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The Effect of Fermented Papaya Preparation on Radioactive Exposure

Eitan Fibach[a,1] and Eliezer A. Rachmilewitz[b]

[a] *Department of Hematology, Hadassah - Hebrew University Medical Center, Jerusalem, Israel; and*
[b] *Department of Hematology, The E. Wolfson Medical Center, Holon, Israel*

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Exposure to ionizing radiation causes cellular damage, which can lead to premature cell death or accumulation of somatic mutations, resulting in malignancy. The damage is mediated in part by free radicals, particularly reactive oxygen species. Fermented papaya preparation (FPP), a product of yeast fermentation of *Carica papaya Linn*, has been shown to act as an antioxidant. In this study, we investigated the potential of FPP to prevent radiation-induced damage. FPP (0–100 μg/ml) was added to cultured human foreskin fibroblasts and myeloid leukemia (HL-60) cells either before or after irradiation (0–18 Gy). After 1–3 days, the cells were assayed for: intracellular labile iron, measured by staining with calcein; reactive oxygen species generation, measured with dichlorofluorescein diacetate; apoptosis, determined by phosphatidylserine exposure; membrane damage, determined by propidium iodide uptake; and cell survival, determined by a cell proliferation assay. DNA damage was estimated by measuring 8-oxoguanine, a parameter of DNA oxidation, using a fluorescent-specific probe and by the comet assay. These parameters were also assayed in bone marrow cells of mice treated with FPP (by adding it to the drinking water) either before or after irradiation. Somatic mutation accumulation was determined in their peripheral red blood cells, and their survival was monitored. FPP significantly reduced the measured radiation-induced cytotoxic parameters. This finding suggests that FPP might serve as a radioprotector, and its effect on DNA damage and mutagenicity might reduce the long-term effects of radiation, such as primary and secondary malignancy.

INTRODUCTION

Exposure to ionizing radiation causes cellular damage, which may lead to acute effects, including lethality, or long-term effects encompassing organ injury (*1*) and carcinogenesis (*2*). The damage is mediated in part by free radicals, particularly reactive oxygen species (ROS) (*3*). Several compounds, among them antioxidants (*4–6*), have been shown in preclinical studies to be radioprotectors by reducing the damage in normal tissues; however, only amifostine has been approved for clinical use (*7*).

Fermented papaya preparation (FPP), a product of yeast fermentation of *Carica papaya Linn*, is a food supplement rich in amino acids and carbohydrates (*8*). FPP has been shown to have anti-inflammatory and immunoregulatory as well as antioxidative potential. The latter is mediated by scavenging ROS (*8*), by upregulating expression of genes related to the oxidative status, such as glutathione peroxidase, superoxide dismutase and catalase (*9*), as well as by chelating excess iron (*10*). Free iron species, such as the intracellular labile iron pool (LIP), participate in chemical reactions that generate ROS (Haber-Weiss and Fenton reactions) (*11*). The composition of FPP's principal components was previously reported (*8*), but the active ingredient(s) have not been defined. It is conceivable that the activity depends on synergy among different ingredients in addition to other components in its environment (*12*).

Oxidative stress-induced damage is implicated in a variety of diseases such as cancers, hemolytic anemias, diabetes, arthritis, cardiovascular dysfunctions and neurodegenerative disorders (e.g., stroke, Alzheimer's disease and Parkinson's disease). These conditions could potentially benefit from treatment with FPP. For example, neuroprotective potential evaluated in an Alzheimer's disease cell model showed that the toxicity of the β-amyloid can be significantly modulated by FPP. Apoptotic pathways such as the c-jun N-terminal kinase and p38-mitogen activated protein kinase are preferentially activated by pro-inflammatory cytokines and oxidative stress resulting in cell differentiation and apoptosis. FPP has been shown to modulate the H_2O_2-induced ERK, Akt and p38 activation with the reduction of p38 phosphorylation as well as reduced the extent DNA damage [for review see (*8*)].

In the current study, we investigated the potential of FPP to prevent various aspects of radiation-induced short- and long-term damage. The potential of FPP to ameliorate LIP accumulation, ROS generation, membrane and DNA damage, apoptosis as well as lethality was first demonstrated in cultured human cells. Then, because bone marrow failure, reflected by cytopenia, is a severe clinical outcome

[1] Address for correspondence: Department of Hematology, Hadassah University Hospital, Ein-Kerem, POB 12,000, Jerusalem 91120, Israel; e-mail: Fibach@yahoo.com.

of radiotherapy, the above parameters were analyzed in bone marrow cells as well. A significant protecting effect was found in irradiated mice that were treated with FPP. To investigate the long-term effect of FPP on mutagenesis, we measured the accumulation of somatic mutations in the X-linked gene, phosphatidyl inositol glycan class A (PIG-A). This mutation causes a deficiency in glycosylphosphatidyl inositol (GPI), which normally anchors various proteins to the cell surface. We used the GPI-negative phenotype as a surrogate biomarker of genetic instability (*13*).

The results of this study showed that FPP can ameliorate the short-term, cytotoxic effects of radiation exposure as well as the long-term effect on mutagenicity. Preventing radiation-induced DNA damage and mutation accumulation with FPP treatment may be of potential benefit in the prevention of primary and secondary tumors due to radiation exposure.

MATERIALS AND METHODS

Cells

Human normal foreskin fibroblasts, grown as adherent monolayers, and human myeloid leukemic HL60 cells, grown in suspension, were cultured in alpha media supplemented with 10% fetal bovine serum (Sigma-Aldrich® LLC, St. Louis, MO) and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells were subcultured by 1:10 dilution in fresh media; fibroblasts were first treated with trypsin-EDTA solution (Sigma-Aldrich).

Mice

Female C3H mice were bred at the Sharett Institute animal facility (Hadassah Hospital, Jerusalem, Israel). Mice were housed in a specific pathogen-free facility on a 12-h light/dark cycle with standard laboratory chow and water *ad libitum*. One-month-old mice were used for experiments. Blood (20 μl) was drawn from the tail vein and bone marrow cells were obtained from the femoral bones. The study was approved by the Institutional Animal Care and Use Committee.

Irradiation

Cultured cells and mice were irradiated with a precalibrated cobalt-60 Gammacell® 220 (MDS Nordion™ Inc., Ottawa, Canada), at a rate of 50 cGy/min at room temperature. Cells in monolayer (fibroblasts) or suspension (HL60) were irradiated in serum-containing media. Mice were immobilized prior to irradiation in a plastic restraining cage.

FPP Treatment

Fermented papaya preparation, a product of yeast fermentation of *Carica papaya Linn*, was supplied as sachets containing 3-g powder by Osato Research Institute (Gifu, Japan). The composition of its principal components has been previously reported (*8*). The FPP was dissolved in water, sterilized through a 0.22 μm filter and added to the media of the cultured cells or to the drinking water of the mice, at the indicated doses, either immediately before or after irradiation.

Cell Analyses

After cultured cells and mice were irradiated and treated with FPP (as detailed in Results), cells were harvested from the cultures and mouse bone marrow and stained for various parameters.

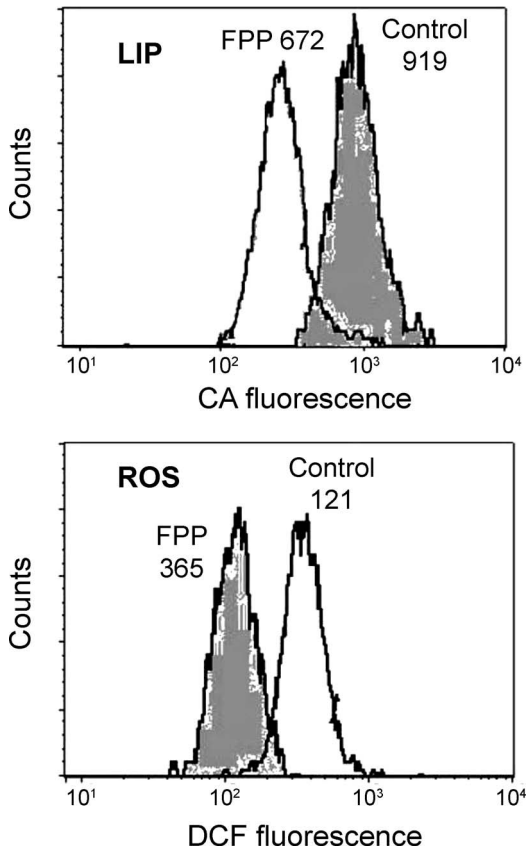

FIG. 1. The *in vitro* effect of FPP on the labile iron pool (LIP) and reactive oxygen species (ROS) of cultured cells. HL60 cells were incubated with (FPP) or without (Control) 100 μg/ml FPP for 2 days. Cells were then harvested and stained with calcein acetoxymethyl ester (CA) for LIP, and with dichlorofluorescin diacetate (DCF) for ROS. Cell fluorescence was measured by flow cytometry. Fluorescence distribution histograms and the mean fluorescence channel of each histogram of one representative experiment (out of 4 experiments) are shown. The results show that FPP decreases DCFH fluorescence and increases CA fluorescence, indicating reduction in both ROS and LIP, respectively. Technical details are described in Materials and Methods. Statistical difference between FPP-treated and untreated cells is $P < 0.05$ for both markers.

Labile Iron Pool and Reactive Oxygen Species

Cellular LIP was determined by staining with 0.25 μ*M* calcein acetoxymethyl ester (CA) (Sigma-Aldrich) (*14*). CA enters viable cells and becomes fluorescent upon hydrolysis by esterases; its fluorescence is quenched by binding of cytosolic LIP (*15*). The CA fluorescence is inversely proportional to the amount of LIP. Cellular ROS were measured by staining with 0.2 m*M* 2′-7′-dichlorofluorescin diacetate (DCF, Sigma-Aldrich) (*16*). DCF enters viable cells by cellular esterases, producing a nonfluorescent compound that is trapped inside the cells. Its oxidation by ROS produces the fluorescent compound, 2′-7′-dichlorofluorescein (*17*). The DCF fluorescence is proportional to the amount of ROS. Both staining procedures were done by incubating the cells with the reagents for 15 min at 37°C.

Apoptosis and Membrane Damage

Apoptosis was determined by measuring phosphatidylserine (PS) on the cell surface using FITC-conjugated Annexin V as previously described (*18*). Membrane damage was measured by propidium iodide (PI) staining (*19*)

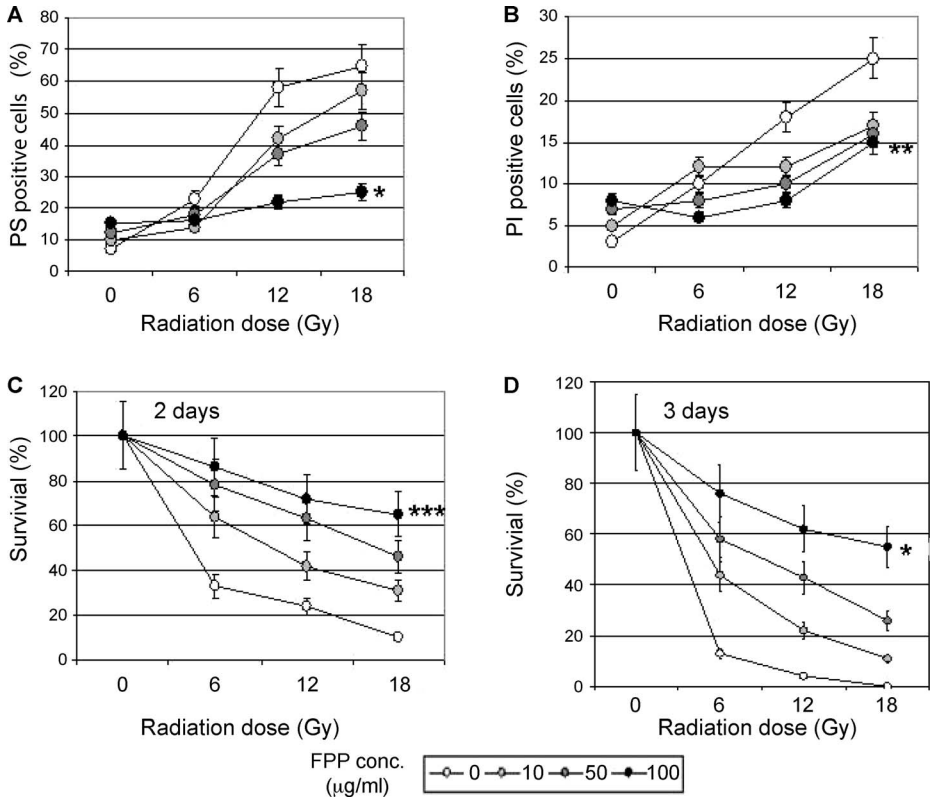

FIG. 2. The *in vitro* effect of FPP on radiation-induced toxicity. The human leukemia HL60 cells growing in suspension, and normal human foreskin fibroblasts growing as adherent monolayers, were irradiated and FPP immediately added (both at the indicated doses). After 24 h, panel A: phosphatidylserine (PS) and panel B: propidium iodide (PI) staining (markers of apoptosis and membrane damage, respectively) were determined in HL60 cells. Cell survival was determined in fibroblasts after 2 days (panel C) and 3 days (panel D). Results of 5 independent experiments are shown as the mean ± SD of the percentage of positively stained cells (panels A and B) and the percentage of total (taken as 100%) staining (panels C and D). Statistical differences between 18 Gy irradiated cells, treated with 100 μg/ml FPP compared with untreated cells, are denoted as follows: $*P < 0.001$, $**P < 0.01$, $***P < 0.05$.

DNA Oxidation

Oxidative DNA damage was determined by measuring 8-oxoguanine levels in cells. Cells were fixed and permeabilized, then stained with a FITC-labeled protein conjugate specific for 8-oxoguanine. The presence of oxidized DNA was demonstrated by a green/yellow fluorescence of the cells (*20*).

GPI Phenotype Analysis

Peripheral red blood cells (RBC) were stained with fluorescent (FITC-conjugated) antibodies to the GPI-anchored markers, CD59 and CD55, as previously shown in patients with paroxysmal nocturnal hemoglobinuria (*21*).

Flow Cytometry

The fluorescence of stained cells was analyzed by flow cytometry using a FACSCalibur™ flow cytometer (Becton-Dickinson, Immunofluorometry Systems, Mountain View, CA). CaliBRITE™-3 beads (Becton-Dickinson) were used to calibrate the FACS. Cells were passed at a rate of ~1,000/s, using saline as the sheath fluid. A 488 nm argon laser beam was used for excitation. For LIP, ROS, PS and PI analyses, list mode data of 30,000 cultured cells and nucleated marrow cells was acquired. The latter cells were analyzed after hemolysis of the RBC. CD55/59 was measured on 10^6 RBC. In this case, the samples were not hemolyzed and the RBC were gated based on forward light scatter and side light scatter (*16*). To confirm the identity of the gated cells as RBC, blood cells were stained for the erythroid-specific marker glycophorin A. All the gated cells were positive for this marker. Cell fluorescence was measured using logarithmic amplification. For each parameter the percentage of positive cells and the mean fluorescence channel of the relevant population were calculated using the FACS-equipped CellQuest™ software (Becton-Dickinson). In each assay unstained cells, both treated and untreated, served as control.

Cell Survival

Cell survival was estimated by CellTiter 96® Aqueous Non-Radioactive Cell Proliferation assay for cell vitality (Promega Inc., Madison WI).

DNA Damage

DNA damage was determined using an alkaline single-cell gel electrophoresis (comet assay) according to the protocol of Singh *et al.* (*22*) and as described elsewhere (*23*). Briefly, cells were mixed with 2% low-melting agarose at 37°C and applied as drops on a frosted microscope slide precoated with 1% standard agarose. Each drop was covered with a cover slip and allowed to solidify. The cover slips were then removed and the slides were immersed in ice-cold lysis solution (2.5 *M* NaCl, 100 m*M* EDTA, 10 m*M* Trizma® and 1% Triton™ X-100, pH 10) for 1 h at 4°C in darkness. The slides were then incubated

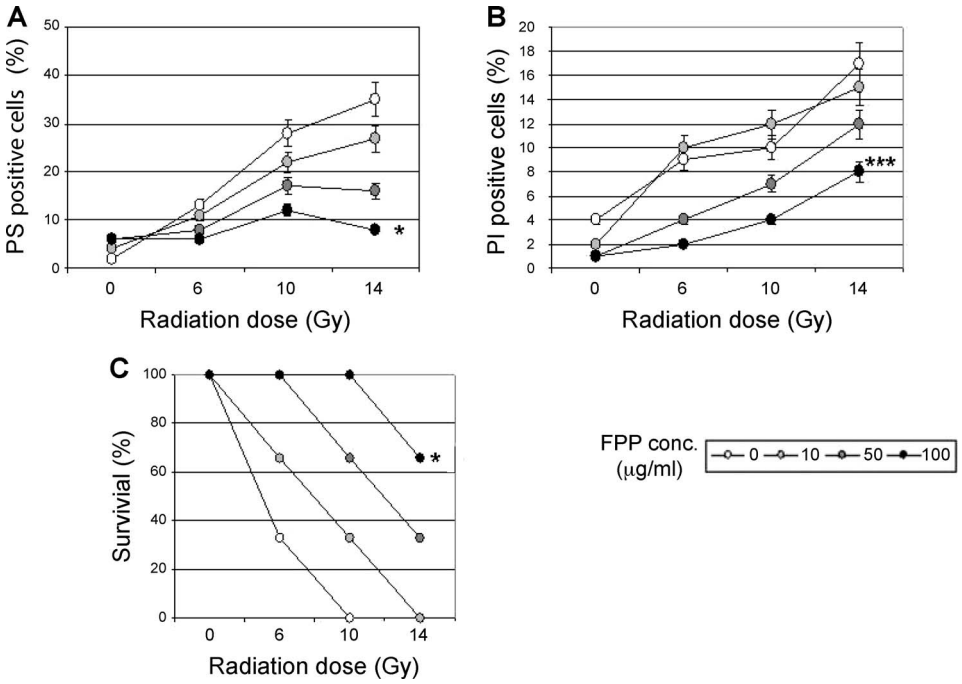

FIG. 3. The *in vivo* effect of FPP on radiation-induced toxicity. After whole-body irradiation, mice were given FPP in drinking water (both at the indicated doses). After 3 days, mice were sacrificed, and their femoral marrows were analyzed for the percentage of phosphatidylserine (PS) (panel A) and propidium iodide (PI) (panel B) positively stained cells. Other mice were observed for 3 weeks and their survival determined (panel C). The results, mean ± SD, of one experiment (6 mice per group) are shown. Statistical differences between 14 Gy irradiated mice that were treated with 100 μg/ml FPP compared with untreated mice, are denoted as follows: $*P < 0.001$, $***P < 0.05$.

for 20 min in ice-cold electrophoresis buffer (0.3 *M* NaOH and 1 m*M* EDTA, pH > 13) to allow DNA unwinding, after which electrophoresis was performed for 30 min (0.7 V/cm and 300 mA). Slides were then washed with neutralization buffer (0.4 *M* Tris HCl, pH 7.5) at 4°C and stained with SYBR® Green II (Sigma–Aldrich), covered with a coverslip and examined under a fluorescence microscope. About 150 randomly chosen images were acquired from two independent gels at a 400× magnification. The DNA damage was quantified in terms of percentage of DNA in comet tail (percentage tail DNA).

Statistics

Comparisons between groups were performed using Student's *t* test and $P < 0.05$ was considered as significant.

RESULTS

Effect of FPP Treatment on Radiation-Induced Toxicity

In vitro. The effects of FPP treatment on the LIP and ROS were determined after the addition of different concentrations of FPP to cultures of human normal foreskin fibroblasts and myeloid leukemia HL60 cells. Figure 1 shows the effect of 100 μg FPP added for 2 days to HL60 cultures. The fluorescence histograms and the indicated mean fluorescence channels show that FPP treatment reduces both the intracellular ROS and LIP. Similar results were obtained with human fibroblasts (data not shown).

The effect on radiation-induced cell toxicity was determined by measuring membrane damage and apoptosis as well as cell survival of irradiated cultures. Figure 2 shows the results of apoptosis, determined by measuring the percentage of PS positive cells (Fig. 2A), and membrane damage, measured by PI staining (Fig. 2B) of HL60 cells irradiated and treated with FPP. Figure 2C and D shows the effect on cell survival, assayed by a cell proliferation assay, after 2 and 3 day exposure of human skin fibroblast to different concentrations of FPP. Taken together, the results show that FPP protects, in a dose-dependent manner, radiation-induced toxicity in both cell lines.

In vivo. Mice were irradiated with 0–14 Gy. After irradiation, mice were provided with FPP (0–100 μg/ml in the drinking water). After 3 days, some mice were sacrificed and bone marrow cells were obtained and analyzed for PS exposure and PI staining (Fig. 3A and B, respectively). Mouse survival was determined after 3 weeks (Fig. 3C). The results show a dose-dependent FPP reducing effect on apoptosis and membrane damage of bone marrow cells as well as a protective effect on mice survival.

Effect of FPP Treatment on Radiation-Induced DNA Damage

In vitro. Cultured fibroblasts were irradiated at 0–18 Gy and 0–100 μg/ml FPP was added either immediately before or after irradiation. After 2 days, the cells were assayed for DNA oxidation (8-oxoguanine) (Fig. 4) and DNA damage (the comet assay) (Fig. 5). Figure 4 depicts histograms with respect to FITC fluorescence of untreated cells, irradiated

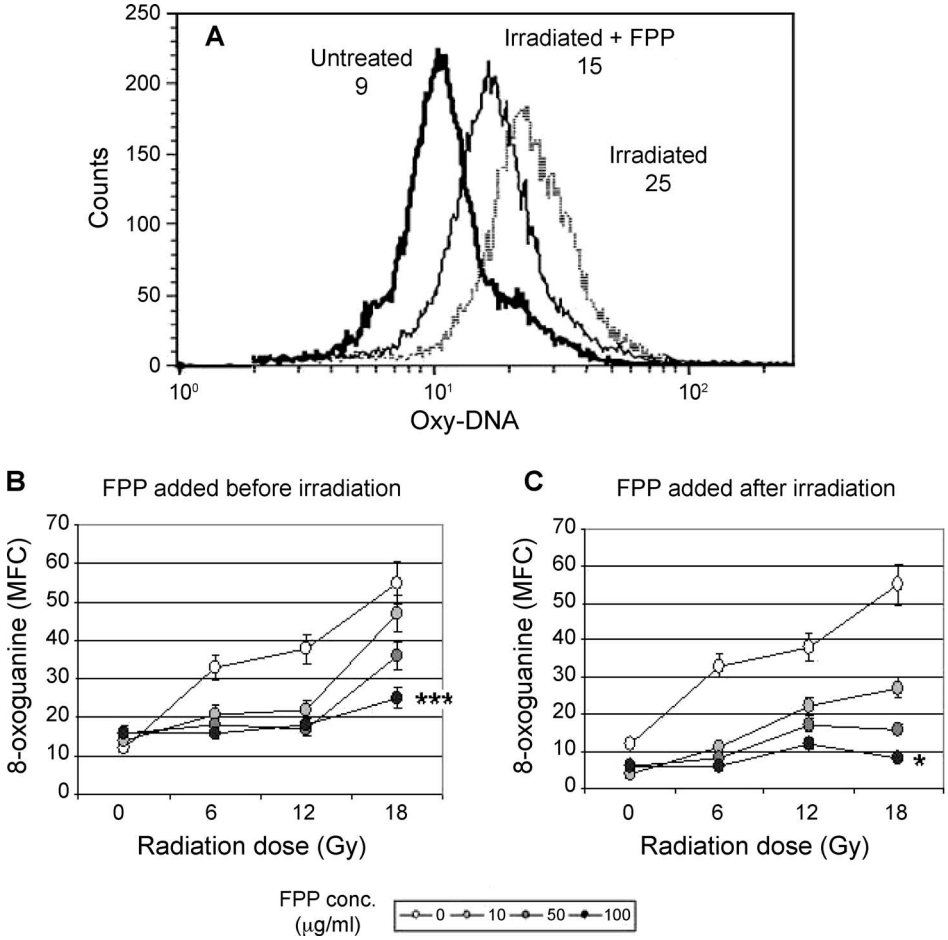

FIG. 4. The *in vitro* effect of FPP on radiation-induced DNA oxidation (8-oxoguanine). Panel A: Cultured human skin fibroblasts were untreated, irradiated (6 Gy) or irradiated and immediately treated with FPP (100 μg/ml). Two days later, the cells were harvested, washed, fixed, permeabilized and stained with a FITC-labeled protein conjugate specific for 8-oxoguanine. The figure shows fluorescence cell distribution histograms and their mean fluorescence channels of a representative experiment, indicating 8-oxoguanine content in the DNA. Panels B and C: Human fibroblasts were irradiated at the indicated doses. FPP, at various doses, was added either immediately before (panel B) or after (panel C) irradiation. Two days after irradiation, the cells were harvested and stained for 8-oxoguanine. The results of 5 independent experiments, presented as the mean ± SD of the mean fluorescence channel, show an increase by exposure to radiation and a decrease by FPP treatment, both in a dose-related fashion. Statistical differences between 18 Gy irradiated cells that were treated with 100 μg/ml FPP compared with untreated cells, are denoted as follows: $*P < 0.001$, $***P < 0.05$.

cells and cells irradiated and treated with FPP. The results show an increase in fluorescence, i.e., 8-oxoguanine content in the DNA after irradiation, which was ameliorated by FPP. Cells treated with FPP alone (without irradiation) were similar to untreated cells (not shown).

To investigate DNA damage, a single-cell gel electrophoresis (the comet assay) was used to detect damage/repair of DNA (*24*). The results are expressed as tail DNA, representing the percentage of migrated DNA from the total nuclear DNA. Figure 5 shows photographs of representative fields of untreated cells (Fig. 5A), irradiated cells (Fig. 5B) or cells irradiated and treated with FPP (Fig. 5C). Tail DNA can be observed (shown by arrows) only in Fig. 5B, indicating radiation-induced DNA breakage, but not in Fig. 5C, indicating protection by FPP.

In vivo. Mice were irradiated with 0–14 Gy. After irradiation, mice were treated with FPP (0–100 μg/ml in the drinking water). After 3 days, mice were sacrificed, and bone marrow cells were obtained and assayed for DNA oxidation (8-oxoguanine) and DNA damage (the comet assay). The results (Fig. 6) indicate that both parameters were increased by radiation exposure and decreased by FPP treatment.

Accumulation of a Somatic Mutation

GPI-negative phenotype was used as a surrogate biomarker of genetic instability. Three months after radiation exposure and treatment with various doses of FPP, peripheral blood samples were obtained and the RBC were labeled with fluorescent antibodies to GPI-markers

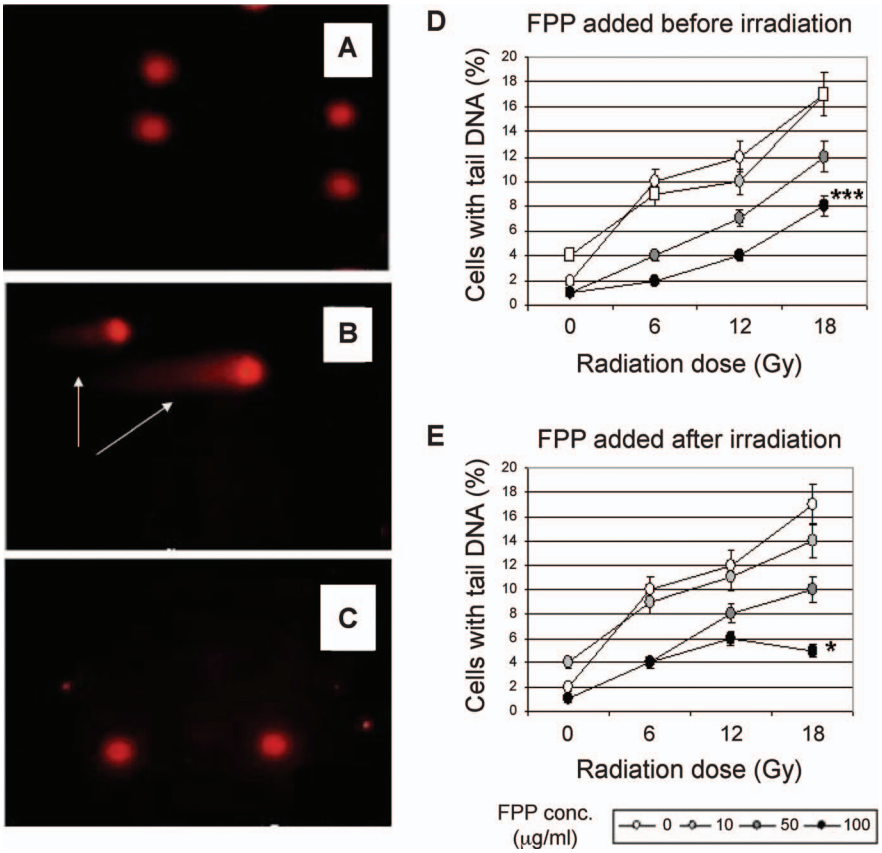

FIG. 5. The *in vitro* effect of FPP on radiation-induced DNA damage (the comet assay). Human fibroblasts were untreated (panel A), 6 Gy irradiated (panel B) and 6 Gy irradiated and treated with FPP (100 μg/ml) (panel C). After 2 days, the cells were harvested, washed and analyzed for DNA damage by the comet assay. Microphotographs of representative fields are shown. ''Tail DNA'' can be observed (shown by arrows) only in panel B, indicating that radiation-induced DNA breakage, which was prevented by FPP treatment (panel C). Panels D and E: FPP treatment at the indicated doses, was added either immediately before (panel D) or after (panel E) irradiation of human fibroblasts. Two days after irradiation, the cells were harvested and stained for tail DNA. DNA damage was assessed based upon percentage tail DNA. The results of 5 independent experiments, presented as the mean ± SD, show increased tail DNA after irradiation that was decreased by FPP, both in a dose-related fashion. Statistical differences between 18 Gy irradiated cells that were treated with 100 μg/ml FPP compared with untreated cells are denoted as follows: $*P < 0.001$, $***P < 0.05$.

(CD55, CD59). The results (Fig. 7) show that the frequency of GPI-negative RBC was increased by radiation exposure and decreased by FPP treatment, both in a dose-dependent fashion.

DISCUSSION

Acute exposure to ionizing radiation may occur after an accident at a nuclear facility such as, Chernobyl or Fukushima, acts of warfare (Hiroshima, Nagasaki) or terrorism, and to a lesser degree, medical procedures such as radiology and radiotherapy or exposure to environmental radiation such as during intercontinental flights (*25*). Such exposure can lead to a variety of short-term acute effects, including lethality, or long-term late effects encompassing organ injury (*1*) and carcinogenesis (*2*). Studies of chemical and biological radiation protectors or mitigators (*7, 26*) are ongoing to protect against these toxicities.

Radiation-induced toxicity is mediated in part by free radicals, particularly ROS (*3*), which can interact with cellular components such as proteins, lipids and DNA, causing short- and long-term damage. Several antioxidants have been shown to be radioprotective and to reduce the damage to normal tissues (*4–6*). Since FPP has been shown to have antioxidant activity, in the current study we investigated its potential as a radioprotector. We first demonstrated that FPP treatment reduces the LIP and ROS in cultured cells (Fig. 1). We then studied its protective effect on radiation-induced toxicity *in vitro* and *in vivo*. The results showed that FPP treatment reduced radiation-induced membrane damage, apoptosis and DNA damage and improved the survival of cultured cells as well as bone marrow cells of irradiated mice (Figs. 2–6).

Radiation exposure has short- and long-term effects on the DNA. We measured the effects of FPP treatment on short-term DNA damage and repair *in vitro* and *in vivo* using the 8-oxoguanine and comet assays. The former assay

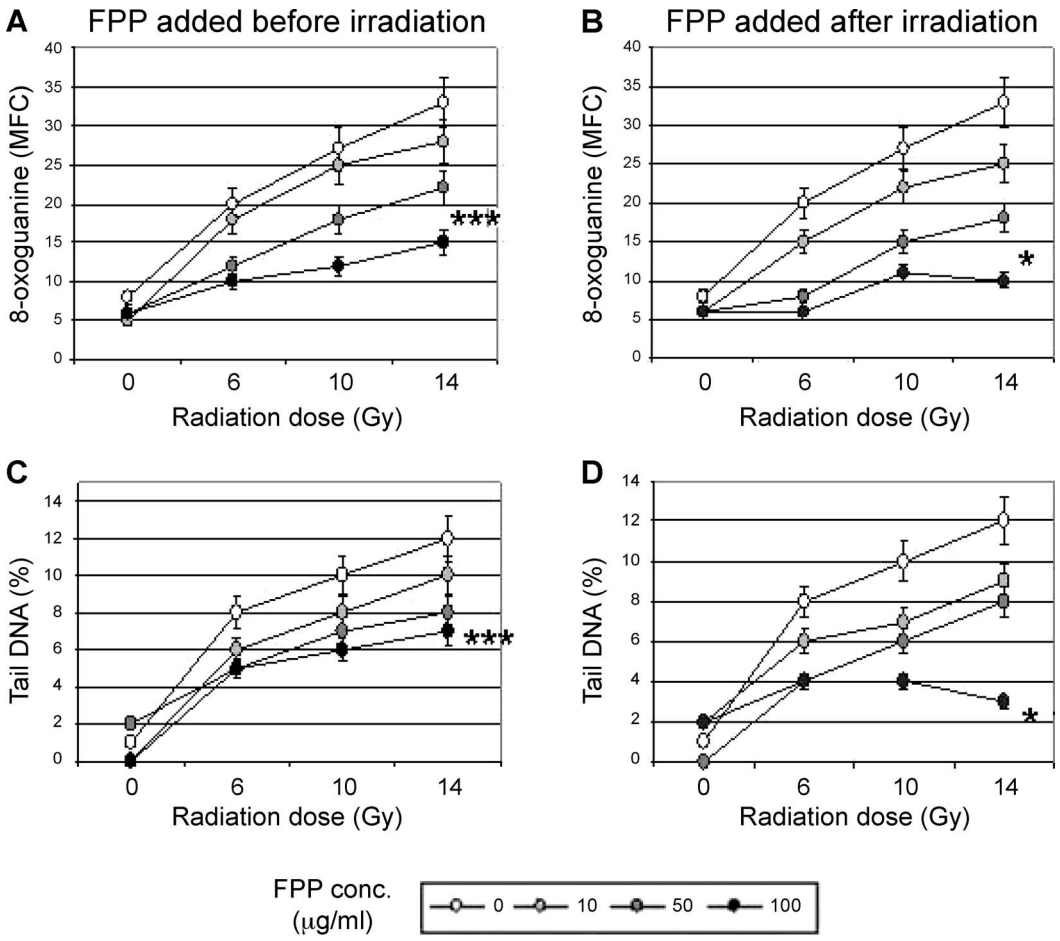

FIG. 6. The *in vivo* effect of FPP on radiation-induced DNA damage. Mice were fed different doses of FPP, starting either 1 day before (panels A and C) or immediately after (panels B and D) irradiation. After 3 days, the marrow cells were analyzed for 8-oxoguanine (panels A and B) and DNA damage (panels C and D). The results of one experiment, 5 mice per group, are presented as the mean ± SD of the mean fluorescence channel (panels A and B) and of the percentage tail DNA (panels C and D). The results show an increase by irradiation and a decrease by FPP, both in a dose-related fashion. Statistical differences between 14 Gy irradiated mice that were treated with 100 μg/ml FPP compared with untreated are denoted as follows: $*P < 0.001$, $***P < 0.05$.

detects the most common DNA adduct formed after irradiation (*20*). A previous study has shown that elderly patients given supplementation of FPP for 3 months had decreased 8-oxoguanine in their lymphocyte DNA (*27*). The latter assay detects various forms of acute DNA damage (e.g., single-strand breaks, double-strand breaks and DNA crosslinks) (*28–30*). Both assays measure various changes in the DNA at the time of analysis but not the ability of the cell to correctly repair these changes at later times.

Long-term radiation-induced DNA changes, if not repaired, may be associated with mutagenicity leading to cancer. Carcinogenesis is a multistep process driven by sequential occurrence and accumulation of mutations in oncogenes and tumor suppressor genes. Exposure to ionizing radiation is a well known inducer of genetic instability, which leads to accumulation of mutations for both initiation and progression of carcinogenesis (*31*). To evaluate genetic instability, we adapted a flow cytometry assay currently employed for diagnosis of paroxysmal nocturnal hemoglobinuria (*32*). In this disease, somatic mutations in the PIG-A gene of a hematopoietic stem cell render its progeny (all blood cell types) deficient in GPI, which normally anchors various proteins to the cell surface. We measured PIG-A mutations by determining the frequency of GPI-negative RBC, after labeling with fluorescent antibodies to GPI-markers (CD55, CD59), as a surrogate biomarker of genetic instability (*32*).

The GPI approach, as it has been utilized previously (*33–35*) for analysis of genetic instability has several theoretical and practical advantages. 1. Procedure measures directly a ''loss of function'' (GPI deficiency) due to a mutation in the PIG-A gene. 2. GPI is not essential for RBC production; the mutant cells remain viable and lend themselves to analysis (although they turn sensitive to activated complement-mediated lysis. 3. The PIG-A gene is chromosome X-linked. Since only one functional X-chromosome exists in male or female cells, a single mutated chromosome is sufficient to cause GPI deficiency. Thus, GPI deficiency is

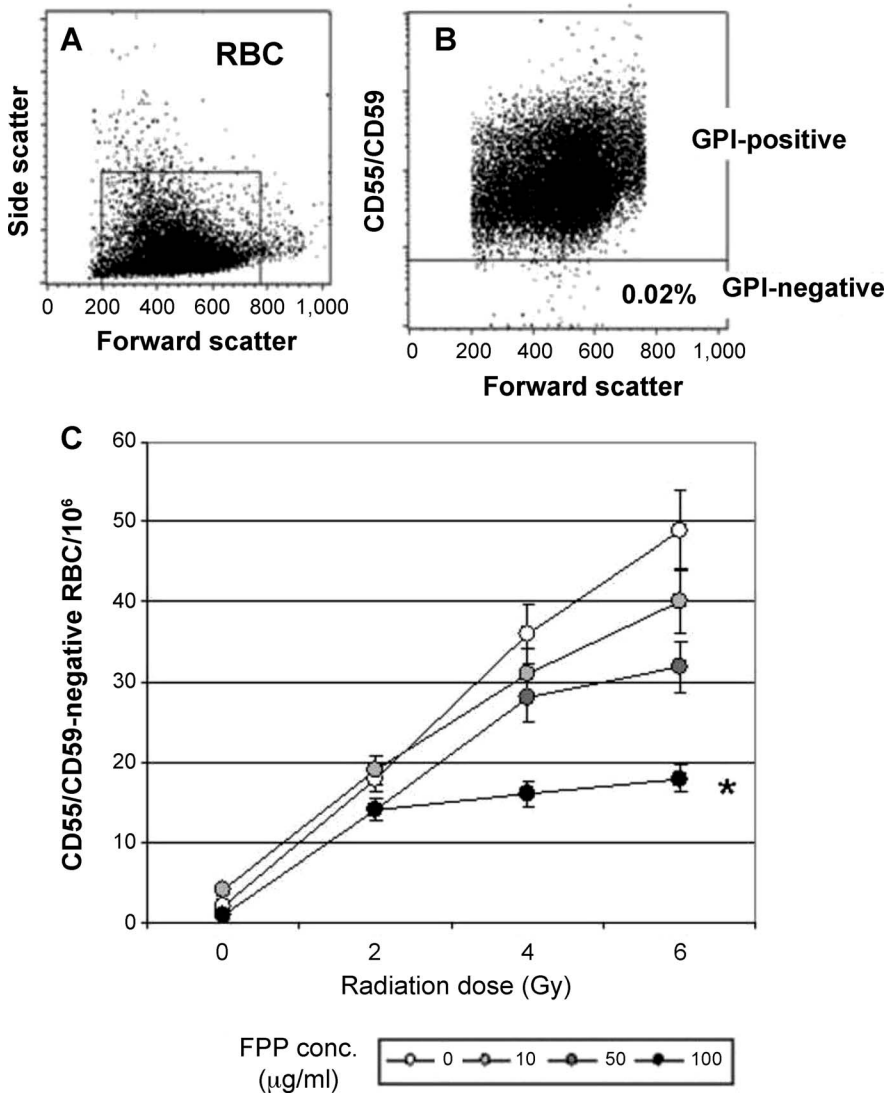

FIG. 7. The effect of FPP on mutation accumulation. After 0–6 Gy irradiation, mice were fed continuously with FPP. The frequency of CD55/CD59 red blood cells (RBC) was determined by flow cytometry after staining with FITC-conjugated anti-CD59/CD55 antibodies. One $\times 10^6$ RBC were gated based on their size and granularity (FSC vs. SSC) (panel A) and analyzed for CD55/CD59 expression (panel B). The results show that in this particular sample, 0.2% of the RBC were negative for CD55/CD59. Panel C: Frequency (mean $\pm$ SD) of CD55/CD59 negative RBC in nonirradiated and irradiated mice (one experiment, 5 mice per group) treated with different doses of FPP for 3 months. The results show an increase by irradiation and a decrease by FPP, both in a dose-related fashion. Statistical difference between 6 Gy irradiated mice that were treated with 100 μg/ml FPP compared with untreated mice is $*P < 0.001$.

equivalent to a PIG-A mutation. 4. RBC are readily available in large numbers, and as single cells they can be analyzed directly by flow cytometry. 5. Flow cytometry enables multiparameter analysis of specific populations; e.g., both lineage (RBC)-specific and GPI-anchor markers can be simultaneously measured. 6. The fast rate of data acquisition by this methodology permits analysis of a large number (millions) of cells, thus, very rare mutant cells can be detected and their frequency measured.

The results showed (Fig. 7) that the rate of GPI-mutation accumulation is accelerated by irradiation, in agreement with previous studies [e.g., (*36*)], and that treatment with FPP has a dose-dependent ameliorating effect. The GPI-negative phenotype has no proven relationship to carcinogenesis, and serves in this study as a surrogate marker to genetic instability, but FPP treatment may have a similar effect on mutations in oncogenes and tumor suppressor genes that are closely associated with carcinogenesis. It should be noted that FPP treatment upregulates not only the expression of genes of key enzymes involved in the redox state, but also of the DNA repair gene 8-oxoguanine glycosylase (*9*).

We suggest that FPP might be considered as a radiation protector or mitigator. The results of this study show that FPP treatment can be effective when administered prior to, as well as shortly after radiation exposure and thus might

serve as a potential countermeasure for radiological mass casualties. As an application in radiotherapy, a radioprotector should improve the therapeutic ratio with relative ease of delivery and with minimal toxicity, as has been demonstrated with FPP treatment in several clinical studies (*8*). A radioprotector should also protect vital normal radiosensitive tissues, which we confirmed in this current study by FPP analysis, by analyzing hematopoietic cells, since bone marrow failure, reflected by cytopenia, is a severe clinical outcome of radiotherapy. Whether FPP is selective in protecting normal tissues versus tissue was not investigated in the current work, and warrants careful additional study.

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