Title:

The potential benefits of nicaraven to protect against radiation-induced injury in hematopoietic stem/progenitor cells with relative low dose exposures

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Abstract

Nicaraven, a hydroxyl radical-specific scavenger has been demonstrated to attenuate radiation injury in hematopoietic stem cells with 5 Gy γ-ray exposures. We explored the effect and related mechanisms of nicaraven for protecting radiation injury induced by sequential exposures to a relatively lower dose γ-ray. C57BL/6 mice were given nicaraven or placebo within 30 minutes before exposure to 50 mGy γ-ray daily for 30 days in sequences (cumulative dose of 1.5 Gy). Mice were victimized 24 hours after the last radiation exposure, and the number, function and oxidative stress of hematopoietic stem cells were quantitatively estimated. We also compared the gene expression in these purified stem cells from mice received nicaraven and placebo treatment. Nicaraven increased the number of c-kit$^+$ stem/progenitor cells in bone marrow and peripheral blood, with a recovery rate around 60~90% of age-matched non-irradiated healthy mice. The potency of colony forming from hematopoietic stem/progenitor cells as indicator of function was completely protected with nicaraven treatment. Furthermore, nicaraven treatment changed the expression of many genes associated to DNA repair, inflammatory response, and immunomodulation in c-kit$^+$ stem/progenitor cells. Nicaraven effectively protected against damages of hematopoietic stem/progenitor cells induced by sequential exposures to a relatively low dose radiation, via complex mechanisms.

Introduction

Acute exposure to ionizing radiation at a dose range of 0.7 to 4 Gy is well defined to develop symptoms associated with hematopoietic and immune damages [1]. It has also reported the increased incidence of cancer risk in nuclear workers who classically exposed to low doses of extraordinary rates [2]. Furthermore, medical doctors and technicians have been shown high incidence of chromosomal disruptions and hematopoietic malignancies even if with exposure to less than 50 mGy [3-5]. Although many chemical and biological compounds were widely tested for protecting against radiation injury [6,7], there is only amifostine, a scavenger of reactive oxygen species (ROS) had been approved clinically as a cytoprotective adjuvant to alleviate the side effects for patients who get radiotherapy [8]. Unfortunately, the administration of amifostine is limited due to its accumulative toxicity and side effects, including nausea, vomiting, hypotension, and allergic reactions [9]. Therefore, it is highly required to develop new agents of less or no toxicity for protecting against the potential health threats from natural, incidental, and occupational exposures to relative low dose radiations.

Nicaraven (1,2-bis (nicotinamido)-propane), synthesized chemical compound known as a hydroxyl radical-specific scavenger [10], had previously been found to amend the survival of mice exposed to a lethal dose of γ-ray [11], and to protect against radiation-induced cell death [12,13]. We have recently demonstrated that the administration of nicaraven to mice soon after the exposure to a high dose of γ-ray attenuates the radiation-induced injury of hematopoietic stem/progenitor cells, via reduction of DNA damage of cells and inhibition of inflammation [14]. Taking the safety profile of nicaraven and the potential long-term healthy risk of occupational radiation exposures in consideration [2, 15], it will be so interesting to confirm the prophylactic effect of nicaraven on radiation-induced injury, especially on these immature stem/progenitor cells after the exposure to relatively lower dose.

By daily exposure of healthy adult mice to 50 mGy γ-ray for 30 days (cumulative

dose of 1.5 Gy), we investigated the prophylactic effect of administration of nicaraven against radiation-induced injury in hematopoietic stem/progenitor cells, and further to understand the relevant mechanisms.

Materials and Methods

Animals

We used 8- to 12-week-old male C57BL/6 mice (SLC, Japan) for the present study. All experiments were approved by the Institutional Animal Care and Use Committee of Nagasaki University (No. 1108120943), and the animal procedures were performed in accordance with institutional and national guidelines.

Radiation exposure and nicaraven administration

Whole-body radiation was done by exposing the mice to γ-rays with a ^{137}Cs source in PS-3100SB γ-ray irradiation system (Pony Industry Co., Ltd. Osaka, Japan) [16]. To explore the effect and relevant mechanisms of nicaraven for protecting radiation-induced injury in hematopoietic stem/progenitor cells, mice were daily exposed to 50 mGy γ-rays for 30 days in sequences (cumulative dose of 1.5 Gy). Intraperitoneal injection with 100 mg/kg nicaraven [14] (Nicaraven group, n=6) or saline only (Placebo group, n=6) was done within 30 minutes before each exposure. Mice were victimized 24 hours after the last exposure, and samples of blood and bone marrow cells were collected for the subsequent experiments.

Measurements of stem cells in the peripheral blood and bone marrow

Samples of heparinized peripheral blood were collected, and the number of mononuclear cells in the blood was counted using a NucleoCounter (Chemotetec A/S, Denmark). To measure the c-kit-positive (c-kit$^+$) stem/progenitor cells, we isolated the nucleated cells from the peripheral blood by density gradient centrifugation [17], and then

labeled with a PE-conjugated anti-mouse CD117 (c-kit) antibody (eBioscience) for 30 minutes. Respective isotype control was used as a negative control. After washing, quantitative flow cytometry was performed using a FACSCalibur (Becton Dickinson) [17]. We analyzed the acquired data using Cell Quest software (Becton Dickinson).

Bone marrow cells were collected from the femur and tibia, and the mononuclear cells were isolated by density gradient centrifugation. The c-kit$^+$ cells in the collected bone marrow mononuclear cells were measured as described above.

Colony-forming assay

To evaluate the function of stem/progenitor cells from peripheral blood and bone marrow, a colony-forming assay was performed by using mouse methylcellulose complete medium, according to the manufacturer's instructions (R&D System). Briefly, $1x10^5$ peripheral nucleated cells or $3x10^4$ bone marrow mononuclear cells were mixed well with 1 ml of medium, plated in 3-cm culture dishes, and then incubated at 37°C in a 5% CO_2 incubator. The formation of colonies was observed under a microscopy, and the number of colonies was counted after 9 days (for bone marrow mononuclear cells) or 12 days (for peripheral blood nucleated cells) of incubation. The average number of colonies from duplicated assays was used for the statistical analyses.

Purification of c-kit$^+$ stem/progenitor cells

The c-kit$^+$ stem/progenitor cells were purified by using the magnetic cell sorting system (autoMACS, Miltenyi Biotec, Auburn, CA). Briefly, freshly collected bone marrow mononuclear cells were incubated with microbeads-conjugated anti-mouse CD117 (c-kit) antibody (Miltenyi Biotec) for 30 minutes. After washing, c-kit$^+$ cells were separated by passing a MACS column. The purity of the c-kit$^+$ cells collected by the autoMACS was about 90%, and the viability was more than 99%.

Detection of intracellular ROS

To elucidate the relevant mechanism, we measured the intracellular ROS level based on the oxidation of 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA, Molecular Probes Inc.) to form the fluorescent compound 2',7'-dichlorofluorescein (DCF), as described previously [16,18]. Briefly, freshly separated c-kit$^+$ cells and c-kit-negative cells were seeded in 96-well culture plates ($1x10^4$ cells/100 µl/well) and incubated at 37°C in a 5% CO_2 incubator for 24 hrs. Cells were then loaded with 10 µM CM-H2DCFDA at 37°C for 30 minutes. After the cells were washed, the fluorescence intensities of the wells were measured using the (ARVO X3 plate reader, Perkin Elmer).

Mouse Molecular Toxicology Pathway Finder RT2 Profiler PCR array

To compare the gene expression, RNA was isolated from pelleted c-kit$^+$ cells by using RNeasy Mini Kit and RNAse free DNase (Qiagen, Hilden, Germany). The concentration of RNA was determined using Nano Drop (Thermo Scientific, Wilmington, DE, USA), and 1 mg of RNA was used to generate cDNA using RT2 First Strand Kit (SABiosciences, a Qiagen Company). Mouse Molecular Toxicology Pathway Finder RT2 Profiler PCR array was done according to the manufacturer's instructions (SABiosciences). This PCR array profiled the expression of 370 genes representative of the 13 biological pathways involved in response to toxic drugs (*Supporting Table 1*) and exhibited good reproducibility of data [19]. The fold change of expression was calculated using SABiosciences' web-based data analysis program. Results represent the mean of four independent samples from nicaraven and placebo.

Statistical analyses

Data were represented as means ± SD. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by post hoc test (Dr. SPSS II,

Chicago, IL). The p value less than 0.05 were accepted as significant**.**

Results

Nicaraven mitigated the radiation-induced reduction of peripheral blood nucleated cells and c-kit$^+$ stem/progenitor cells

Compared with the placebo treatment, the administration of nicaraven increased the number of nucleated cells in the peripheral blood (6.3±1.2 x10^5 *vs.* 4.1±0.4 x10^5, p<0.001; ***Fig 1A***), but it was still measured as only 60% of the level of non-irradiated healthy mice (11.0±1.3 x10^5, p<0.01; ***Fig 1A***). The exposure to 50 mGy for 30 days also decreased the percentage of c-kit$^+$ cells in the peripheral blood to around 50% of the level of non-irradiated healthy mice (p<0.05, ***Fig 1B***). However, the administration of nicaraven increased the percentage of c-kit$^+$ cells in the peripheral blood (0.053±0.02% *vs.* 0.024±0.01% in Placebo, p<0.05; ***Fig 1B***), which resulted to keep the c-kit$^+$ cells at a comparable level of non-irradiated healthy mice (0.058±0.02%, p=0.7; ***Fig 1B***).

Nicaraven alleviated the radiation-induced reduction of bone marrow mononuclear cells and c-kit$^+$ stem/progenitor cells

Compared with the placebo treatment, the administration of nicaraven increased the number of mononuclear cells (69.5±5.0 x10^6 *vs.* 55.2±9.0 x10^6, p<0.05; ***Fig 2A***), and even higher than the non-irradiated healthy mice (63.8±3.2 x10^6 *vs.* 69.5±5.0 x$10^{6,}$ p<0.05; ***Fig 2A***). However, the percentage of c-kit$^+$ cells in the nicaraven-treated mice were still measured as almost 55% of the non-irradiated healthy mice (1.9±0.1% *vs.* 3.7±0.6%, p<0.001, ***Fig 2B***), although it was higher than that of in placebo treatment (1.4±0.2%, p<0.01, ***Fig 2B***).

Nicaraven enhanced the function of hematopoietic stem/progenitor cells

The functional assessment of hematopoietic stem/progenitor cells was done by

colony-forming assay *in vitro*. The formation of colonies was also clearly detectable within 12 days from peripheral blood nucleated cells. By quantitatively counting, we found that the total number of colonies formed from peripheral blood nucleated cells was higher in the Nicaraven group than Placebo group (8.0±2.1 *vs.* 5.0±1.0, $p<0.05$; ***Fig 3A***). The potency of colonies formation from peripheral blood nucleated cells of the nicaraven-treated mice was kept almost the same as that of the non-irradiated healthy mice (7.3±1.2, $p=0.52$; ***Fig 3A***)

The formation of different types of colonies was obviously observed from bone marrow mononuclear cells after 9 days of culture. Although we did not measure the overall size, colonies was bigger in size in the Nicaraven group than of Placebo group. Also, the total number of colonies resulting from bone marrow mononuclear cells was higher in the Nicaraven group than of Placebo group (75.8±2.7 *vs.* 52.2±6.0, $p<0.001$; ***Fig 3B***). However, the size and number of colonies resulting from the nicaraven-treated mice were quite similar as that of the non-irradiated healthy mice (81.8±6.6, $p=0.08$; ***Fig 3B***).

Nicaraven diminished slightly the levels of intracellular ROS

As nicaraven is well recognized as a hydroxyl radical-specific scavenger, we measured the level of intracellular ROS in purified c-kit$^+$ cells. Unexpectedly, the intracellular ROS levels in both of c-kit$^+$ stem/progenitor cells and c-kit-negative matured bone marrow mononuclear cells were measured very similar among groups, although the ROS level was slightly decreased by nicaraven treatment ***(Fig 4)***.

Complex mechanisms involved in the protective effect of nicaraven to radiation injury

We used a Mouse Molecular Toxicology Pathway Finder RT2 Profiler PCR array to screen the gene expression changes. Among the total of 370 genes included in the array, 208 genes were detected to be up- or down-regulated over two-fold by the nicaraven administration in comparison with placebo treatment (*Supporting Table*). The top 20 genes

were listed up in tables (*Table 1 and 2*). We noticed that the most of top 20 genes with up- or down-regulation by nicaraven treatment were functionally associated with DNA damage repair (*Brca2*, *Prkdc* and *Ercc2*), ER stress gene (*Ube2g2*), cell apoptosis (*Bcl2l1*, *Bid* and *Bad*), inflammatory response (*TNF*, *IL1a*), immunomodulation (*C3, CD36*), and beta oxidation *(ACOX1)*, suggested complex mechanisms were involved in the protective effect of nicaraven to radiation injury.

Discussion

In this study, we have demonstrated that the administration of nicaraven can effectively mitigate the damage of hematopoietic stem/progenitor cells in mice exposed to successive relatively low dose of γ-ray (daily 50 mGy for 30 days). We have further found that the protective effect of nicaraven against radiation-induced injury to hematopoietic stem/progenitor cells likely associated with complex mechanisms included the inhibition of inflammation, immunomodulation and enhancement of DNA repair, rather than a simple radical scavenger reducing ROS.

A high dose ionizing radiation can directly lead to DNA double-strand break to kill the cells. It has also generally accepted that the exposure to even relative low dose of radiation may trigger the generation of ROS, which indirectly induce DNA damage and genomic instability [20]. Therefore, quickly scavenging the ROS by radical scavengers was considered as one of the effective approach for attenuating radiation-induced injury, especially that occupationally exposed to a relatively low dose radiation. Our study showed that nicaraven has a prophylactic effect on protecting c-kit$^+$ hematopoietic stem/progenitor cells against radiation injury in mice exposed to a relatively low dose radiation, although the peripheral blood nucleated cells and the percentage of c-kit$^+$ cells in bone marrow in mice received nicaraven treatment was not recovered to the levels of healthy mice.

On the mechanism, agreed well with the result of recent study in mice received

high dose radiation [14], surprisingly, the ROS level was only slightly decreased in these purified c-kit$^+$ stem/progenitor cells from mice received nicaraven when compared to those received placebo treatment. This again suggests that nicaraven protection against radiation injury seems to be associated with other mechanism rather than as simple as a radical scavenger. To further understand the potential mechanisms of the protective effect of nicaraven against radiation injury, we purified c-kit$^+$ stem/progenitor cells from mice received nicaraven or placebo treatment and then performed a PCR array analysis to compare the gene expression profile related to molecular toxicology pathways. Among the top 20 of up- and down-regulated genes, many genes are associated with DNA repair/ stress responses, inflammatory/ immunomodulation, cell apoptosis/ necrosis, and beta oxidation.

Within these genes that associated with DNA damage repair (*Supporting Fig 1*), the most up-regulated gene of *BRCA2* involved in homologous recombination repair pathway, which contributes to an error-free DNA repair that play an essential role in maintaining genome stability and tumor suppression [21,22]. Although, the most down-regulated gene of *OGGI1* has been demonstrated to remove damaged bases from the DNA, especially in repair of DNA damages induced by oxidative stress [23]. Although, nicaraven treatment slightly decreased the ROS level in these purified c-kit$^+$ stem/progenitor cells, it was exciting to investigate whether nicaraven decrease the DNA damage induced by oxidative stress , including a technical challenging to monitor the ROS level within the c-kit$^+$ stem/progenitor cells *in situ*. Also, nicaraven up-regulated the expression of many genes associated with apoptosis (*Supporting Fig 2*), including *BCL2L1*, *BID* and *BAD* that required for radiation-induced apoptosis [24]. It is known that the defect of apoptosis cause genomic instability [25, 26]. Also, some DNA repair molecules induce apoptosis in response to DNA lesions, these genes associated with apoptosis and DNA repair work together to create a complex biological network for maintaining genomic stability [27]. In other words, nicaraven treatment not only reduces

cell death, but also helps to commit these c-kit$^+$ stem/progenitor cells with DNA damage into apoptosis. However, many necrotic genes were down-regulated by nicaraven treatment (*Supporting Fig 3*).

Nicaraven treatment decreased the expressions of many genes associated with inflammatory response (*Supporting Fig 4*), including *TNF and IL1a,* in the top of the down-regulated genes. Furthermore, the decrease of immune function is highly observed after radiotherapy or occupationally exposures to even low level of ionizing radiation [28]. Interestingly, nicaraven up-regulated the expression of *C3*gene, it plays a central role in complement system and innate immunity [29]. Nicaraven treatment also up-regulated *ACOX1,* a gene involving the first step in beta oxidation process of fatty acid to produce ATP supporting cell withstand and survival [30,31], also playing roles in redox equilibrium [32]. Otherwise, nicaraven up-regulated other genes, such as *Ube2g2* that involved in ER stress recovery and supporting cell survival under stress [33].

This study has some limitations. The first, although complex mechanisms, including DNA repair, inflammatory response, immunomodulation, and energy supply were likely involved in nicaraven for protection against radiation injury, further study will be needed to identify the precise mechanism. The second, we only measured the percentages of c-kit$^+$ cells within bone marrow mononuclear cells and peripheral blood nucleated cells. However, it is still unclear whether the protective effect of nicaraven will be varied among different subtypes of stem/progenitor cells, such as the rare population of long-term hematopoietic stem cells (Lin-/Sca1+/c-Kit+, LSK cells). Otherwise, it is also important to compare the protective effect against radiation injury and the side effect of nicaraven with other drugs, including amifostine that is already approved for clinical application [8].

In summary, we have confirmed that the prophylactic effect of nicaraven on protecting c-kit$^+$ hematopoietic stem/progenitor cells against radiation injury in mice exposed daily to 50 mGy γ-ray for 30 days (cumulative dose of 1.5 Gy). Although the

complex molecular mechanism was not completely understood, the excellent safety profile of nicaraven provides potential possibility as prophylactic treatment for protecting against radiation injury, especially for those whom occupationally exposed to relatively low dose radiation.

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None

Author contributions

T.L. conceived and designed the experiments

O.G., Y.U., S.G., C.G., L.L., E.A., E.M., Y.O., T.L. performed the experiments and analyzed the data

H.A., T.L. wrote the main manuscript text.

All authors reviewed the manuscript.

Additional Information

The authors have not competing financial interests.

References

1. B.J. Chen, D. Deoliveira, I. Spasojevic, et al, Growth hormone mitigates against lethal irradiation and enhances hematologic and immune recovery in mice and nonhuman primates, *PLoS One* 5 (2010) e11056.
2. E. Cardis, M. Vrijheid, M. Blettner, et al, Risk of cancer after low doses of ionising radiation: retrospective cohort study in 15 countries, *B.M.J.* **331** (2005)7508-7577.
3. F. Maffei, S. Angelini, G.C. Forti, et al, Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation. *Mutat. Res.* **547** (2004) 91-99.
4. E. Vañó, L. González, F. Beneytez , F. Moreno, Lens injuries induced by occupational exposure in non-optimized interventional radiology laboratories, *Br. J. Radiol.* **71** (1998) 728-733.
5. M.S. Linet, D.M. Freedman, A.K. Mohan, et al, Incidence of hematopoietic malignancies in US radiologic technologists, *Occup. Environ. Med.* **62** (2005) 861-867.
6. J.F. Weiss, M.R. Landauer, History and development of radiation-protective agents, *Int. J. Radiat. Biol*. **85** (2009) 539-573.
7. C.K. Nair, D.K. Parida, T. Nomura, Radioprotectors in radiotherapy, *J. Radiat. Res.* **42** (2001) 21-37.
8. J. Bourhis, P. Blanchard, E. Maillard, et al, Effect of amifostine on survival among patients treated with radiotherapy: a meta-analysis of individual patient data, *J. Clin. Oncol.* **29** (2011) 2590-2597.
9. J.M.Yuhas, J.M. Spellman, F. Culo, The role of WR -2721 in radiotherapy and/or chemotherapy, *Cancer Clin. Trials* **3**, 211-216 (1980).
10. T. Akimoto, Quantitative analysis of the kinetic constant of the reaction of N, N'-propylenedinicotinamide with the hydroxyl radical using dimethyl sulfoxide and deduction of its structure in chloroform, *Chem. Pharm. Bull.* **48** (2000) 467-476.

11. Y. Masana, T. Yoshimine, T. Fujita, M. Maruno, E. Kumura, T. Hayakawa, Reaction of microglial cells and macrophages after cortical incision in rats: effect of a synthesized free radical scavenger, (+/-)-N, N'-propylenedinicotinamide (AVS), *Neurosci. Res.* **23** (1995) 217-221.
12. Y. Mori , H. Takashima , H. Seo , et al, Studies on nicaraven as radio protector-free radical scavenging effect and the inhibition of the cellular injury, *Nihon Igaku Hoshasen Gakkai Zasshi* **53** (1993) 704-712.
13. M. Watanabe, N. Akiyama, H. Sekine, M. Mori, Y. Manome, Inhibition of poly (ADP-ribose) polymerase as a protective effect of nicaraven in ionizing radiation- and ara-C-induced cell death, *Anticancer Res.* **26** (2006) 3421-3427.
14. M. Kawakatsu , Y. Urata , R. Imai , et al, Nicaraven attenuates radiation induced injury in hematopoietic stem/progenitor cells in mice, *PLoS One* **8** (2013) e60023.
15. T. Asano, K. Takakura, K. Sano, et al, Effects of a hydroxyl radical scavenger on delayed ischemic neurological deficits following aneurysmal subarachnoid hemorrhage: results of a multicenter, placebo-controlled double-blind trial, *J. Neurosurg.* **84** (1996) 792-803.
16. T. Yoshida, S. Goto, M. Kawakatsu, Y. Urata, T.S. Li, Mitochondrial dysfunction, a probable cause of persistent oxidative stress after exposure to ionizing radiation, *Free Radic. Res.* **46** (2012) 147-153.
17. T.S. Li , S. Ikeda , M. Kubo, et al, Diabetic impairment of C-kit bone marrow stem cells involves the disorders of inflammatory factors, cell adhesion and extracellular matrix molecules, *PLoS One* **6** (2011) e25543.
18. T.S. Li, E. Marbán, Physiological levels of reactive oxygen species are required to maintain genomic stability in stem cells, *Stem Cells* **28** (2010) 1178-1185.
19. E. Arikawa, Y. Sun, J. Wang, et al, Cross-platform comparison of SYBR Green real-time PCR with TaqMan PCR, microarrays and other gene expression measurement technologies evaluated in the MicroArray Quality Control (MAQC) study, *B.M.C.*

Genomics **9** (2008) 328.

20. E.I. Azzam, J.P. Jay-Gerin, D. Pain, Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury, *Cancer Lett*. **327** (2012) 48-60.
21. N. Siaud, M.A. Barbera, A. Egashira, et al, Plasticity of BRCA2 function in homologous recombination: genetic interactions of the PALB2 and DNA binding domains, *PLoS Genet.* **7** (2011) e1002409.
22. W.K. Holloman, Unraveling the mechanism of BRCA2 in homologous recombination, *Nat. Struct. Mol. Biol.* **18** (2013) 748-754.
23. D.R. Denver, S.L. Swenson, M. Lynch, An evolutionary analysis of the helix-hairpin-helix superfamily of DNA repair glycosylases, *Mol. Biol. Evol.* **20** (2003) 1603-1611.
24. R.C. Taylor, S.P. Cullen, S.J. Martin, Apoptosis: controlled demolition at the cellular level, *Nat. Rev. Mol. Cell Biol.* **9** (2008) 231-241.
25. A.L. Barabasi, Z.N. Oltvai, Network biology: understanding the cell's functional organization, *Nat. Rev. Genet.* **5** (2004) 101-113.
26. D.A. Nelson, T.T. Tan, A.B. Rabson, D. Anderson, K. Degenhardt, E. White, Hypoxia and defective apoptosis drive genomic instability and tumorigenesis, *Genes Dev.* **18** (2004) 2095-2107.
27. M.A. Castro, R.J. Dalmolin, J.C. Moreira, J.C. Mombach, R.M. de Almeida, Revolutionary origins of human apoptosis and genome-stability gene networks, *Nucleic Acids Res.* **36** (2008) 6269-6283.
28. A. Godekmerdan, M. Ozden, A. Ayar, M.F. Gursu, A.T. Ozan, Serhatlioglu S. Diminished cellular and humoral immunity in workers occupationally exposed to low levels of ionizing radiation, *Arch. Med. Res.* **35** (2004) 324-328.
29. B.J. Janssen, E.G. Huizinga, H.C. Raaijmakers, et al, Structures of complement component C3 provide insights into the function and evolution of immunity, *Nature* **437** (2005) 505-511.
30. Y. Jiao, L.S. Zan, Y.F. Liu, H.B. Wang, Molecular characterization, polymorphism of

the ACOX1 gene and association with ultrasound traits in Bos taurus, *Genet. Mol. Res.* **10** (2011) 1948-1957.

31. Z. Jia, Z. Pei, Y. Li, L. Wei, K.D. Smith, P.A. Watkins, X-linked adrenoleukodystrophy: role of very long-chain acyl-CoA synthetases, *Mol. Genet. Metab.* **83** (2004) 117-127.
32. M. Baarine, P. Andréoletti, A. Athias, et al, Evidence of oxidative stress in very long chain fatty acid -Treated oligodendrocytes and potentialization of ROS production using RNA interference-directed knockdown of ABCD1 and ACOX1 peroxisomal proteins, *Neuroscience* **213** (2012) 1-18.
33. L. Yan, W. Liu, H. Zhang, et al, Ube2g2-gp78-mediated HERP polyubiquitylation is involved in ER stress recovery, *J. Cell Sci.* **127** (2014) 1417-1427.

Table 1: The top 20 genes that up-regulated by nicaraven treatment.

Rank	Symbol	Gene Name	Fold increased	Gene Function
1	Brca2	Breast cancer 2	94.092	DNA Repair
2	Ube2g2	Ubiquitin-conjugating enzyme E2G 2	84.800	ER stress
3	C3	Complement component 3	79.672	Inflammatory and immunity
4	Acox1	Acyl-Coenzyme A oxidase 1, palmitoyl	51.840	Beta Oxidation
5	Mlx	MAX-like protein X	47.373	Phospholipidosis
6	Ercc2	Excision repair cross-complementing rodent repair deficiency, complementation group 2	32.809	DNA Repair
7	Dnajc5	DnaJ (Hsp40) homolog, subfamily C, member 5	26.465	Heat Shock Response
8	Esr1	Estrogen receptor 1 (alpha)	23.039	Cholestasis
9	Bad	BCL2-associated agonist of cell death	22.565	Apoptosis
10	Bid	BH3 interacting domain death agonist	22.409	Apoptosis
11	Nploc4	Nuclear protein localization 4 homolog (S. cerevisiae)	18.844	ER stress
12	Smpd1	Sphingomyelin phosphodiesterase 1, acid lysosomal	16.749	Phospholipidosis
13	Icam1	Intercellular adhesion molecule 1	13.232	Cholestasis
14	Dnajc6	DnaJ (Hsp40) homolog, subfamily C, member 6	12.346	Heat shock Response
15	Ephx1	Epoxide hydrolase 1, microsomal	12.176	Phospholipidosis
16	Prkdc	Protein kinase, DNA activated, catalytic polypeptide	12.009	DNA Repair
17	Mttp	Microsomal triglyceride transfer protein	11.600	Steatosis
18	Bcl2l1	Bcl2-like 1	11.361	Apoptosis
19	H2-Eb1	Histocompatibility 2, class II antigen E beta	9.822	Cholestasis
20	Cd36	CD36 antigen	7.341	Immunity

Table 2: The top 20 genes that down-regulated by nicaraven treatment.

Rank	Symbol	Gene Name	Fold decreased	Gene Function
1	TNF	Tumor necrosis factor	-137.568	Inflammatory and immunity
2	Cd86	CD86 antigen	-63.734	Inflammatory and immunity
3	Hsf2	Heat shock factor 2	-48.976	Heat shock Response
4	Cd19	CD19 antigen	-48.976	Inflammatory and immunity
5	Cd80	CD80 antigen	-47.968	Inflammatory and immunity
6	Il1a	Interleukin 1 alpha	-41.184	Inflammatory and immunity
7	Hspa1l	Heat shock protein 1-like	-35.853	Heat shock Response
8	Dpysl4	Dihydropyrimidinase-like 4	-35.115	Necrosis
9	Tff3	Trefoil factor 3, intestinal	-29.733	Steatosis
10	Hspb6	Heat shock protein, alpha-crystallin-related, B6	-29.733	Heat shocks Response
11	Ogg1	8-oxoguanine DNA-glycosylase 1	-27.550	DNA Repair
12	Cyp1a2	Cytochrome P450, family 1, subfamily a, polypeptide 2	-11.827	Cytochrome P450s
13	Fmo3	Flavin containing monooxygenase 3	-11.267	Cytochrome P450s
14	Lig4	Ligase IV, DNA, ATP-dependent	-11.112	DNA Repair
15	Jph3	Junctophilin 3	-9.945	Necrosis
16	Aldh2	Aldehyde dehydrogenase 2, mitochondrial	-9.409	Steatosis
17	Retn	Resistin	-8.363	Steatosis
18	Atp6v1g2	ATPase, H+ transporting, lysosomal V1 subunit G2	-7.749	Necrosis
19	Sel1l	Sel-1 suppressor of lin-12-like (C. elegans)	-7.642	ER stress
20	Ercc1	Excision repair cross-complementing rodent repair deficiency, complementation group 1	-7.180	DNA Repair

Figure legends

Fig 1. *The number of nucleated cells and stem/progenitor cells in the peripheral blood of mice after radiation exposure and nicaraven treatment.* The number of nucleated cells in the peripheral blood (**A**) was directly counted, and c-kit$^+$ stem/progenitor cells (**B**) in the peripheral blood were measured in the fraction of the nucleated cells by flow cytometry.

Fig 2. *The number of mononuclear cells and stem/progenitor cells in the bone marrow of mice after radiation exposure and nicaraven treatment.* The number of collected bone marrow mononuclear cells from each mouse was directly counted (**A**) and the c-kit$^+$ stem/progenitor cells were measured by flow cytometry (**B**).

Fig 3. *Colony-forming assay*. The formation of different types of colonies (>50 cells) was directly counted under microscopy. Data indicates the number of colonies formed from a total of $1x10^5$ peripheral blood nucleated cells after 12 days of incubation (**A**), or $3x10^4$ bone marrow mononuclear cells after 9 days of incubation (**B**).

Fig 4. *Intracellular ROS in the bone marrow stem/progenitor cells in the bone marrow of mice after radiation exposure and nicaraven treatment.* The intracellular ROS in the purified c-kit$^+$ stem/progenitor cells (**A**) and c-kit-negative matured cells (**B**) were detected as the fluorescence intensity.

Fig 1.

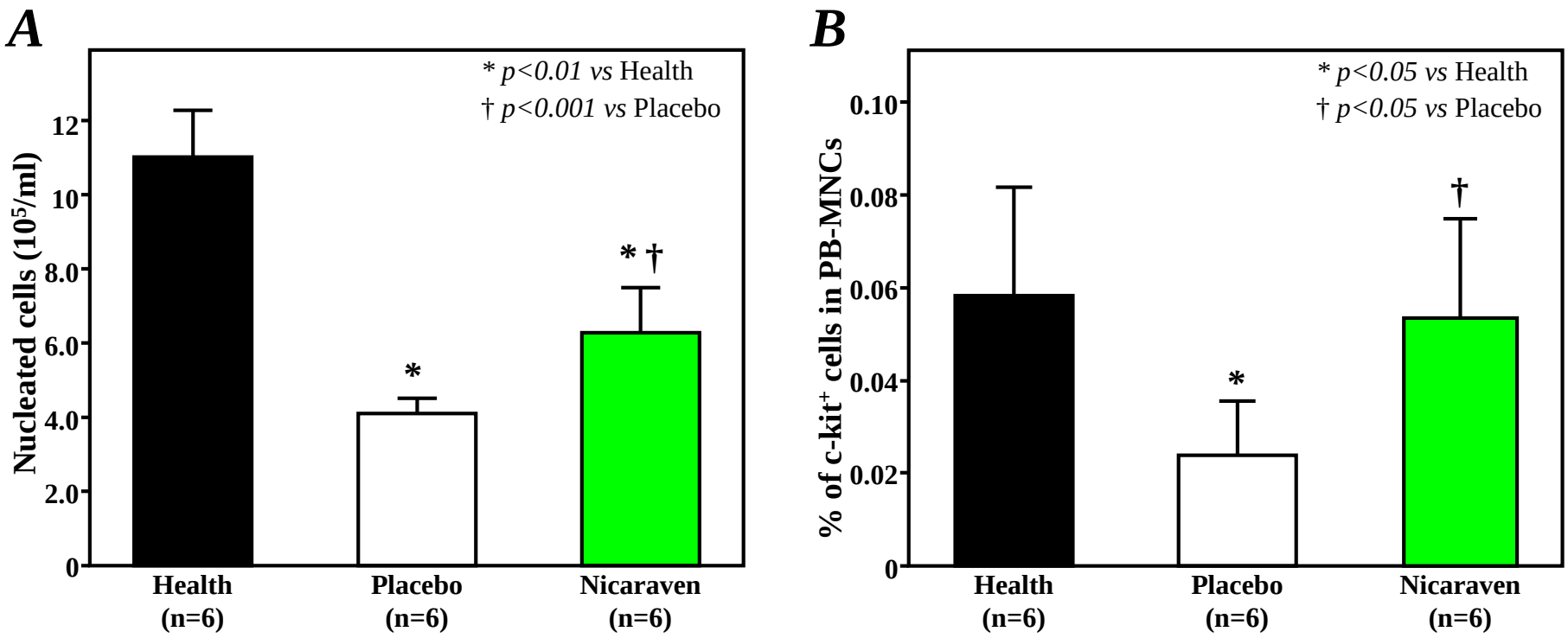

A
* p<0.01 vs Health
† p<0.001 vs Placebo
Nucleated cells (10⁵/ml)
0
2.0
4.0
6.0
8.0
10
12
*
* †
Health (n=6)
Placebo (n=6)
Nicaraven (n=6)
B
* p<0.05 vs Health
† p<0.05 vs Placebo
% of c-kit⁺ cells in PB-MNCs
0
0.02
0.04
0.06
0.08
0.10
*
†
Health (n=6)
Placebo (n=6)
Nicaraven (n=6)

Fig 2.

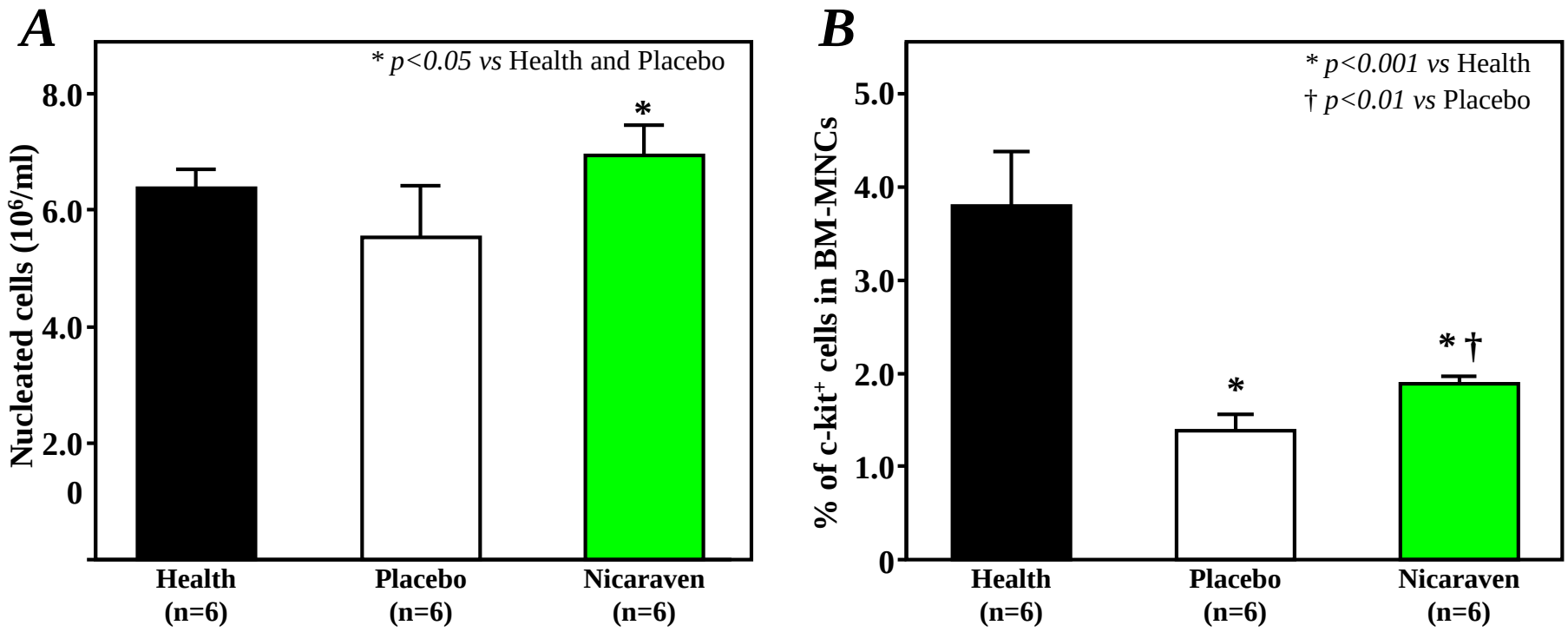

A
* p<0.05 vs Health and Placebo
Nucleated cells (10^6/ml)
8.0
6.0
4.0
2.0
0
*
Health (n=6)
Placebo (n=6)
Nicaraven (n=6)
B
* p<0.001 vs Health
† p<0.01 vs Placebo
% of c-kit+ cells in BM-MNCs
5.0
4.0
3.0
2.0
1.0
0
*
* †
Health (n=6)
Placebo (n=6)
Nicaraven (n=6)

Fig 3.

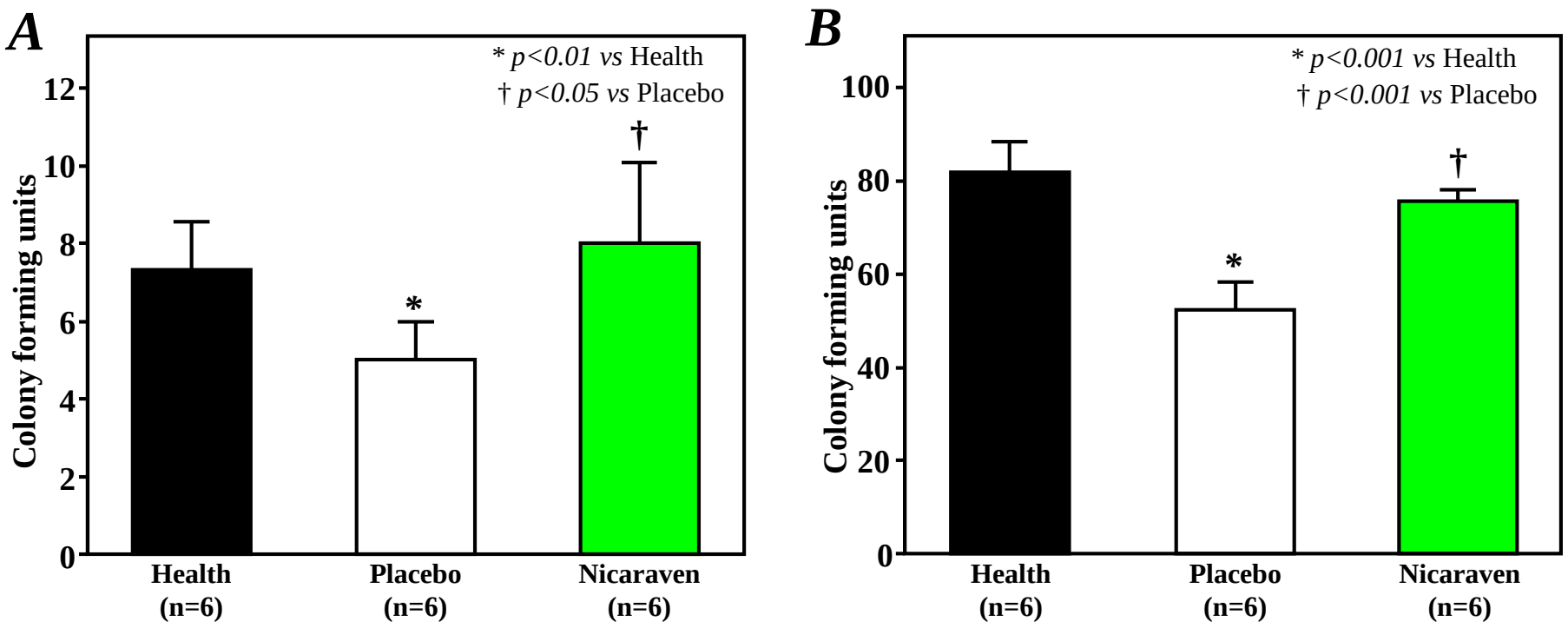
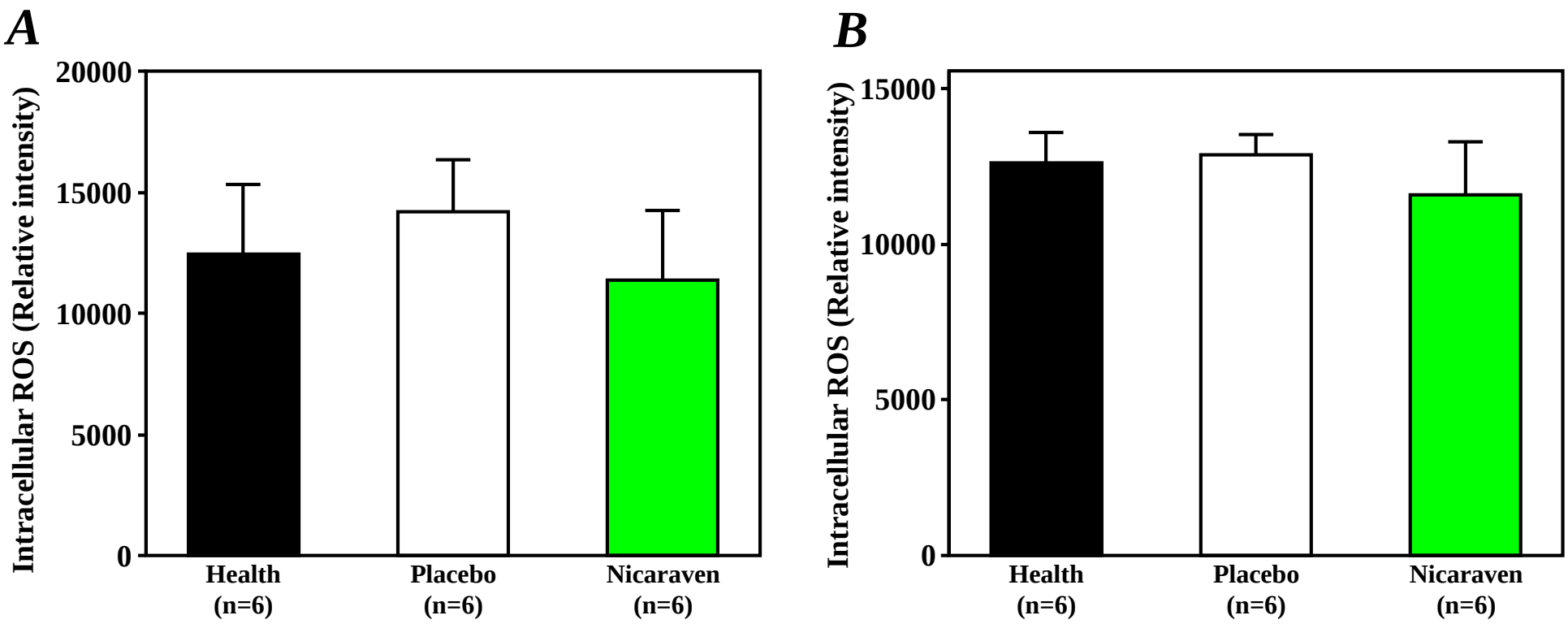

A
* p<0.01 vs Health
† p<0.05 vs Placebo
Colony forming units
0
2
4
6
8
10
12
*
†
Health (n=6)
Placebo (n=6)
Nicaraven (n=6)
B
* p<0.001 vs Health
† p<0.001 vs Placebo
Colony forming units
0
20
40
60
80
100
*
†
Health (n=6)
Placebo (n=6)
Nicaraven (n=6)

Fig 4.


A
Intracellular ROS (Relative intensity)
20000
15000
10000
5000
0
Health
(n=6)
Placebo
(n=6)
Nicaraven
(n=6)
B
Intracellular ROS (Relative intensity)
15000
10000
5000
0
Health
(n=6)
Placebo
(n=6)
Nicaraven
(n=6)