 Therefore, the radiosensitization by pitavastatin was assumed probably to be due to the inhibition of NF-κB activation and translocation, rather than cell damage or growth inhibition. Note, however, that another mechanism of HMG-CoA reductase inhibition should be...
considered. Pitavastatin may decrease the production of mevalonate derivatives such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. These isoprenoids are responsible for Ras prenylation, a step required for their translocation to the cell membrane, which is necessary for cell signaling.  

In the present study, we found an antiautophagic function of NF-κB in glioma cells. Compared with the antiapoptotic mechanism of NF-κB, little is known about the impact of this factor on autophagy. Recently, it has been shown that NF-κB directly and indirectly represses autophagy through mammalian target of rapamycin signaling, which may be a downstream signaling of NF-κB.13,23,31,44,48 Interestingly, inhibitors of mammalian target of rapamycin signaling induce apoptosis in some types of cancer cells, whereas they trigger autophagy in other settings as well as in malignant glioma cells.23,34,44 Moretti et al.31 have reported that Bax/Bak double-knockout cells

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**Fig. 8.** Immunofluorescence photomicrographs showing the NF-κB p50 of A172 cells with and without pitavastatin treatment. Nuclear factor-κB p50 was localized primarily in the cytoplasm of control A172 cells (A), in the nucleus 1.5 hours after irradiation in cells not pretreated with pitavastatin (B), and in the cytoplasm 8 hours after irradiation in cells without pitavastatin pretreatment (C). In contrast, NF-κB remained in the cytoplasm 1.5 (D) and 8 hours (E) after irradiation in cells pretreated with 0.1 μM pitavastatin. Fluorescein-conjugated second antibody to anti–NF-κB, original magnification ×1000.
are more radiosensitive than wild-type cells, and they assumed that the inhibition of apoptosis leads to the induction of autophagy. Other studies have also shown that the impairment of NF-kB activation resulted in the accumulation of reactive oxygen species, a potential stimulator of autophagy.22,23 In contrast, NF-kB activation mediates the repression of autophagy in Ewing sarcoma cells treated with tumor necrosis factor-α and suggests that autophagy amplifies apoptosis when associated with a death signaling pathway.13 In addition, Fabre et al.24 have reported that NF-kB inhibition leads to an autophagic response followed by apoptotic cell death in leukemia cells.

Conclusions

The precise network that controls the possible cross-talk between apoptosis and autophagy remains unknown; however, NF-kB, one of the major regulators of apoptosis, may play an important role in controlling autophagy. In particular, glioma cells may have a defect in their apoptotic machinery, and the therapeutic targeting of autophagy may better clinical outcomes following radiotherapy for glioma. Although the exact involvement of NF-kB in malignant glioma cells must be fully clarified, we believe that NF-kB is an appropriate molecular target in novel therapies for malignant glioma.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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