Published in final edited form as:

Health Phys. 2014 June ; 106(6): 720–726. doi:10.1097/HP.000000000000108.

CIPROFLOXACIN INCREASES SURVIVAL AFTER IONIZING IRRADIATION COMBINED INJURY: γ -H2AX FORMATION, CYTOKINE/CHEMOKINE, AND RED BLOOD CELLS

Juliann G. Kiang^{1,2,3,*} and Risaku Fukumoto¹

¹Radiation Combined Injury Program, Armed Forces Radiobiology Research Institute, Bethesda, MD, U.S.A

²Department of Radiation Biology, Uniformed Services University of the Health Sciences, Bethesda, MD, U.S.A

³Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, U.S.A

Abstract

Exposure to ionizing radiation alone (radiation injury, as abbreviated RI) or combined with traumatic tissue injury (radiation combined injury, as abbreviated CI) is a crucial life-threatening factor in nuclear and radiological accidents. It is well-documented that RI and CI occur at the molecular, cellular, tissue, and system levels. However, their mechanisms remain largely unclear. It has been observed in dogs, pigs, rats, guinea pigs, and mice that radiation exposure combined with burns, wounds, or bacterial infection results in greater mortality than radiation exposure alone. In our laboratory, we found that B6D2F1/J female mice exposed to 9.75 Gy ⁶⁰Co- γ photon radiation followed by 15% total body surface area wounds experienced 50% higher mortality (over 30-day observation period) compared to irradiation alone. CI enhanced DNA damages, amplified iNOS activation, induced massive release of pro-inflammatory cytokines, overexpressed MMPs and TLRs and aggravated sepsis that led to cell death. In the present study, B6D2F1/J mice received CI were treated with ciprofloxacin (CIP, 90 mg/kg *p.o.*, *q.d.* within 2h after CI through day 21). At day 1, CIP treatment significantly reduced CI-induced γ -H2AX formation. At day 10, CIP treatment not only significantly reduced cytokine/chemokine concentrations, including IL-6 and KC (i.e., IL-8 in human), but also enhanced IL-3 production compared to vehicle-treated controls. CIP also elevated red blood cell counts, hemoglobin levels and hematocrits. At day 30, CIP treatment increased 45% survival after CI (i.e., 2.3-fold increase over vehicle treatment). The results suggest that CIP may prove to be an effective therapeutic drug for CI.

*For correspondence contact: Juliann G. Kiang, Radiation Combined Injury Program, Armed Forces Radiobiology Research Institute, Bethesda, MD 20889-5603, U.S.A., or juliann.kiang@usuhs.edu.

The views, opinions and findings contained in this report are those of the authors and do not reflect official policy or positions of the Armed Forces Radiobiology Research Institute, the U.S. Department of Defense, the National Institutes of Health, or the United States Government.

There is no conflict of interest.

Keywords

radiation; wound; ciprofloxacin; γ -H2AX; cytokine; RBC; WBC; bone marrow

INTRODUCTION

Injuries induced by ionizing radiation alone or in combination with trauma from blast and thermal energy exposure are expected after the detonation of radiation dispersal devices or nuclear weapons. *In vivo* (Kiang et al. 2012b, Fukumoto et al. 2013) and *in vitro* (Kiang et al. 2009, Fukumoto and Kiang, 2011) studies indicate that radiation injury (RI)-induced DNA strand breaks activated signal transduction pathways, elevated cytokine/chemokine concentrations in the peripheral blood, and increased systemic bacterial infection, thereby leading to cell death and multiple organ dysfunction and failure. Traumatic injury followed by RI (*i.e.*, combined injury abbreviated as CI) enhanced histopathological responses to RI (Kiang et al. 2010 and 2012b). Because the responses to RI and CI occurred at molecular, cellular, tissue and system levels, the complexity made very difficult to identify countermeasures for radioprotection, radiomitigation, or radiotherapy.

We previously reported an experimental model for CI, in which 15% total body surface area (TBSA) dorsal skin wound trauma increased RI-induced cell death, tissue damage, organ dysfunction, and finally mortality in mice (Kiang et al. 2010). In B6D2F1/J female mice exposed to 9.75 Gy ^{60}Co - γ -photons at 0.4 Gy/min alone (RI) or in combination with wound trauma (CI) were applied. Within hours, ionizing irradiation induces DNA strand breaks that lead to ataxia telangiectasia mutated (ATM) phosphorylation. As a result, the histone H2AX is phosphorylated within seconds, which is termed γ -H2AX and radiation dose-dependent (Rogakou et al., 2000; Houtgraaf et al., 2006). It is evident that the γ -H2AX formation is correlated with DNA strand breaks (Scherthan et al., 2008). During the course of 10 days after CI, these mice also experienced greatly increased concentrations of pro-inflammatory cytokines/chemokines in serum (Kiang et al. 2010 and 2012b), rapid loss in hematological parameters, including lymphocytes and granulocytes (Kiang et al. 2012b), overexpression of MMPs and TLRs (Kiang, 2012a), and gastrointestinal injury as well as systemic endogenous bacterial translocation from the intestinal tract and subsequent sepsis (Kiang et al. 2010). At day 30, skin wound trauma increased 50% mortality after RI (Kiang et al. 2012b).

Ciprofloxacin (CIP) is an FDA-approved fluoroquinolone (FQ), which is widely used as an antimicrobial. CIP has been included in the Strategic National Stockpile, which is maintained by the U.S. Department of Health and Human Services, to control bacterial infection during a national emergency such as a nuclear detonation or other radiological incident. Our laboratory was the first to report that CIP enhanced bone marrow repopulation and limited cell death in small intestine via apoptosis and autophagy (Fukumoto et al., 2013). In this communication, we report that CIP significantly reduced γ -H2AX formation and levels of pro-inflammatory cytokines, while it potentiated IL-3 production. CIP also elevated RBC counts, hemoglobin levels and hematocrits. These CIP-mediated changes may have contributed to improve 30-day survivability in CI-mice compared to vehicle-treated control animals.

MATERIALS AND METHODS

Animals

Research was conducted in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care-International (AAALACI). All procedures involving animals were reviewed and approved by the AFRRRI Institutional Animal Care and Use Committee (IACUC). Euthanasia was carried out in accordance with the recommendations and guidelines of the American Veterinary Medical Association.

Female B6D2F1/J mice were purchased from Jackson Laboratory (Bar Harbor, ME) and were used at average weights of 20–25-g. Male mice were not used in this study because of problems associated with aggression, which in these experiments could lead to further damage to wound sites and enhanced infection. All mice were randomly assigned to experimental groups. Rooms were provided with 10–15 changes per hour of 100% fresh air conditioned to $72 \pm 2^\circ\text{F}$ with a relative humidity of $50 \pm 20\%$. Mice were maintained on a 12-h light/dark, full-spectrum light cycle with no twilight and freely reached food.

Radiation injury

Mice were placed in well-ventilated acrylic restrainers and given 9.25 Gy ($\text{LD}_{70/30}$ for survival study), 8.5 Gy (non-lethal for $\gamma\text{-H2AX}$ study), and 9.75 Gy ($\text{LD}_{70/30}$ for cytokine and blood cell studies) of whole-body ^{60}Co γ -photon radiation delivered at a dose rate of approximately 0.4 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 mice were placed in the same acrylic restrainers, taken to the radiation facility, and restrained for the time required for irradiation.

Wound trauma

Four or five days prior to the experiments, hair of the dorsal surface was removed using electric clippers under anesthesia by isoflurane inhalation. On the day of experiments, within 1 h after irradiation, mice were under anesthesia by methoxyflurane inhalation; an experimental wound approximately 15% total body surface area was administered. Sham-wounded mice were treated identically to other groups except without wounding. Mice were first irradiated and then wounded. All mice, including controls, received an intraperitoneal injection of 0.5mL sterile isotonic 0.9% NaCl solution as fluid therapy immediately after combined injury or sham injury to avoid radiation-induced dehydration. After injuries mice were returned to clean cages and provided with proper food and acidified water.

Preparation and administration of ciprofloxacin

Veterinary-, oral-use ciprofloxacin (CIP) tablets (500 mg/each) (Dr. Reddy's laboratories, Hyderabad, India) were used to prepare fresh solution every week. Tablets were ground, dissolved in sterile water (vehicle) and after a brief centrifugation sterile-filtered using 0.22 μm CN (cellulose nitrate) filter system (Corning; Corning, NY). Each dose of 0.2mL ciprofloxacin solution delivered 90 mg/kg based on average body weight. All mice received

0.2mL of either ciprofloxacin or vehicle via oral route once per day for 21 days, starting within 2h after CI and through day 21.

Experimental Design

For studies of survival (N=10 per group, repeated once) and γ -H2AX formation (N=8 per group), animals were divided into 8 groups: sham+vehicle, sham+CIP, wound+vehicle, wound+CIP, radiation (RI)+vehicle, RI+CIP, radiation+wound (CI)+vehicle, and CI+CIP. Because CIP was effectively improved survival and reduced the γ -H2AX formation after CI but not RI, and because CIP did not affect the 100% survival of wounded mice, for measurements of cytokine/chemokine concentrations and blood cell counts in peripheral blood, animals were mainly divided into 4 groups (N=5 per group): sham+vehicle, sham+CIP, CI+vehicle, and CI+CIP. The reduction of animal groups in the latter experiment was encouraged by the AFRRI IACUC to meet the 3R guidelines of the American Veterinary Medical Association.

Survival

Thirty day-Survival after CI and therapy with CIP was evaluated. The gross appearance, general health, and survival of each mouse were followed by visual inspection daily.

Bone marrow cell staining

Bone marrow cells were collected from both femurs of euthanized mice at day 1 after RI or CI. Cells were washed with cold PBS twice. According to the manufacturer's protocol provided by BD Biosciences, approximately 1×10^6 total bone marrow cells were incubated with 1 μ l of Live/Dead Fixable Dead Cell Stain-Near-Infrared (Invitrogen) and $<1 \mu$ g / ml of Mouse Fc Block (BD Biosciences) in PBS on ice for 30 min and washed with PBS. The cells were then incubated with a cocktail of Mouse Hematopoietic Lineage Flow Cocktail-eFluor450 (e-Bioscience), anti-mouse-c-kit-Cy5(e-Bioscience), and anti-mouse-Sca-1-phycoerythrin-Cy5.5(e-Bioscience), and then washed again with PBS, fixed with 1.5% paraformaldehyde for 20 min on ice, washed with PBS, permeabilized and stained with perm/wash buffer (BD Biosciences) containing 7% v/v anti-phospho-H2AX-FITC (Millipore) and Mouse Fc block (BD Biosciences), and washed with 1x perm/wash buffer (BD Biosciences).

Flow cytometry for γ -H2AX measurement

Stained bone marrow cells with no less than 5,000 cells were analyzed on a LSR II cytometer (BD Biosciences) equipped with 407, 488 and 633 nm lasers, emission filters for near-infrared, FITC, PE-Cy5.5, APC, and Cy5, and a high-throughput sampler in Biomedical Instrumentation Center (BIC) in USUHS. Data were analyzed with FACSDiva software (BD Biosciences). Analyses were performed at the Uniformed Services University of the Health Sciences Flow Cytometry Core Facility, Bethesda, Maryland (Kiang et al., 2012b).

Cytokine/chemokine measurements

Whole blood samples (0.7–1.0 mL) were collected by terminal cardiac puncture from mice anesthetized by methoxyflurane at day 10 after CI. CapiJect tubes (Terumo; Somerset, NJ) were used to separate sera by centrifugation at 3,500 xg for 90 sec and stored at –70°C until assayed. Cytokine concentrations were analyzed using the Bio-Plex™ Cytokine Assay (Bio-Rad; Hercules, CA) following the manufacturer's directions. Concentrations were expressed in pg/mL unless otherwise noted (Kiang et al. 2010).

Assessment of blood cell profile in peripheral blood

Blood samples (0.5 mL) were collected by terminal cardiac puncture from mice anesthetized by methoxyflurane at day 10 after CI in EDTA-containing tubes (Sarstedt Inc., Newton, NC) and assessed with the ADVIA 2120 Hematology System (Siemens, Deerfield, IL). Differential analysis was conducted using the peroxidase method and the light scattering techniques recommended by the manufacturer.

Statistical Analysis

Eight mice groups were used for each survival experiment, and it was repeated to gain statistical significance (total N= 10/group, repeated once). Survival analyses were performed using the log-rank test. All other results are expressed as means ± SEM. One-way ANOVA, two-way ANOVA, Studentized-range test, and χ^2 test were used for comparison of groups; 5% was used as the level of significance.

RESULTS

Ciprofloxacin significantly increased survival of CI-mice

Either sham operation or skin wounds (15% total body surface area) alone with vehicle or CIP treatment did not result in mortality over a 30-day observation period. CIP treatment increased 10% survival of RI-mice (from 40% to 50%), which was not statistically significant. However, skin wound following irradiation resulted in an early onset of mortality at day 10 and 35% survival of CI-mice, whereas treatment with CIP, however, delayed the onset by 2 days and significantly increased 45% survival above the vehicle group after CI (from 35% to 80% survival, $P<0.05$, Fig. 1), with a 2.3-fold increase in survival.

Ciprofloxacin reduced γ -H2AX formation in bone marrow cells of CI-mice

Because it is evident that RI and CI increased the γ -H2AX formation in lineage-positive bone marrow cells (Kiang et al., 2012b), the γ -H2AX formation was measured in bone marrow cells at day 1 after CI in the presence of CIP. Because Sca1 (Holmes and Stanford, 2007) and c-Kit (Yarden et al., 1987) are cell surface proteins promoting cell proliferation and differentiation in gastrointestinal tissues, cells were also stained with either Sca1 or c-Kit. As shown in Fig. 2, wounding alone, compared to sham groups, did not affect γ -H2AX basal levels. CIP treatment did not alter their basal levels either. RI and CI significantly increased γ -H2AX formation. CIP treatment effectively inhibited γ -H2AX formation only

in lineage-negative cells regardless of the presence of sca1 or c-Kit proteins on the cell membrane.

Ciprofloxacin modulated cytokine/chemokine concentrations in sera of CI-mice

CI is known to increase concentrations of cytokines/chemokines in serum. We measured cytokines in sera of CI-mice at day 7 and showed that CI increased serum concentrations of IL-6, KC, G-CSF, Rantes, IL-1 α , IL-13, TNF- α , IL-1 β , IL-9, IL-10, GM-CSF, eotaxin and MIP-1 α/β (Kiang et al. 2010) and returned to their baseline except KC and G-CSF in surviving CI-mice at day 30 after CI (Kiang et al., 2012b), suggesting that cytokine/chemokine concentrations in peripheral blood were important indicators for survival. Because in CI-mice, the onset of mortality started at day 10 and CIP treatment delayed the onset (Fig. 1), cytokine/chemokine concentrations in peripheral blood were measured at day 10 in CI-mice treated with CIP to investigate whether CIP reduced their increases caused by CI. CIP treatment mitigated CI-induced increases in IL-6, KC (*i.e.*, IL-8), G-CSF and Rantes, but did not alter the CI-induced increase in GM-CSF. CIP significantly increased the IL-3 concentration in CI-mice (Fig. 3).

Ciprofloxacin elevated RBC counts but not WBC counts and platelet counts after CI

CI induced RBC, WBC and platelet depletion (Kiang et al. 2012b). We found that in surviving CI-mice at day 30 after CI, the RBC count returned to the basal level but the white blood cell (WBC) count was still approximately 10 times lower than the baseline (Kiang et al., 2014), suggesting the RBC count was important indicator for survival. Because in CI-mice, the onset of mortality started at day 10 and CIP treatment delayed the onset, RBC counts in peripheral blood were measured at day 10 in CI-mice treated with CIP to investigate whether CIP accelerated its recovery. As shown in Fig. 4, CIP mitigated RBC depletion, hemoglobin decreases, and hematocrit decreases caused by CI. We did not find recovery in numbers of WBCs and platelets in CI-mice treated with CIP at this time point.

DISCUSSION

There is a pressing need to find countermeasures to treat RI and CI. At present, none of countermeasures yet evaluated to treat either condition has been approved by the U.S. FDA for the use in humans based upon evaluation in two suitable laboratory animal species. In the search for appropriate CI-treatment targets, it is necessary to understand the mechanisms underlying CI. Our research has been investigating many pathophysiological changes that occurred during the period of 30 days after CI, which identified increased DNA damage, stress-response gene expressions (Kiang et al., unpublished data), serum cytokine and chemokine concentrations, ileal damage, bacterial translocation (Kiang et al., 2010), and blood-cell depletion (Kiang et al. 2012a and b). Some of these changes were great enough to distinguish CI from any other single injuries, and offered us multiple treatment targets or parameters to monitor prospective countermeasure development.

CIP is a second-generation fluoroquinolone (FQ). The spectrum of antimicrobial activity of CIP is due to the specificity in inhibition directed to bacterial DNA gyrase and topoisomerase IV, particularly in Gram(–) species (Hooper 2002). The pharmacokinetics

and pharmacodynamics in humans have been well documented. It is well tolerated, distributes rapidly in tissues, and has similar half-lives in mice and humans (Craig 2004), and is readily available in several formulations for oral, intravenous, intraperitoneal, and topical routes. These factors meet the needs for the management of bacterial infection in a mass-casualty scenario. Although it has been used after the anthrax attack in 2001 (Stephens and Marvin 2010), it has never been tested for a treatment of CI.

CIP were shown *ex vivo* to enhance cytokines IL-3 and GM-CSF in mouse splenocytes that gave rise to enhanced hematopoiesis (Shalit et al. 1997), while CIP, together with a mitogen, induced hyper-production of IL-2 in human peripheral lymphocytes (Riesbeck 2002). Although the exact mechanism is elusive, it is possible that transcription factors, which are common to increased cytokines, such as activator protein-1 (Riesbeck 2002) or NF- κ B (Fukumoto et al., 2013), are activated by those FQs. The topoisomerase II inhibitory effect of CIP may be a benefit in treating enhanced inflammation induced by systemic bacterial infection (Verdrengh and Tarkowski 2003) caused by CI.

In the present study, we investigated whether CIP mitigated CI through its effects on survival, γ -H2AX formation, cytokine/chemokine levels, RBC recovery, and bacterial elimination of surviving mice after CI. CIP significantly reduced γ -H2AX formation in lineage-negative bone marrow cells regardless of the presence of Sca1 or c-Kit, suggesting that Sca1 or c-Kit were not required for CIP exerting its actions and that CIP recovered lineage-negative bone marrow cells (*i.e.*, mesenchymal stromal cells) that might have helped radiation-sensitive tissue repair timely (Kolf et al., 2007). It is possible that the inhibitory effect of CIP on topoisomerase II (used for DNA repair) might be an alternative explanation for the reduced γ -H2AX levels measured at day1 after CI. For instance, cells remaining blocked in S-phase so that the γ -H2AX formation cannot occur or cells being dying because of insufficient ways of repair due to inhibited topoisomerase-II cannot be excluded. It is elusive why CIP did not reduce the γ -H2AX formation in all bone marrow cells after RI and in lineage-positive bone marrow cells (*i.e.* hematopoietic stem cells) after CI. The further study in these regards to unfold the complexity will surely be eagerly anticipated.

In the sera, CIP effectively reduced CI-induced pro-inflammatory concentrations of cytokines IL-6, KC (*i.e.*, IL-8 in human), G-CSF, and Rantes. Continuous, high expression of IL-6, IL-8, and Rantes are known to cause serious secondary damage to the organs rather than its benefits obtained by mild and transient expression (Neta et al., 1992). IL-3 is an essential mediator for myeloid lineage development and has been reported to be elevated by CIP treatment (Shalit et al. 1997). In our study, CIP significantly increased IL-3 concentrations, RBC counts, hemoglobin levels, and hematocrits only in CI-animals. IL-3 in combination with erythropoietin (EPO) did not help ameliorate anemia in myelodysplastic patients (Musto et al. 2001) that was distinctly different from the CI-induced anemia shown in this report. Therefore, the observation of accelerated bone-marrow recovery by CIP after CI (Fukumoto et al. 2013) warrants future studies on the association of the IL-3, EPO and red blood cell production, perhaps mediated by activation of the HIF-1 α /NF- κ B transduction pathway.

We previously reported that CIP limited physical damages occurring in the ileum after CI. Analysis on cell-death type-specific markers revealed that after CI villous epithelial cells underwent apoptosis while crypt cells displayed elevated formation of autophagosomes. CIP therapy effectively reduced both types of cell death, the consequences resulting from activation of caspase-3 pathway and LC3 increases, respectively. In CI mice, we detected both systemic Gram(+) (*Enterococcus faecalis*, *Staphylococcus xylosum*, *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Enterococcus gallinarum*, *Bacillus cereus*, *Rothia mucilaginosa*) and Gram(−) (*Escherichia coli*, *Klebsiella oxytoca*, *Enterobacter cloacae*) bacterial infection. CIP treatment eliminated all Gram(−) bacteria (Fukumoto et al. 2013). It should bear in mind that the data presented in this report obtained at the specified time points after CI provide limited information on CIP mechanisms. However, taking these data together with the previous publications, orally administered CIP, therefore, offers advantages to be developed further as a drug to treat CI in a mass-casualty scenario.

CONCLUSION

In summary, CIP significantly increased survival after CI. This effect could be associated with its capability of reducing γ -H2AX formation, modulating the serum cytokine/chemokine profile, elevating RBC counts, hemoglobin levels, and hematocrits. Therefore, the results suggest that CIP may prove to be beneficial for treating critical sequelae of CI.

Acknowledgments

We thank Mr. True M. Burns, Mr. Bradley R. Garrison, Dr. Lynnette H. Cary, Ms. Joan T. Smith, and Dr. Thomas B. Elliott for their technical contribution. Research was supported by NIH/NIAID R33 AI080553 to JGK.

References

- Craig WA. Proof of concept: performance testing in models. Clin Microbiol Infect. 2004; 10 (Suppl 2): 12–17. [PubMed: 14759229]
- Fukumoto R, Cary LH, Gorbunov NV, Elliott TB, Kiang JG. Ciprofloxacin modulates cytokine profiles, accelerates bone marrow recovery and mitigates ileum injury after radiation combined with wound trauma. PLoS One. 2013; 8:e58389. (11 pages). [PubMed: 23520506]
- Fukumoto R, Kiang JG. Geldanamycin analog 17-DMAG limits apoptosis in human peripheral blood cells by inhibition of p53 activation and its interaction with heat-shock protein 90 kDa after exposure to ionizing radiation. Radiat Res. 2011; 176:333–345. [PubMed: 21663398]
- Holmes C, Stanford WI. Concise review: stem cell antigen-1: Expression, function, and enigma. Stem Cells. 2007; 25:1339–1347. [PubMed: 17379763]
- Hooper DC. Fluoroquinolone resistance among Gram-positive cocci. Lancet Infect Dis. 2002; 2:530–538. [PubMed: 12206969]
- Houtgraaf JH, Versmissen J, van der Giessen WJ. A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovasc Revasc Med. 2006; 7:165–172. [PubMed: 16945824]
- Kiang JG. Overview of biological effects of irradiation combined injury. NATO-HFM-223-P5:1-18, STO Meeting Proceedings, Biological Effects of Ionizing Radiation Exposure and Countermeasures: Current Status and Future Perspectives; Ljubljana, Slovenia, Oct 8-10, 2012. Published by The NATO Science and Technology Organization (ISBN 978-92-837-0179-8); www.sto.nato.int.
- Kiang JG, Garrison BR, Burns TM, Zhai M, Dews IC, Ney PH, Fukumoto R, Cary LH, Elliott TB, Ledney GD. Wound trauma alters ionizing radiation dose assessment. Cell & Bioscience. 2012b; 2:20. [PubMed: 22686656]

- Kiang JG, Jiao W, Cary LH, Mog SR, Elliott TB, Ledney GD. Wound trauma increases radiation-induced mortality by activation of iNOS pathway and elevation of cytokine concentrations and bacterial infection. *Radiat Res.* 2010; 173:319–332. [PubMed: 20199217]
- Kiang JG, Smith JA, Agravante NG. Geldanamycin analog 17-DMAG inhibits iNOS and caspases in gamma irradiated human T cells. *Radiat Res.* 2009; 172:321–330. [PubMed: 19708781]
- Kiang JG, Zhai M, Liao PJ, Bolduc DL, Elliott TB, Gorbunov NV. Pegylated G-CSF inhibits blood cell depletion, increases platelets, blocks splenomegaly, and improves survival after whole-body ionizing irradiation but not after irradiation combined with burn. *Oxid Med Cell Longv.* 2014; 48:1392
- Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells biology of adult mesenchymal stem cells: regulation of niche, self-removal and differentiation. *Arthritis Res Ther.* 2007; 9:204. [PubMed: 17316462]
- Musto P, Sanpaolo G, D'Arena G, Scalzuli PR, Matera R. Adding growth factors or interleukin-3 to erythropoietin has limited effects on anemia of transfusion-dependent patients with myelodysplastic syndromes unresponsive to erythropoietin alone. *Haematologica.* 2001; 86:44–51. [PubMed: 11146570]
- Neta R, Perlstein R, Vogel SN, Ledney GD, Abrams J. Role of interleukin 6 (IL-6) in protection from lethal irradiation and in endocrine response to IL-1 and tumor necrosis factor. *J Exp Med.* 1992; 175:689–694. [PubMed: 1311016]
- Riesbeck K. Immunomodulating activity of quinolones: review. *J Chemother.* 2002; 14:3–12. [PubMed: 11892896]
- Rogakou EP, Nieves-Neira W, Boon C, Pommier Y, Bonner WM. Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *J Biol Chem.* 2000; 275:9390–9395. [PubMed: 10734083]
- Scherthan H, Hieber L, Braselmann H, Meineke V, Zitzelsberger H. Accumulation of DSBs in gamma-H2AX domain fuel chromosomal aberration. *Biochem Biophys Res Commun.* 2008; 371:694–697. [PubMed: 18457664]
- Shalit I, Kletter Y, Weiss K, Gruss T, Fabian I. Enhanced hematopoiesis in sublethally irradiated mice treated with various quinolones. *Eur J Haematol.* 1997; 58:92–98. [PubMed: 9111589]
- Stephens MB, Marvin B. Recognition of community-acquired anthrax: has anything changed since 2001? *Mil Med.* 2010; 175:671–675. [PubMed: 20882930]
- Verdrengh M, Tarkowski A. Impact of topoisomerase II inhibition on cytokine and chemokine production. *Inflamm Res.* 2003; 52:148–153. [PubMed: 12755380]
- Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO J.* 1987; 6:3341–3351. [PubMed: 2448137]

Fig. 1. CIP increased survival after CI

Mice received 9.25 Gy ^{60}Co γ -photon radiation (RI) followed within 1 h by 15% TBSA skin wound (CI). Mice were given CIP (90 mg/kg *p.o. q.d.*) or vehicle for 21 days starting day 0 2h after CI (N = 10 per group). Experiment was repeated to achieve statistical significance. *P < 0.05 vs. CI+CIP. W: wound; RI: radiation injury at 9.25 Gy; CI: 9.25 Gy + wound; Veh: vehicle; CIP: ciprofloxacin.

Fig. 2. CIP reduced γ -H2AX formation in lineage-negative bone marrow cells of CI-mice

On day 1 after wounding, RI, or CI, whole femoral bone marrow cells were collected (N=8 per group). *P<0.05 vs. CI+vehicle.

RI: 8.5 Gy; CI: 8.5 Gy + wound; CIP: ciprofloxacin; VEH: vehicle. W: wound.

Fig. 3. CIP modulated cytokine/chemokine concentrations in serum of CI-mice

On day 10 after CI, whole blood was collected from surviving mice and the sera were separated. Sera were tested for their cytokine and chemokine concentrations (N = 3–5 per group); P<0.05 vs. Veh and CIP; **P<0.05 vs. CI. CI: 9.75 Gy + wound; CIP: ciprofloxacin; S: sham; V: vehicle.

Fig. 4. CIP ameliorated CI-induced depletion of RBC but not WBC and platelets

On day 10 after CI, whole blood was collected from surviving mice (N = 4–5 per group); * $p < 0.05$ vs. Veh and CIP; ** $P < 0.05$ vs. CI. CI: 9.75 Gy + wound; CIP: ciprofloxacin; S: sham; V: vehicle; RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; WBC: white blood cell; PLT: platelet