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Mitoquinone restores platelet production in irradiation-induced thrombocytopenia

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Abstract

Myelodysplastic syndromes (MDS) are hallmarked by cytopenia and dysplasia of hematopoietic cells, often accompanied by mitochondrial dysfunction and increases of reactive oxygen species (ROS) within affected cells. However, it is not known whether the increase in ROS production is an instigator or a byproduct of the disease. The present investigation shows that mice lacking immediate early responsive gene X-1 (IEX-1) exhibit lineage specific increases in ROS production and abnormal cytology upon radiation in blood cell types commonly identified in MDS. These affected cell lineages chiefly have the bone marrow as a primary site of differentiation and maturation, while cells with extramedullary differentiation and maturation like B- and T-cells remain unaffected. Increased ROS production is likely to contribute significantly to irradiation-induced thrombocytopenia in the absence of IEX-1 as demonstrated by effective reversal of the disorder after mitoquinone (MitoQ) treatment, a mitochondria-specific antioxidant. MitoQ reduced intracellular ROS production within megakaryocytes and platelets. It also normalized mitochondrial membrane potential and superoxide production in platelets in irradiated, IEX-1 deficient mice. The lineage-specific effects of mitochondrial ROS may help us understand the etiology of thrombocytopenia in association with MDS in a subgroup of the patients.

Keywords

Megakaryocytes; Mitoquinone; Reactive Oxygen Species; Thrombocytopenia

Introduction

Myelodysplastic syndromes (MDS) are a diverse set of progressive hematopoietic stem cell disorders hallmarked by ineffective hematopoiesis and potential advancement to acute myeloid leukemia (AML). Although several clinical studies confirm increased formation of

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Declaration of interest

The authors declare no conflict of interest.

ROS in the patients, it is not clear whether or not the hematopoietic cell lineages affected in MDS are particularly vulnerable to the deteriorative effects of ROS [1–3]. We recently showed that IEX-1-deficient mice developed an MDS-like syndrome characterized by thrombocytopenia, decreased red blood cell counts, and cytogenic anomalies following exposure to a single dose of low-grade irradiation, a phenotype also transmissible by bone marrow transplantation [4]. These findings are in line with multiple clinical studies reporting decreases in IEX-1 expression in CD34⁺ hematopoietic cells of low-risk MDS patients and an increased incidence of MDS post therapeutic irradiation, known as therapeutically initiated MDS (tMDS) [5–7]. Of particular clinical interest are thrombocytopenia and anemia that typically dominate tMDS, both of which are recapitulated in the absence of IEX-1 [4]. Conceivably, any finding allowing for the reversal and/or repair of the ineffective hematopoiesis in this novel model of MDS may hold remarkable potentials for management and delaying the progress of the disease in the future.

IEX-1 expression is found throughout the body, varying in levels in different tissues and organs and can be strongly upregulated in response to stressors such as irradiation, viral infections, growth factors, and steroid hormones [8]. Its main functions are not only regulation of reactive oxygen species (ROS), but also control of the fate of stressed cells [9–11]. Its anti-apoptotic quality lies, at least in part, on maintenance of ROS homeostasis at mitochondria [10, 12]. While a low-level of ROS serve as secondary messengers in signaling cascades regulating cell proliferation and homeostasis, significant increases of ROS enhances susceptibility of cells to apoptosis. We have shown that IEX-1 targets F1F0-ATPase inhibitor (IF1) for degradation and IF1 degradation prevents a rise of ROS triggered by various apoptotic stimuli thereby protecting cells from undergoing apoptosis [10]. In contrast, loss of IEX-1 modestly elevates ROS formation, rendering some cells susceptible to apoptosis, but altering cell-signaling, differentiation, and maturation in others, depending on the level of ROS production in individual cells [4, 13].

Given the role of IEX-1 in maintenance of ROS homeostasis, alongside our and other studies showing IEX-1 down regulation in the genesis of MDS, IEX-1 dysregulation may alter the level of ROS formation at mitochondria, commonly identified within the hematopoietic compartments of MDS subjects [1, 2, 14, 15]. The present study corroborates a significant rise in intracellular ROS within hematopoietic stem cells, common myeloid progenitors, and megakaryocytes in IEX-1 deficient bone marrow (BM) after irradiation. In contrast, hematopoietic cells that mature extra-medullary, such as the lymphoid series, remained unaffected. Strikingly, irradiation-induced thrombocytopenia in IEX-1 knockout (KO) mice was completely reversed by MitoQ, a mitochondria-targeted anti-oxidant, arguing strongly an importance of mitochondrial ROS in differentiation and maturation of platelets from hematopoietic stem cells in the BM. IEX-1 KO mice offer a novel murine model to delineate the role of mitochondrial ROS in thrombopoiesis and an importance of ROS homeostasis within hematopoietic compartments.

Methods

Mice and thrombocytopenia induction and treatment

IEX-1 KO mice on mixed 129Sv/C57BL/6 background (F1) were generated by gene-targeting deletion in our laboratory as previously described [16]. The animals and wild type (WT) control mice on the same genetic background were maintained in pathogen-free animal facilities of Massachusetts General Hospital in compliance with institutional guidelines. To induce thrombocytopenia, KO and WT mice at 8-weeks-old were administered a single dose of 300 Rad (3 Gy) total body irradiation (TBI), using a $^{137}\text{Cesium}$ gamma irradiator. For treatment of the disorder, MitoQ was administered to the animals in drinking water containing 250 μM MitoQ *ad libitum* for 2 weeks, with a change of the drinking water every three days.

Flow cytometric analysis

Single cell suspensions from BM and splenic tissues were prepared by passing the tissue through a 100 μm mesh nylon cell strainer (BD Falcon) and stained with indicated fluorescent-conjugated antibodies obtained from BD Biosciences unless otherwise indicated, at a concentration recommended by the manufacturer. For examination of hematopoietic stem cells, BM cells were stained with a lineage cocktail of rat anti-mouse antibodies against mature blood cell markers including Mac-1, Gr-1 CD3, CD4, CD8, B220, and Ter-119. The lineage cells were excluded using PE or PE-Cy7-conjugated goat anti-rat antibody. LSK cells were identified as Lin⁻ Sca-1⁺ c-Kit⁺ cells by additional antibody combinations against Sca-1 (clone D7) from Biolegend (San Diego, CA) and c-Kit (clone 2B8) from eBioscience (San Diego, CA). Myeloid progenitors were recognized as IL7R α ⁻ Lin⁻ Sca-1⁻ c-Kit⁺ cells by inclusion of another antibody against CD127 from Biolegend, more committed myeloid progenitors with antibody directed at FcGR2/III (clone 93) from eBioscience, and common myeloid progenitor (CMP) as Lin⁻ IL7R α ⁻ c-Kit⁺ Sca-1⁻ CD34⁺ FcR2/III⁻ cells, respectively [17]. Erythropoietic lineages were marked using Ter-119 (Ter-119) antibody, and megakaryocytes and platelets using CD41 (MWReg30) antibody (eBiosciences). Flow cytometric studies were performed on a FACS Aria (BD Bioscience; San Jose, CA) and data were analyzed by FlowJo software (Tree Star; Ashland, OR).

Platelet counts

Blood was collected via tail vein into EDTA-coated microtainer tubes (BD Bioscience) and platelet counts were attained on a HemaTrue veterinary hematology analyzer (Heska Corporation; Loveland, CO).

Transmission electron microscopy (TEM)

BM cells were fixed in Karnovsky's fixative at 4 °C for overnight. After removal of the fixative, sodium cacodylate buffer (0.1 M) was added to the pellets, decanted, followed by addition of agar. The resultant cell pellets were post-fixed in 2% OsO₄ in sodium cacodylate buffer, dehydrated, and embedded in Epon 812 (Tousimis). Ultrathin sections were cut on a microtome (Reichert-Jung Ultracut E), collected on 200 mesh copper grids, stained with uranyl acetate and lead citrate, and examined on a Philips CM-10 transmission electron

microscope (Eindhoven). Images were taken using an undermount XR41M 4 Mpixel cooled camera.

Analyses of cellular ROS and mitochondrial membrane potential and superoxide levels

To measure cellular ROS, cells were incubated for 30 minutes in serum-free RPMI media or phosphate buffered saline (PBS) containing 5 μ M 5-(and-6)-chloromethyl-2',7'-dichlorodihydro-fluorescein diacetate, acetyl ester (CM-H₂DCFDA or DCF) from Sigma. The amount of ROS was quantified by a shift in green mean fluorescence intensity (MFI). To assay mitochondrial membrane potential (MMP) and superoxide in platelets, WT and KO mice were irradiated with 3 Gy TBI as previously described [4]. The irradiated mice were divided equally into two groups, one of which was given water supplemented with 250 μ M MitoQ *ad libitum* immediately after irradiation, whereas the other group was provided with water only. Platelets were isolated 2 weeks later and incubated for 20 minutes in PBS containing 5 μ M JC-1 per manufacturer's instructions (Invitrogen). Superoxide levels were concluded with the use of Mitosox (Invitrogen) after the platelets were incubated at 37 °C for 30 minutes with 5 μ M Mitosox in PBS following manufacturer's instructions. Fluorescent intensities of the resultant cells or platelets were captured on a FACSaria and analyzed as above.

Statistical analysis

Statistical significance was determined by 2-tailed student's t-test for two group comparison or one way ANOVA for comparing multiple groups using Graphpad Prism 5 (Graphpad Software).

Results

Loss of IEX-1 increases ROS formation in the BM of irradiated mice

IEX-1 deficient mice show no gross abnormality under a steady condition [18], but develop an MDS-like disease after a low dose of TBI or BM transplantation, characterized typically with irreversible thrombocytopenia [4]. In accordance with this, we identified a significant and persistent increase in ROS formation in IEX-1 deficient BM cells after irradiation, as measured by increasing dichlorofluorescein (DCF-DA) intensity (Figure 1A and B). ROS production was slightly higher in the absence than in the presence of IEX-1 in non-irradiated control mice, but it was without statistical significance, regardless of their age. In contrast to BM, irradiation-induced ROS production was not apparent in splenic cells in a manner independent on IEX-1 expression (Figure 1C). Interestingly, the overall level of ROS production was one order of magnitude lower in the spleen than in the BM, irrespective of IEX-1 expression or irradiation (Figure 1C). Such differences point to anatomy-specific regulation of ROS formation. These results confirms increased ROS formation in the absence of IEX-1, in agreement with a role for IEX-1 in the regulation of mitochondrial ROS homeostasis under stress [10, 13].

Loss of IEX-1 increases ROS in a cell lineage- and anatomy-specific fashion

Given the significant increase of ROS in irradiated KO BM cells, we next determined whether the increase was associated with altered differentiation and maturation of specific

cell lineages identified in the mice following irradiation [6, 19]. The investigation began with LSK (Lin⁻ Sca⁺ C-kit⁺) stem cells, due to a discernible increase of cycling in the cells in IEX-1 KO mice undergoing similar irradiation treatment described previously [4]. As expected, LSK cells from KO mice, but not from WT mice, generated a significantly higher amount of ROS post irradiation (Figure 2A). Increases in intracellular ROS have been shown to affect the ability of hematopoietic stem cells to maintain quiescence [20, 21]. Therefore, more active cycling of LSK cells previously reported in IEX-1 KO mice following irradiation is probably ascribed to increased ROS formation within the cells [4]. Downstream, significant changes in ROS production were also found in the common myeloid and lymphoid progenitors (CMP and CLP) between KO and WT mice after irradiation (Figure 2B and C). The difference in CLP was seen even before irradiation (Figure 2C).

We next evaluated an increase of ROS formation specifically in cell populations commonly affected in MDS. As can be seen in Figure 2D, ROS levels were significantly elevated by radiation in erythroblasts (CD71⁺TER119⁺) in both WT and KO mice, with a prominent effect on KO mice. This effect was BM specific and no such an increase was observed in splenic erythroblasts (Figure 2E). In line with this, ROS levels in KO peripheral RBCs (TER119⁺) were also elevated significantly when compared to WT counterparts in both irradiated and non-irradiated mice, with a predominant impact in irradiated mice (Figure 2F).

A similar trend was seen within megakaryocytic series, in which ROS levels in CD41⁺ cells were increased in KO mice post TBI (Figure 2G). Similar to erythroblasts, this effect was also specific for BM compartment, and splenic megakaryocytes sustained stable levels of ROS before and after radiation and in the presence or absence of IEX-1 (Figure 2G). Not surprisingly, while irradiation elevated ROS levels in circulating platelets in the presence or absence of IEX-1, platelets exhibited a KO specific increase in ROS production prior and after irradiation (Figure 2H). As CLP progenitors also displayed aberrant levels of ROS following irradiation, we looked at ROS levels in peripheral lymphocytes, revealing no significant increases of ROS within peripheral lymphocytic B220⁺ B cells and CD4⁺ or CD8⁺ T cells, notably all of which undergo extra-medullary maturation (Figure 2I). These lymphoid cells, opposed to platelets and RBCs, showed no alterations in either their numbers or phenotypes in the absence or presence of IEX-1, in both irradiated and BM transfer models of MDS [4]. These results emphasize cell- and tissue-specific effects of IEX-1 deficiency on ROS generation.

Mitochondria-targeted anti-oxidant reverses TBI-induced thrombocytopenia

Our previous BM transplant studies confirmed that stress-induced thrombocytopenia in IEX-1 KO mice resulted from an intrinsic defect in hematopoietic stem cells [4]. To further delineate the defect, WT and KO mice were treated with 250 μ M MitoQ in drinking water *ad libitum* for two weeks, starting at five months post-TBI, when irreversible thrombocytopenia was evident in the mice [4]. Within two weeks of MitoQ treatment, platelet levels were reversed from thrombocytopenia to above normal levels in irradiated KO mice (Figure 3A). The treatment augmented platelet production in WT mice as well,

albeit to a lesser extent. This may be explained by the fact that WT mice had a small increase in ROS production post-TBI, which potentially hindered thrombopoiesis, though at yet compensated and undetectable levels. Additionally, in our previous study a significant increase in the number of megakaryocytes within BM compartment in IEX-1 KO mice post-TBI was shown [4], presumably as a compensatory mechanism for thrombocytopenia frequently seen in MDS and other diseases [22]. This increase in the number of megakaryocytes was almost completely normalized by MitoQ treatment, as suggested by a similar number of CD41⁺ megakaryocytes in both irradiated WT and KO mice post-MitoQ, in sharp contrast to significant differences in the mice prior to MitoQ treatment (Figure 3B). Normalization of the number of megakaryocytes in IEX-1 KO BM may be attributed to a negative feedback response to increased platelet counts following MitoQ treatment (Figure 3A). The normalization in the number of platelets and megakaryocytes was concurrent with decreases of ROS in the cells in both WT and KO mice following MitoQ treatment (Figure 3C and D).

There was no difference in the number of peripheral RBCs post MitoQ treatment (Figure 3E), which is probably due to an insignificant loss of RBCs in this period of time in KO mice after irradiation [4]. A decline in RBC counts was a gradual phenomenon reaching significance only 7 months post-TBI, in contrast to the thrombocytopenia that was evident within three weeks post-TBI. The relatively modest effect on erythropoiesis, as opposed to thrombocytopenia, may also result from strong, stress-induced extra-medullary erythropoiesis in the spleen during an increased need of RBCs. These findings demonstrate an importance of mitochondrial ROS in the regulation of thrombopoiesis within the BM.

MitoQ restores defective megakaryocyte demarcation system exacerbated by pathologic increases of ROS

Pro-platelet formation precedes platelet production, and it is a highly sensitive and finely orchestrated process dependent upon compartmentalized apoptosis alongside upregulation of proplatelet genes [23, 24]. After endomitosis is completed, megakaryocytes begin to produce and align a demarcation system (DMS), consisting of a series of tubular invaginations throughout the megakaryocytic cytoplasm as shown by TEM of megakaryocytes from control WT mice (Figure 4, WT control). These invaginations yield pro-platelets that are extended and sheared from the cell, giving rise circulating platelets. IEX-1 KO mice exhibited a defect in the DMS within megakaryocytes (Figure 4, KO control), generating an irregular and misconjoined DMS that gave rise to a fewer and much less complex network of proplatelets and an overall decrease in platelet production [4]. Irradiation of the mice resulted in further dilated and fragmentation of DMS tubules, with abnormal morphology of granules (Figure 4, KO irradiated). These changes were also seen, albeit at a much lesser extent, within WT megakaryocytes post-irradiation (Figure 4, WT irradiated). In contrast, a majority of mature megakaryocytes from KO mice MitoQ treated for two weeks displayed fine, continuous tubular invaginations throughout the cytoplasm resembling the DMS seen in megakaryocytes of non-irradiated WT mice (Figure 4, KO irradiated/MitoQ). The finding underscores the pivotal regulation of DMS formation and platelet biogenesis by mitochondrial ROS.

MitoQ restores mitochondrial membrane potential and lowers superoxide production in platelets

To assure a specificity of MitoQ in restoration of mitochondrial function in platelets of irradiated, IEX-1 KO mice, we looked at the levels of mitochondrial membrane potential (MMP) and superoxide production in platelets using JC-1 and MitoSox, respectively, before and after MitoQ treatment, two agents specifically measuring mitochondrial functions [25, 26]. At a standing state, platelets of IEX-1 KO mice exhibited significantly higher levels of mitochondrial superoxides (Figure 5A), concomitant with a decrease in the levels of mitochondrial membrane potential as denoted by a decreased JC-1 ratio (Figure 5B). JC-1 dye accumulates within the mitochondria in a membrane potential-dependent manner and shifts its color from green to red fluorescence. This potential-dependence color-shift is recorded as a ratio of red to green fluorescence, increasing proportionally with MMP. Increases in mitochondrial superoxide and decreases in MMP were induced by radiation in both WT and KO mice with more predominant effects on the latter (Figure 5A and B). The alterations were specifically corrected by the post-irradiative use of MitoQ, in a good agreement with a role for IEX-1 in the maintenance of mitochondrial function under stress.

Discussion

From this study, we report that irradiation increases ROS formation within the hematopoietic compartment in IEX-1 KO mice at a level significantly higher than WT mice. Treatment with MitoQ, a mitochondria-specific antioxidant, drastically lowered intracellular ROS levels in megakaryocytes giving rise to increased platelet production in the mice. These findings are of highly clinical significance in development of novel therapeutic drugs to prevent thrombocytopenia in association with MDS. The protection of tissues from ROS-mediated damage through the use of antioxidant supplements is far from being a novel concept. For example, clinical studies have shown increased levels of ROS within the vascular smooth muscle cells of hypertensive patients, in association with upregulation of NADPH oxidase, a membrane-bound enzyme complex [27]. In accordance with this, treatment of antioxidants such as Vitamin C has been shown to reduce blood pressure in hypertensive patients [28]. With all antioxidant therapeutics, location of abnormal ROS production within both tissues and cells appears to be crucial in determining a success of a given anti-oxidant drug. The idea of a mitochondria-specific antioxidant is quite contemporary medically and the results achieved to date have been promising with human trials showing success in multiple mitochondria-associated diseases [29, 30]. Several studies have highlighted mitochondrial dysfunction in MDS patients, findings consistent with a high incidence of the disease in the elderly, whose mitochondria are degenerating due to aging [14, 31, 32]. Our investigation suggests that MitoQ may have potentials to improve platelet production and slow down the progress of MDS in this subgroup of patients.

ROS has been shown to be crucial not only in MDS but also in multiple other hematological malignancies. These effects are probably ascribed to the evolutionarily conserved role of ROS in cell differentiation and maturation and intimate relationships of ROS formation with oxidative phosphorylation at mitochondria. Consistent with this, higher levels of ROS were found in cells like erythrocytes and megakaryocytes undergoing maturation within the

notably subnormoxic O₂ level in the BM. A modest increase in the level of ROS is associated with augmented proliferation and homing of hematopoietic stem cells as demonstrated by a number of studies [33–36]. These findings raise an intriguing possibility that cells matured in BM niches may be highly susceptible to aberrant increases in ROS. In support, disruption of ROS homeostasis has been shown to impede the quiescence of hematopoietic stem cells, a paradigm corroborated by our current investigations [21, 37, 38]. Alongside the current research, another recent publication showed that IEX-1 was a key to up regulate NF- κ B and to activate Erk signaling resulting in repairing DNA breaks induced by gamma-irradiation in hematopoietic stem cells [39]. The two studies, one employing TPO receptor and the other using IEX-1 knockout mice by us, converge in support of a role for IEX-1 in maintaining homeostasis of hematopoietic stem cells under stress. Moreover, megakaryocyte proliferation, differentiation and maturation and platelet shedding are impacted profoundly by ROS, often in a BM specific manner [40]. ROS are considered upstream of the expansionary signaling cascades, as treatment of megakaryocytes with diphenyliodonium, a NADPH-1 (NOX1) inhibitor prevented the activation of AKT, STAT3 and STAT5, hindering megakaryocyte differentiation and proliferation [41]. Likewise, over-expression of nuclear factor erythroid 2 (NF-E2), a redox-sensitive transcription regulator, promotes megakaryocytic maturation [42]. However, too much ROS as occurs following irradiation of IEX-1 KO mice may lead to premature apoptosis compartmentally during proplatelet formation, in light of a pivotal role for anti-apoptosis in proplatelet biogenesis and well-established anti-apoptotic function of IEX-1. In this regard, megakaryocytes deficient in pro survival gene Bcl-xL undergo a premature apoptosis, leading to reduced platelet shedding, a phenotype subsequently rescued by the deletion of both Bak and Bax [43]. Such findings truly hold implications in studying human diseases where aberrant apoptotic death of megakaryocytes has been suspected [44, 45].

We previously showed that lack of IEX-1 predisposed mice to MDS induced by irradiation. The finding suggests a pathogenic role of IEX-1 deregulation in a subgroup of MDS patients, and it is consistent with the notion that deregulation of a key conserved cellular function can perturb multiple differentiation steps involved in hematopoiesis, leading to MDS development, in light of an importance of IEX-1 in regulation of evolutionarily conserved mitochondrial functions. This study extends the previous investigation showing that excess production of ROS at mitochondria can hinder platelet biogenesis, contributing to MDS in the animal model. Whether or not an increase in mitochondrial ROS production also affects RBC differentiation and maturation remains to be investigated, which is another abnormality identified in irradiated, IEX-1 KO mice [4]. However, it is clear from the current investigation that platelet biogenesis relies on the ability of IEX-1 to sustain ROS at low levels to protect against stress-induced disruption of platelet demarcation systems and/or premature apoptosis of megakaryocytes. The fact that MitoQ restores platelet biogenesis in the absence of IEX-1 and rescues mice from irradiation induced thrombocytopenia sheds novel insight into the etiology of thrombocytopenia in association with inadequate function of mitochondria.

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Figure 1. Loss of IEX-1 increases ROS production in BM. (A) Representative histograms showing ROS levels in IEX-1 KO and WT BM cells 8-months post-irradiation (grey line) as opposed to non-irradiated control BM cells (black line). Mean fluorescence intensity (MFI) of DCF is summarized in BM cells (B) and splenic cells (C) of WT (black) and IEX-1 KO (white) mice. Histograms are representative results of two experiments, $n = 8$ for WT and KO mice in each experiment.

Figure 2.

Increases of ROS in specific hematopoietic populations post irradiation. Irradiation elevated ROS formation in KO LSK stem cells (A), common myeloid progenitors (CMP) (B), and common lymphoid progenitors (CLP) (C), but not in corresponding WT counterparts. A higher level of ROS formation was also seen in KO CLP compared to WT CLP in the absence of irradiation. Irradiation-induced enhancement of ROS formation was also evidenced in BM erythroblasts (EBLAST) (D), red blood cells (RBCs) (F), BM megakaryocytes (G), and circulating platelets (H), but not in splenic resident erythroblasts (E), splenic megakaryocytes (G), or peripheral T or B cell populations (I). Data shown are means $\pm$ standard deviations (SD) of two experiments, $n = 8$ for WT and KO mice in each experiment.

Figure 3. MitoQ restores platelet biogenesis in irradiated KO mice. (A) Platelet counts and (B) CD41⁺ megakaryocytes in BM in irradiated WT and KO mice before and after MitoQ treatment. ROS levels in CD41⁺ megakaryocytes in BM (C) and platelets (D) before and after MitoQ treatment. Red blood cell counts before TBI, 5-months post-TBI and after MitoQ treatment are shown in (E). Data shown are mean $\pm$ SD of two experiments, $n = 7$ for WT and 8 for KO mice in each experiment.

Figure 4.

TEM reveals a healthy DMS in MitoQ-treated megakaryocytes. Megakaryocytes were prepared from control WT and KO mice or the mice 2 weeks post-irradiation with or without MitoQ treatment, followed by subjecting the cells for TEM analysis. Original magnification 7920 $\times$ in the upper panel, in which outlined areas are enlarged in the bottom 24,700 $\times$. Each photograph is representative of eight mice examined.

Figure 5.

MitoQ ameliorates mitochondrial functions in platelets of irradiated mice. (A) Increases in mitochondrial superoxide, denoted by increased Mitosox staining, were noted in platelets isolated from KO mice (white) over WT mice (black) prior to 3 Gy TBI, which were further elevated 2 wks after 3 Gy TBI. The abnormality was corrected by MitoQ treatment for 2 wks in both WT and KO mice with more predominant effects on KO mice. (B) MMP was measured by JC-1 fluorescence ratios. It was diminished in platelets of KO mice significantly at a standing state and profoundly 2 wks post-irradiation compared to WT counterparts. MitoQ treatment for 2 weeks post-irradiation greatly restored MMP in both WT and KO mice. $n = 6$ in each group.