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Ciprofloxacin as a potential radio-sensitizer to tumor cells and a radio-protectant for normal cells: differential effects on γ -H2AX formation, p53 phosphorylation, Bcl-2 production, and cell death

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Abstract

Ionizing radiation increases cell mortality in a dose-dependent manner. Increases in DNA double strand breaks, γ -H2AX, p53 phosphorylation, and protein levels of p53 and Bax also occur. We investigated the ability of ciprofloxacin (CIP), a widely prescribed antibiotic, to inhibit DNA damage induced by ionizing radiation. Human tumor TK6, NH32 (p53^{-/-} of TK6) cells, and human normal peripheral blood mononuclear cells (PBMCs) were exposed to 2–8 Gy ⁶⁰Co- γ -photon radiation. γ -H2AX (an indicator of DNA strand breaks), phosphorylated p53 (responsible for cell-cycle arrest), Bcl-2, apoptotic proteins, and cell death were measured. Ionizing irradiation increased γ -H2AX amounts in TK6 cells (p53^{+/+}) within 1 hr in a radiation dose-dependent manner. CIP pretreatment and post-treatment effectively inhibited the increase in γ -H2AX. CIP pretreatment reduced Bcl-2 production but promoted p53 phosphorylation, caspase-3 activation and cell death. In NH32 cells, CIP failed to significantly inhibit the radiation-induced γ -H2AX increase, suggesting that CIP inhibition involves in p53-dependent mechanisms. In normal healthy human PBMCs, CIP failed to block the radiation-induced γ -H2AX increase but effectively increased Bcl-2 production but blocked the phospho-p53 increase and subsequent cell death. CIP increased Gadd45 α , and enhanced p21 protein 24 hr postirradiation. Results suggest that CIP exerts its effect in TK6 cells by promoting p53 phosphorylation and inhibiting Bcl-2 production and in PBMCs by inhibiting p53 phosphorylation and increasing Bcl-2 production. Our data are the first to support the view that CIP may be effective to protect normal tissue cells from radiation injury, while enhancing cancer cell death in radiation therapy.

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Conflict of Interest The authors declare no conflict of interest.

Keywords

radiation; γ -H2AX; p53; p21; Gadd45; Bax; Bcl-2; caspase-3; cell viability

Introduction

Ionizing irradiation is used to eliminate cancer cells while normal cells next to them are also exposed to it resulting in cell damage. Ionizing irradiation increases cell mortality in a dose-dependent manner [1]. Within 1 hr after exposure, ionizing irradiation induces DNA double strand breaks (DSBs) that lead to ataxia telangiectasia mutated (ATM) phosphorylation. As a result, a histone variant H2AX is phosphorylated beginning within seconds, which is termed γ -H2AX [2, 3]. γ -H2AX accumulates at sites of DNA damage and form foci which can span mega-base regions [4–7]. γ -H2AX is frequently used as a DNA damage biomarker. The rate of DSB repair correlates with the rate of loss of γ -H2AX foci [8].

γ -H2AX triggers the CHK2 signal-transduction pathway that activates p53 and CDC25 [9]. DSB-induced phosphorylated ataxia-telangiectasia-mutated (ATM) can also directly phosphorylate p53 [9]. Phosphorylated p53 transcriptionally activates the CDK inhibitor p21 and arrests the cell cycle at G1/S [10]. The phosphorylated p53 also transcriptionally induces Gadd45, and Bax. Four hr after irradiation, increases in protein levels of p53, p21, and Bax are found [11]. Bcl-2 was included in this study because of its anti-apoptotic capability [12] as opposed to Bax that is pro-apoptotic [13].

Ciprofloxacin (CIP) is a FDA-approved drug for clinical use, a widely prescribed antimicrobial agent and a known topoisomerase-II inhibitor. It is very safe, even in high concentrations [14]. It is listed in the Strategic National Stockpile for emergency use against *Bacillus anthracis* and all U.S. Food and Drug Administration requirements for human use of CIP have been fulfilled. In our previous work, we observed that CIP improved 30-day survival after irradiation followed by wound trauma, modulated cytokine profile in serum, and mitigated bone marrow damage and small intestinal injury in mice in addition to its capability of eliminating Gram-negative bacteria [15, 16]. The observation that CIP modulates cytokine levels is consistent with findings from other laboratories [17]. Furthermore, it is indicated that CIP has anti-proliferative activity in several cancer cell lines [18]. We, therefore, investigated *in vitro* the ability of CIP to inhibit DNA damage and subsequent gene expression responses induced by ionizing radiation in human blood cells. Herein, we report that gamma radiation significantly increased γ -H2AX, p53 phosphorylation, p21, Bcl-2 in human tumor cells (TK6 cells) and normal healthy peripheral blood mononuclear cells (PBMCs). CIP treatment effectively inhibited γ -H2AX and Bcl-2 production and promoted p53 phosphorylation, caspase-3 activation, and cell death in TK6 cells, while CIP treatment significantly increased Bcl-2 production and blocked p53 phosphorylation and cell death in human normal PBMCs.

Materials and Methods

Drug

Ciprofloxacin (CIP) was purchased from Sigma-Aldrich Co. (St. Louis, MO) and prepared in sterile water.

Cell culture

Human B lymphoblastoid cell line TK6 (p53^{+/+}) and human NH32 (p53^{-/-} of TK6 cells) were generous gifts from Dr. James Mitchell. Human peripheral blood mononuclear cells (PBMCs) were purchased from AllCells (Emeryville, CA). Cells were grown in RPMI 1640 medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Invitrogen), 2 mM L-glutamine (Invitrogen), 100 U/ml penicillin and 100 mg/ml streptomycin (Quality Biological Inc., Gaithersburg, MD) and maintained in a humidified 37°C incubator with continuous 5% CO₂ supply. TK6 and NH32 cells were fed twice a week.

Irradiation

Cells were placed in 6-well plates and exposed to various doses of ⁶⁰Co gamma-photon radiation delivered at a dose rate of approximately 0.6 Gy/min. Dosimetry was performed using the alanine/electron paramagnetic resonance system. Calibration of the dose rate with alanine was traceable to the National Institute of Standards and Technology and the National Physics Laboratory of the United Kingdom. Sham-irradiated cells were exposed to the same treatments as irradiated cells, except for irradiation.

Cell viability

Cell viability was determined using the trypan blue dye exclusion assay [1]. A 10 µl volume of cell suspension was combined with 10 µl of 0.4% trypan blue solution (Sigma Chemical Co., St Louis, MO), gently mixed, and allowed to stand for 5 minutes at room temperature. A 10 µl volume of the stained cell suspension were placed in a CountessTM cell counting chamber slides (Invitrogen, Eugene, Oregon) and the number of viable (unstained) and dead (stained) cells counted using a CountessTM automatic cell counter (Invitrogen).

Flow cytometry

Flow cytometry measured γ-H2AX (an indicator of DNA double-strand breaks or implication of gene repair) and phosphorylated p53 on serine residue at position 15 (arrest cell-cycle). About 10⁵ cells were fixed in fixation buffer, washed, and stained with FITC-conjugated antibody against γ-H2AX and PE-conjugated antibody against phosphorylated p53, using permeabilization buffer following the manufacturer's protocol (Millipore, Billerica, MA). Non-specific IgG was used as a control antibody. Stained cells were analyzed using a Guava EasyCyte MiNi flow cytometer and Guava software (Millipore).

Western blotting

To investigate levels of p53 phosphorylation, Gadd45, Bax, p21, Bcl-2, caspase-3, IgG, and actin, cells were removed from the 6-well plates and pelleted by centrifugation at 750 × g for 10 min. Cell pellets were resuspended in 100 µL Na⁺ Hanks' solution containing protease

inhibitors and sonicated. The total protein in the cell lysate was determined with Bio-Rad reagent (Bio-Rad, Richmond, CA, USA). Aliquots containing 20 µg of protein in Tris buffer (pH=6.8) containing 1% sodium dodecyl sulfate (SDS) and 1% 2-mercaptoethanol were resolved on SDS-polyacrylamide slab gels (Novex precast 4–20 % gel; Invitrogen). After electrophoresis, proteins were blotted onto a PVDF nitrocellulose membrane (type NC, 0.45 µm; Invitrogen), using a Trans-Blot Turbo™ Transfer System (Bio-Rad, Richmond, CA) and the manufacturer's protocol. After blocking the nitrocellulose membrane by incubation in Tris-buffered saline-0.5% tween20 (TBST) containing 3% nonfat dried milk for 90 min at room temperature, the blot was incubated for 60 min at room temperature with antibodies directed to p53 phosphorylation, Gadd45, Bax, p21, Bcl-2, actin (Santa Cruz Biotechnology), and caspase-3 (Epitomics, CA, USA) at a concentration of 1 µg/ml in TBST - 3% dried milk. The blot was then washed 3 times (10 min each) with TBST before incubating the blot for 60 min at room temperature with a 1000X dilution of species-specific IgG peroxidase conjugate (Santa Cruz Biotechnology) in TBST. The blot was washed 6 times (5 min each) in TBST before detection of peroxidase activity using the Enhanced Chemiluminescence Plus (Amersham Life Science Inc., Arlington Heights, IL, USA). Actin and IgG levels were not altered by radiation; we, therefore, used them as controls for protein loading. Protein bands of interest were quantitated densitometrically and normalized to IgG or actin.

Immunofluorescent staining and confocal microscopic analyses of suspension cells

Cells harvested at specific time points were fixed in 10% formalin. After fixation, cells were embedded first in HistoGel™ (Thermo Fisher Scientific Inc., Rockville, MD) according to the manufacturer's instruction, then in paraffin, and sectioned transversely. Unstained paraffin sections were used for immunofluorescent staining. Paraffin sections on slides were treated with Target Retrieval Solution and Protein Block Serum-Free (Dako North America, Inc., Carpinteria, CA) according to the manufacturer's instruction, and stained with anti-phospho-Histone H2A.X antibody (Cell Signaling Technology®, Danvers, MA) followed by Alexa Fluor® 647 Goat Anti-Rabbit IgG (Life Technologies Corporation, Grand Island, NY) antibody with washing between and after in phosphate-buffered saline (PBS) with 0.1% Tween® 20. Resulting slides were briefly rinsed with PBS, desalted by dipping in distilled-deionized water, and sealed with coverslips in mounting medium with 4',6-diamidino-2-phenylindole (DAPI, Life Technologies Corporation, Grand Island, NY). A Zeiss LSM710 laser scanning confocal microscope (Carl Zeiss MicroImaging, Thornwood, NY) with an EC Plan-Neofluar 40x/0.75 objective was used to scan the signals.

Statistics

Data are presented as mean ± sem. One-way ANOVA, two-way ANOVA, studentized-range test, Bonferroni's inequality, and Student's t-test were used for comparisons of groups with 5% as a significant level.

Results

Ciprofloxacin inhibited gamma-photon radiation-induced γ -H2AX increases in TK6 cells but not in PBMCs

Because the rate of DSB repair correlates with the decreased number of γ -H2AX foci [8], γ -H2AX formation was measured in TK6 cells using flow cytometry. As shown in Fig. 1, radiation increased γ -H2AX formation in a radiation dose-dependent manner. Because 8 Gy displayed a drastically increased γ -H2AX formation, this radiation dose was used for the rest of experiments with TK6 cells. CIP pretreatment 24 hr (Fig. 1b) or 3 hr (Fig. 2a) prior to irradiation inhibited γ -H2AX formation (Fig. 1b). CIP treatment 5 min or 30 min after irradiation also effectively inhibited the γ -radiation-induced increases in γ -H2AX formation in TK6 cells (Figure 2b). The radiation-induced γ -H2AX formation increase was confirmed by γ -H2AX foci using immunofluorescent stainings. The increase was effectively inhibited by CIP pretreatment (Figure 2c).

It is evident that radiation activates the p53 signal transduction pathway resulting in cell apoptosis *in vitro* [19]. p53 gene knockout cells display radio-resistance [11]. To determine whether CIP inhibition of radiation-induced γ -H2AX formation was mediated by p53 protein, NH32 cells, a p53 gene knockout cell line, were irradiated in the presence or absence of CIP. Figure 3 depicts that radiation increased the γ -H2AX formation that CIP was unable to significantly inhibit.

To see if human normal blood cells also responded to γ -irradiation like these tumor cells, human PBMCs were irradiated. Similar to TK6 cells, radiation increased the γ -H2AX formation in human PBMCs (Fig. 4a). However, CIP pretreatment 24 hr prior to irradiation failed to inhibit γ -H2AX formation (Fig. 4b). The increased formation of γ -H2AX after irradiation was confirmed by γ -H2AX foci using the immunofluorescent staining. Unlike TK6 cells, the increase was not inhibited by CIP pretreatment in PBMCs (Figure 4c).

Ciprofloxacin potentiated gamma-irradiation-induced p53 phosphorylation, reduced Bcl-2 production and increased cell death in TK6 cells

It is evident that radiation increases p53 phosphorylation [3, 11, 20]. To seek the effect of CIP on p53 phosphorylation using FacScan cytometry, TK6 cells were treated with CIP and analyzed by flow cytometric analysis. CIP pretreatment at 24 hr or 3 hr effectively potentiated p53 phosphorylation (Fig. 5a and b), whereas CIP posttreatment at 10 min significantly inhibited p53 phosphorylation (Fig. 5c). p53 phosphorylation was also observed using Western blot analysis (Figure 5d and e). CIP pretreatment also reduced Bcl-2 production in irradiated cells (Figure 5d and e).

Our laboratory has demonstrated that p53^{-/-} cells were resistant to radiation [19] and caspase-3 played a central role in apoptosis [1]. Therefore, caspase-3 activation was evaluated in CIP-pretreated cells. Fig. 6a shows that irradiation decreased caspase-3 inactive form and increased its active form. This alteration was enhanced further by CIP. In irradiated cells, CIP upregulated p21 and Gadd45- α but reduced Bax protein levels (Figure 6a).

Simultaneously, cell death was evaluated. We found that CIP sensitized cells to radiation-induced death in TK6 cells. CIP significantly rendered cell death in non-irradiated cells as well (Figure 6b).

Ciprofloxacin inhibited gamma-irradiation-induced p53 phosphorylation and increased Bcl-2 production and reduced cell death in PBMCs

To seek the effect of CIP on p53 phosphorylation, PBMCs were treated with CIP and analyzed by flow cytometric analysis, PBMCs were treated with CIP 24 hr at various doses prior to various doses of irradiation. Irradiation increased p53 phosphorylation in a dose-dependent manner. Unlike TK6 cells, Fig. 7a shows that CIP pretreatment alone slightly yet significantly increased p53 phosphorylation. However, CIP pretreatment at 50 μ M and 200 μ M in PBMCs significantly diminished the radiation-induced increase in p53 phosphorylation. Because 4 Gy produced a significant increase in p53 phosphorylation in PBMCs, 4 Gy was selected for the following experiments with PBMCs. The radiation-induced increase in p53 phosphorylation and CIP inhibition on this increase were confirmed by Western blotting analysis (Figure 7b and c). Radiation significantly increased Bcl-2 levels. In the presence of CIP, Bcl-2 protein level was further increased (Figure 7b and c).

We next looked at the levels of several proteins whose exposures were regulated by phosphorylated p53. CIP pretreatment increased p21, Gadd45- α , Bax, caspase-3 but not caspase-3 in irradiated PBMCs (Figure 8a). Because PBMCs were non-dividing cells, they died quickly after few days and became difficult to collect sufficient numbers of viable cells. Therefore, we measured cell viability only 1 hr and 24 hr after irradiation. Fig. 8b shows that the radiation-induced increase in cell death was also attenuated by CIP.

Discussion

CIP, a fluoroquinolone antibiotic, is well known and widely prescribed to treat a variety of bacterial infections. Research into CIP led to the discovery of its immunomodulatory properties. Several laboratories reported that CIP exerts immunomodulatory effects in rodent models and human clinical trials [21, 22], improving a wide spectrum of conditions including thrombocytopenia [23–25], Crohn's disease [26, 27], rheumatoid arthritis [28, 29] and chemotherapy-induced neutropenia [30]. These favorable improvements are unrelated to its antimicrobial activity, but rather are ascribed to two general immunomodulatory actions that fluoroquinolones may share: stimulation of hematopoiesis by enhanced IL-3 and GM-CSF production [15, 31, 32] and reduction of inflammation mediated by IL-1, IL-6, and TNF- α [8, 15].

In addition to the immunomodulatory properties of CIP, CIP displays anti-neoplastic activity in many cancer cell lines [18]. It is reported that CIP inhibited the ability of Metnase not only to cleave DNA but also to repair DNA [33], because Metnase is in a complex with topopolymerase II and strongly enhances topopolymerase II activity [34].

We, herein, report that CIP pretreatment effectively inhibited radiation-induced increases in γ -H2AX formation but promoted p53 phosphorylation, reduced Bcl-2 production, and increased cell death in TK6 cells. The observation of CIP inhibition on the radiation-induced

increases in γ -H2AX formation is not consistent with the report with A549 human carcinoma cells [33]. The discrepancy can be due to different types of cells and different means of inducing γ -H2AX formation. Nevertheless, the CIP enhancement on TK6 cell death is in agreement with other laboratories [18, 35]. A significant decrease in Bcl-2 levels was observed in irradiated cells pretreated with CIP, suggesting that the CIP enhancement of TK6 cell death is mediated by inhibition of Bcl-2. Despite CIP pretreatment inhibiting DNA strand breaks caused by irradiation in TK6 cells, CIP-induced the p53 activation accompanied with the Bcl-2 inhibition, which might be a significant mechanism that led to increased cell death.

In contrast to what was found in TK6 cells, in normal human PBMCs, CIP did not alter the radiation-induced γ -H2AX formation. It is elusive why CIP exerts its action differently between normal healthy cells and tumor cells. It is possible that activities, structures or interactions between Metnase and topopolymerase II in these two types of cells are distinct, which requires further studies to be unfolded. CIP significantly reduced p53 phosphorylation and increased Bcl-2 levels. CIP also increased subsequent Gadd45 α , Bax, and p21 levels. The latter indeed is important for inhibiting CDK2/Cyclin-A/E to suppress induction of extra centrosomes after irradiation [36]. It has been demonstrated that p53 phosphorylation increases apoptosis [37, 38]. Nevertheless, it is evident that ionizing radiation induces apoptosis via both p53-dependent and p53-independent mechanisms [39, 40]. More importantly, p53 phosphorylation can occur in either presence or absence of ATM [41]. Therefore, we measured only p53 phosphorylation instead of ATM phosphorylation. We have reported that NH32 cells (the p53^{-/-} cells) were less sensitive to ionizing radiation resulting in attenuation of mortality [42], suggesting that the p53-dependent apoptosis is absent in NH32 cells. Inhibition of p53 phosphorylation, stimulation of Bcl-2 protein amount, and reduced cell death after irradiation in CIP-treated cells suggest that CIP may increase cell survival in normal cells by suppressing p53 and upregulating Bcl-2. The possibility of CIP affecting the cellular response to radiation via p53-independent mechanisms cannot be excluded.

This laboratory has reported that CIP significantly increased *in vivo* survival, altered the serum cytokine and chemokine profile, accelerated bone-marrow recovery, ileal cell death, and prevented systemic bacterial infection in irradiated mice followed by skin-wound trauma [15, 16]. These effects could be mediated by its capability to inhibit NF- κ B and caspase-3 [14]. The pharmacokinetics [43] and pharmacodynamics [44] of CIP in humans have been well documented. CIP is well tolerated, distributes rapidly in tissues, and has similar half-lives in mice [45–47] and humans [43]. The CIP efficacy on survival improvement [48] was not observed with amoxicillin or levofloxacin treatment [49], suggesting the specificity of CIP. The *in vitro* human cell data from this report, together with above *in vivo* mouse results [15], suggest that CIP may prove to be beneficial for treating critical sequelae of ionizing irradiation beginning at the step of p53 by inhibition of p53 phosphorylation.

CIP offers several advantages supporting the idea of further development as a drug to treat radiation-associated injuries: (1) it is included in the Strategic National Stockpile for bacterial infection control; (2) it can be taken orally enabling patients to self-administer; (3)

it possesses not only antimicrobial, but also favorable immunomodulatory activity; and (4) it is inexpensive.

In summary, CIP pretreatment significantly inhibited radiation-induced increases in γ -H2AX formation, but promoted p53 phosphorylation and inhibited Bcl-2 levels in TK6 cells. CIP posttreatment inhibited these parameters and elevated the subsequent cell death. In human normal PBMCs, CIP failed to inhibit radiation-induced increases in γ -H2AX formation, but significantly inhibited p53 phosphorylation, increased Bcl-2 levels, and reduced subsequent cell death. CIP also elevated p21 and Gadd45 α levels in both types of irradiated cells. Therefore, these results suggest that CIP may prove to be beneficial as an effective countermeasure for treating critical sequelae associated to radiation in normal healthy cells and cancerous cells.

In conclusion, CIP exerted its actions in normal human blood cells differently from tumor cells. Figure 8 shows a diagram proposing an idea that is very important in radiation-induced cell death. The idea is that acute ionizing irradiation increases γ -H2AX formation, p53 phosphorylation, Gadd45 α , and p21 in both tumor cells and normal healthy cells. Moreover, radiation increases Bcl-2 levels in normal healthy cells but does not alter Bcl-2 and Bax levels in tumor cells. CIP inhibits γ -H2AX formation and promotes p-53 phosphorylation and inhibits Bcl-2 basal levels to increase cell death in irradiated TK6 cells, while CIP inhibits p53 phosphorylation and increases Bcl-2 production to reduce cell death in normal healthy cells. Despite the need for more evidence, our data are the first to support the idea that CIP displays different actions towards normal human blood cells and tumor cells after irradiation.

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Fig. 1. CIP inhibits γ -radiation-induced increases in γ -H2AX formation in TK6 cells

Cells were treated with 100 M CIP 24 hr prior to irradiation (n=3). γ -H2AX was measured 1 h after irradiation using FacScan. a. Radiation increases γ -H2AX formation in human TK6 cells. b. CIP inhibits radiation-induced γ -H2AX formation. Veh: vehicle; CIP: ciprofloxacin

*P<0.05 vs. Veh+0 Gy

^P<0.05 vs. Veh+respective radiation dose

Fig. 2. CIP treatment inhibited γ -radiation-induced increases in γ -H2AX in TK6 cells

Cells were treated with 200 M CIP 3 hr before (a) or 5 min or 30 min after (b) 8 Gy (n=3).

The γ -H2AX formation was measured 1hr after irradiation using FacScan. CIP treatment alone resulted in $2.1 \pm 0.34\%$, not different from vehicle treatment $1.7 \pm 0.15\%$. (c) Cells were treated with 200 M CIP 3 hr before 8 Gy. The γ -H2AX foci was detected using immunofluorescent technique. DAPI (in blue color) represented nuclei; Alexa Fluor® 647 (in Pink color) represented γ -H2AX foci in the nucleus. The scale bar is 20 μ m. Veh: vehicle; CIP: ciprofloxacin

*p<0.05 vs. Veh+0 Gy

^P<0.05 vs. Veh+8 Gy

Fig. 3. CIP did not inhibit radiation-induced γ -H2AX formation in p53 knockout NH32 cells NH32 cells (p53-null TK6 cells) were treated with CIP at either 100 M or 200 M 24 hr before irradiation (n=3). γ -H2AX was measured 1 hr after γ -irradiation.

*P<0.05 vs. Veh+0 Gy. Veh: vehicle; CIP: ciprofloxacin

Fig. 4. CIP did not inhibit γ -radiation-induced increases in γ -H2AX in human normal PBMCs Cells were treated with CIP 24 hr prior to γ -irradiation at 2, 4, or 6 Gy (n=3). γ -H2AX was measured 1 hr after irradiation. (a) Radiation increased γ -H2AX formation in human PBMCs using FacScan. (b) CIP did not inhibit radiation-induced γ -H2AX formation in human PBMCs using FacScan. (c) Cells were treated with 200 M CIP 24 hr before 4 Gy. The γ -H2AX foci was detected using imunofluorescent technique. DAPI (in blue color) represented nuclei; Alexa Fluor®647 (in Pink color) represented γ -H2AX foci in the nucleus. The scale bar is 20 μ m. IR: irradiation; Veh: vehicle; CIP: ciprofloxacin
*p<0.05 vs. 0 Gy treated with Veh or CIP at respective concentration

Fig. 5. CIP altered γ -radiation-induced increases in phospho-p53 and Bcl-2 levels in TK6 cells
 Cells were treated with CIP 24 hr before (a), 3 hr before (b), or 10 min after (c) 8 Gy γ -irradiation (n=3). Phospho-p53 was measured 1 hr after irradiation using FacScan. (d) Cells were treated with 200 μ M CIP 3 hr before 8 Gy. Cell pellets were collected 24 hr after irradiation and cell lysates were prepared for Western blot analysis. Representative gels were presented. (e) Specified protein was quantitated densitometrically and normalized with IgG. Veh: vehicle; CIP: ciprofloxacin
 Panel a–c: *p<0.05 vs. Veh+0 Gy; ^p<0.05 vs. Veh+8 Gy.
 Panel e: *p<0.05 vs. Veh+0 Gy; ^p<0.05 vs. Veh+8 Gy.

Fig. 6. CIP altered protein levels and increased cell death in irradiated TK6 cells

Cells were treated with 200 μ M CIP 3 hr prior to 8 Gy γ -irradiation (n=3). (a)

Representative gels for each interested protein. Cells were collected 24 hr after irradiation

and their lysates were prepared for Western blot analysis. (b) Cell viability was evaluated at

different time points after irradiation. Veh: vehicle; CIP: ciprofloxacin

*p<0.05 vs. Veh+0 Gy at respective day

^p<0.05 vs. Veh+8 Gy at respective time point

Fig. 7. CIP inhibited γ -radiation-induced increases in phospho-p53 and increased Bcl-2 levels in normal human PBMCs

(a) Cells were treated with CIP 24 hr prior to 2, 4, or 6 Gy γ -irradiation (n=3). Phospho-p53 was measured 1 hr after irradiation using FacScan. (b) Representative gels were presented. Cells were treated with 200 μ M CIP 24 hr before 4 Gy. Cell pellets were collected 24 hr after irradiation and cell lysates were prepared for Western blot analysis. (c) Specified protein was quantitated densitometrically and normalized with actin. Veh: vehicle; CIP: ciprofloxacin

Panels a and c: *p<0.05 vs. Veh+0 Gy. ^P<0.05 vs. Veh+4 Gy.

Fig. 8. CIP altered protein levels and decreased cell death in irradiated PBMCs

Cells were treated with 200 μ M CIP 24 hr prior to 4 Gy γ -irradiation (n=3). (a)

Representative gels for each interested protein. Cells were collected 24 hr after irradiation

and their lysates were prepared for Western blot analysis. (b) Cell viability was evaluated at

1 hr and 1d after irradiation. Veh: vehicle; CIP: ciprofloxacin

*p<0.05 vs. Veh+0 Gy

[^]p<0.05 vs. Veh+4 Gy

Fig. 9. A proposed diagram with steps that CIP may exert its actions

Acute ionizing irradiation increases γ -H2AX formation, p53 phosphorylation, Gadd45 α , and p21 in both tumor cells and normal healthy cells. Moreover, radiation increases Bcl-2 levels in normal healthy cells but does not alter Bcl-2 levels in tumor cells. CIP inhibits γ -H2AX formation and promotes p-53 phosphorylation and inhibits Bcl-2 basal levels to increase cell death in irradiated TK6 cells, while CIP inhibits p53 phosphorylation and increases Bcl-2 production to reduce cell death in normal healthy cells. CIP: ciprofloxacin; $\uparrow$: increase; $\downarrow$: decrease; —: no change; $\top$ or $\vdash$ or $\dashv$: inhibition; $\vdash$: stimulation