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Evaluation of Potential Ionizing Irradiation Protectors and Mitigators Using Clonogenic Survival of Human Umbilical Cord Blood Hematopoietic Progenitor Cells

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Abstract

We evaluated the use of colony formation (CFU-GM, BFU-E, and CFU-GEMM) by human umbilical cord blood (CB) hematopoietic progenitor cells for testing novel small molecule ionizing irradiation protectors and mitigators. Each of 11 compounds was added before (protection) or after (mitigation) ionizing irradiation including: GS-nitroxides (JP4-039 and XJB-5-131), the bifunctional sulfoxide MMS-350, the phosphoinositol-3-kinase inhibitor (LY294002), TPP-imidazole fatty acid, (TPP-IOA), the nitric oxide synthase inhibitor (MCF-201-89), the p53/mdm2/mdm4 inhibitor (BEB55), methoxamine, isoproterenol, propranolol, and the ATP sensitive potassium channel blocker (glyburide). The drugs XJB-5-131, JP4-039, and MMS-350 were radiation protectors for CFU-GM. JP4-039 was also a radiation protector for CFU-GEMM. The drugs, XJB-5-131, JP4-039, and MMS-350 were radiation mitigators for BFU-E, MMS-350 and JP4-039 were mitigators for CFU-GM, and MMS350 was a mitigator for CFU-GEMM. In contrast, other drugs that were effective in murine assays: TTP-IOA, LY294002, MCF201-89, BEB55, propranolol, isoproterenol, methoxamine, and glyburide showed no significant protection or mitigation in human CB assays. These data support testing of new candidate clinical radiation protectors and mitigators using human CB clonogenic assays early in the drug discovery process, reducing the need for animal experiments.

Keywords

cord blood; radiosensitivity; radiation mitigation; therapeutics

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Introduction

Radiation protectors reduce radiation toxicity when delivered prior to irradiation, while radiation mitigators are effective when delivered after irradiation but before the onset of symptoms or signs of damage (1–3). Evaluation of the effectiveness of new small molecule radiation protectors and mitigators often begins with *in vitro* assays for clonogenic survival as well as for drug effects on apoptosis and DNA damage repair [4–5]. Further testing of, novel drugs for use in normal tissue protection during clinical radiotherapy or as radiation counter measures requires animal testing, including survival after total body irradiation [3]. While carbamazepine (Tegretol) showed promise as a radiation mitigator in mouse cellular assays and in animal tests [6], it was ineffective with human cells [7], and was found in retrospective human patient outcomes review to not decrease radiotherapy side effects [8–9]. We tested the hypothesis that earlier use of the human CB MNC colony assay to evaluate drugs as radiation protectors and mitigators might identify ineffective compounds and reduce the need for animal testing.

We evaluated several established murine radioprotective and mitigator agents. The small molecule mitochondrial targeted GS-nitroxide, JP4-039, has been shown to mitigate irradiation induced delay in bone wound healing in mice [1], protect against ionizing irradiation-induced esophagitis [2] and act as a radioprotector in mouse and human cell lines [3–4]. XJB-5-131, another mitochondrial targeted GS-nitroxide, was shown to be a radioprotector [5] and neuroprotector [10]. MMS-350 is a novel water-soluble sulfoxide developed as a 2nd generation, selective analog of dimethyl sulfoxide (DMSO) [10], a known radioprotector in mice [11]. A mitochondria-targeted inhibitor of irradiation-induced peroxidase activity of cytochrome c/cardiophilin complexes, triphenylphosphonium imidazole fatty acid (TPP-IOA) has been shown to mitigate radiation induced cell death in mouse cells [12–13], and the phosphoinositide-3 kinase (PI3K) inhibitor LY294002 has been shown to mitigate radiation-induced apoptosis in mouse cells *in vitro* [14].

In addition, we evaluated the mitochondrial targeted nitric oxide synthase (NOS) inhibitor, MCF-201-89 [3] and the p53/mdm2/mdm4 inhibitor, BEB55 [3], which were shown to be effective radiation mitigators for the murine 32D cl 3 hematopoietic progenitor cell line. Following on the data with Carbamazepine [6–7], we tested other ion channel modifying drugs: isoproterenol, propranolol, methoxamine, and glyburide [15]. The effectiveness of each drug as a radiation protector and/or mitigator, was evaluated using colony forming human CB progenitor cells that form CFU-GM, BFU-E, and CFU-GEMM.

Materials and Methods

Drugs

The drugs GS-nitroxides (JP4-039 and XJB-5-131) [1, 3], bifunctional sulfoxide (MMS-350) [11], PI3K inhibitor (LY294002) [14], and the triphenylphosphonium mitochondrial targeted imidazole fatty acid (TPP-IOA) [16] have been described. JP4-039, XJB-5-131, MMS-350 [11], BEB55 and MCF-201-89 [3] were synthesized according to published protocols and used after passing Quality Control by Liquid Chromatography/Mass Spectroscopy Standards (purity >92%) [16]. TPP-IOA [13] was synthesized by Dr. Jeffrey Atkinson (Brock University, St Catharines, Ontario, Canada), LY294002 (Enzo Life Sciences, Farmingdale NY), methoxamine, isoproterenol, propranolol and glyburide (Sigma, St. Louis, MO) were purchased.

Irradiation Survival Curves

Human umbilical cord blood (CB) samples were obtained immediately after delivery in accordance with IRB and institutional guidelines, and placed in 50-mL tubes containing

anticoagulant citrate dextrose solution (ACD-A; Cytosol Labs, Braintree, MA). Low density mononuclear cells (MNC) were isolated by Ficoll-Paque density gradient centrifugation (Pharmacia Biochem, Piscataway, NJ) and irradiated in suspension to doses ranging from 0 to 8 Gy using a ^{137}Cs g-ray source (JL Shepherd, San Fernando, CA, USA). Test compounds were added to cells one hour before irradiation or immediately after irradiation and used at the following concentrations; JP4-039 or XJB-5-131 10 μM in DMSO, MMS-350 at 50, 100 or 200 μM in Iscove's Modified Dulbecco's Medium (IMDM), LY294002 at 0.1, 1, or 10mM, TPP-OFA at 2.5, 5 or 10 μM in DMSO, methoxamine, isoproterenol, propranolol, BEB55, MCF-201-89 and glyburide each at 10 μM in Iscove's Modified Dulbecco's Medium. Cord Blood Mononuclear Cells (MNCs) were plated in triplicate in 0.8% methylcellulose containing IMDM supplemented with recombinant human stem cell factor (rh SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), IL 3 and erythropoietin (Stemcell Technologies, Vancouver, British Columbia, Canada). Colony-forming unit-granulocyte macrophage (CFU-GM), burstforming unit erythroid (BFU-E), and colony-forming unit-granulocyte-erythroid-megakaryocytemonocytes (CFU-GEMM) were scored on day 14. Data were analyzed with linear quadratic and single-hit multitarget models. All experiments were done in triplicate with 3 different cord blood samples.

The radiosensitivity of human cord blood colony forming progenitor cells was measured using a clonogenic assay that quantitates cells, which, after irradiation to each of several doses, survive to undergo at least 7 cell doublings over 7 – 14 days and form colonies of over 50 cells in semisolid medium according to published methods [17]. Colonies were scored at days 7 and 14 and data presented as surviving fraction at each dose and as average. The D_0 is a measure of radiosensitivity and is the dose on the linear portion of the survival curve that reduces the control unirradiated colony number by 37% [17]. The $\tilde{n}$ is a measure of cellular capacity to accumulate sublethal irradiation damage and is calculated by extrapolation of the linear portion of the radiation clonogenic survival curve back to the vertical axis on a log/linear plot) [17].

Statistical Analysis

Comparisons were made with the two-sided two-sample t-test. P-values ≤ 0.05 were regarded as significant.

Results

We evaluated each drug as a radiation protector or mitigator using human CB MNCs in clonogenic irradiation survival curves. The data were compared using full radiation survival curves and then by comparing D_0 and $\tilde{n}$. The D_0 as a measure of radiosensitivity is the dose required to reduce the surviving fraction of cells to 37% of control levels and is on the exponential portion of the radiation dose response survival curve. Radioresistant cells have a higher D_0 than radiosensitive cells. The extrapolation number ($\tilde{n}$) is a measure of the shoulder on the survival curve and is calculated by extending the linear portion of the survival curve back to the ordinate in a log linear scale [17]. The $\tilde{n}$ is a measure of cellular capacity to accumulate sublethal irradiation damage. Radioresistant cells display a larger $\tilde{n}$ value reflecting a larger shoulder on the survival curve. In contrast, irradiation survival curves with a steep slope (low D_0 value) and/or narrow shoulder (low $\tilde{n}$ value) are characteristics of radiosensitivity. [17].

Mitochondrial targeted GS-nitroxides, JP4-039 and XJB-5-131 are ionizing irradiation protectors and mitigators for CB MNCs

We first treated CB MNCs with 10 μM JP4-039 or XJB-5-131 pre- or post-irradiation, and the results are shown in Table 1 and Figure 1. Both compounds were protectors when given before irradiation as shown by an increase in the D_0 to 1.93 ± 0.13 for CFU-GM with XJB-5-131 and 1.99 ± 0.13 with JP4-039 compared to 1.49 ± 0.23 ($p=0.0445$) for control irradiated cells. The drug JP4-039 was also protective and mitigative for CFU-GEMM. The D_0 of CFU-GEMM was significantly increased by JP4-039 added pre-irradiation to 2.04 ± 0.07 from 1.65 ± 0.05 for control 10 irradiated cells ($p=0.0012$) and to 2.00 ± 0.11 ($p=0.0074$) when added post-irradiation. Both JP4-039 and XJB-5-131 were effective mitigators when added after irradiation, as shown by the increased shoulder on the survival curve with a $\bar{n}$ of 3.35 ± 0.36 ($p=0.0012$) for BFU-E with XJB-5-131 and 3.80 ± 0.95 ($p=0.0445$) with JP4-039 compared to 2.91 ± 1.90 for control irradiated cells. JP4-039 was an effective mitigator for CFU-GEMM, increased to 2.0 ± 0.11 from 1.65 ± 0.05 for control irradiated cells ($p=0.0074$).

MMS-350 is an effective ionizing irradiation protector and mitigator for CB MNCs

At drug doses of 50, 100 and 200 μM , MMS-350 was an effective irradiation protector and mitigator for CB MNCs as shown in clonogenic survival curves (Table 2 and Figure 2). MMS-350 (50 μM) was protective when administered before irradiation, as shown by a statistically significant increase in the D_0 for CFU-GM to 1.83 ± 0.09 , $p=0.028$ and CFU-GEMM (2.20 ± 0.06 , $p=0.009$) compared to irradiated control cells (1.48 ± 0.14 , 1.54 ± 0.23 , respectively). There was a significant increase in the D_0 for CFU-GM and CFU-GEMM with 100 μM MMS-350 added post irradiation from 1.48 ± 0.14 (irradiated control cells) to 1.88 ± 0.12 , $p=0.018$ (CFU-GM) and from 1.54 ± 0.23 for irradiated control cells to 2.43 ± 0.07 , $p=0.003$ for CFU-GEMM. When cells were treated with 200 μM MMS350 prior to irradiation, there was an increase in the $\bar{n}$ for CFU-GEMM to 2.41 ± 0.75 from 1.11 ± 0.13 ($p=0.042$) for control irradiated cells. At 200 μM MMS-350 was also mitigative for BFU-E. The D_0 was increased to 1.76 ± 0.11 from 1.56 ± 0.07 , $p=0.057$. Thus, MMS-350 was effective over a wide dose range both as a protector and as a mitigator of human CB MNC.

The phosphoinositol-3 kinase inhibitor LY294002 and the mitochondrial targeted triphenylphosphonium imidazole fatty acid TPP-IOA are not effective radiation protectors or mitiators for CB MNCs

Results of testing the effect of 0.1 μM , 1 μM or 10 μM LY294002 in CB irradiation survival curves are shown in Table 3. LY294002 0.1 μM added post-irradiation, increased the $\bar{n}$ for CFU-GM to 6.48 ± 1.78 from 1.49 ± 0.25 , $p=0.014$ compared to irradiated control cells. Although this data point was significant compared to irradiated control cells, this single effect may have been attributable to chance and its biological significance was questionable. Isolated effects were also observed when CB MNCs were treated with 2.5 or 5.0 μM TPP for 1 h prior to irradiation (Table 4). The D_0 for CFU-GEMM was 2.13 ± 0.18 (2.5 μM TPP) compared to 1.57 ± 0.23 , $p=0.030$ for irradiated control cells and 2.25 ± 0.36 , $p=0.051$ (5 μM TPP) compared to 1.57 ± 0.23 , $p=0.051$ for irradiated control cells. These isolated effects may have been attributed to chance and their biological significance was also questioned.

Isoproterenol, propranolol, methoxamine, and glyburide are not effective radiation protectors or mitigators for CB MNCs

Prior studies showed that the ion channel modulator, carbamazepine was an effective radiation protector and mitigator for mouse but not human cells [6–7]. We next tested the ion channel modifying drugs methoxamine, isoproterenol and propranolol and the ATP

sensitive potassium ion channel blocker glyburide as protectors and mitigators when added pre- or post-irradiation of CB MNC respectively (Table 5). Methoxamine and glyburide showed no evidence of either 12 radioprotection or mitigation. There was a significant reverse effect of radiosensitization in the D_0 for BFU-E with 10 μ M glyburide treatment pre- or post-irradiation ($p=0.047$ and $p=0.048$ respectively); furthermore, there was toxicity of glyburide for CFU-GEMM with no colonies surviving to be scored (Table 5). When CB MNCs cells were treated with isoproterenol preirradiation, the $\bar{n}$ for BFU-E increased from 1.80 ± 0.44 for irradiated control cells to 2.77 ± 0.22 , $p=0.018$. Propanolol added post-irradiation increased in the $\bar{n}$ for BFU-E to 2.79 ± 0.61 , from 1.80 ± 0.44 $p=0.04$. These two localized increases were statistically significant compared to irradiated control cells; however, these isolated effects may have been attributable to chance and their biological significance was questionable.

The nitric oxide synthase inhibitor MCF-201-89, and the Mdm2/Mdm4 inhibitor BEB55 are not effective irradiation mitigators for CB MNCs

The mitochondrial targeted nitric oxide synthase (NOS) inhibitor, MCF-201-89 and the p53/mdm2/mdm4 inhibitor, BEB55, were each added at (10 μ M) to CB MNC post-irradiation (Table 6). Neither compound was effective as a mitigator for human CB MNC. When BEB55 was added post-irradiation there was a reverse effect with significant decrease (radiosensitization) in the $\bar{n}$ for CFU-GM ($p=0.0231$) and decrease in the D_0 for (radiosensitization) BFU-E ($p=0.0386$) compared to control irradiated cells.

Discussion

The search for effective, novel radioprotective and radiation damage mitigating agents has intensified in recent years due to both the need for clinical radiation protectors for radiotherapy and for radiation countermeasures [18]. In clinical radiotherapy, there is need for development of normal tissue radiation protectors in the setting of fractionated radiotherapy and image guided stereotactic radiotherapy, particularly where the normal tissue transit volume is highly susceptible to generation of clinical side effects [17]. In contrast, drugs for use in counterterrorism require administration of agents for radiation mitigation rather than protection [18]. In the scenario of a willful radiation terrorist event, including: the use of a radiologic dispersal device, fission device, or exposure to radioisotopes in the air, drinking water, or through the skin through fallout, the administration of drugs to decrease radiation damage must necessarily follow the radiation event [18]. Radiation mitigators must have low toxicity profiles since agents will be administered to individuals who are not cancer patients and will have varying levels (in some cases undetectable levels) of irradiation exposure. Radiation exposure might be to subtotal body volumes and in some cases, as with injection of radioisotopes, only in specific organ systems.

The discovery of radiation countermeasures has utilized chemical library screening [19–20], siRNA screening [14–15], and small molecule drug screening programs, including those with a computational chemistry or structural biology application for drug design [20]. Use of sophisticated robotic screening methodologies has led to the identification of many small organic [21] and biological [22] candidate radiation mitigator drugs. Once identified each agent is evaluated for effect on irradiation induced DNA damage by a Comet Chip assay [23], or the modulation of irradiation-induced yeast DNA fragmentation [19]. Clonogenic radiation survival curves utilize cell lines representative of the critical target cells for radiation damage (the hematopoietic stem cell and the intestinal stem cell) [3, 24]. A commonly used hematopoietic progenitor cell line is the murine hematopoietic progenitor cell line 32D cl 3 derived from C3H/HEJ mouse bone marrow, which parallels the radiobiology of the multilineage hematopoietic stem cell [3]. The next step in the drug

discovery process has usually been animal testing which can be prohibitively expensive [25] when multiple agents or analogs must be compared.

In the present studies, we confirmed the potential value as radiation protectors and/or mitigators of 3 of 11 drugs tested using the human CB MNCs clonogenic cell assay [26–27]. The mitochondrial targeted antioxidant GS-nitroxides (JP4-039 and XJB-5-131), which were effective radiation protectors and mitigators for the mouse hematopoietic progenitor cell line 32D cl 3 and in total body irradiated C57BL/6J mice [3, 5] were also effective with CB MNCs. These drugs accumulate in mitochondria and are hypothesized to function as antioxidants against irradiation induced reactive oxygen species (ROS) [3, 5].

The water-soluble bifunctional dimethylsulfoxide analog, MMS-350, which can also be used as a vehicle for administration of other drugs [11] demonstrated protective and mitigative properties as a single agent in mouse cells in total body irradiated mice, and was effective with the CB MNC assay. The mechanism of action of MMS-350 is currently being studied, but is hypothesized to function as an antioxidant scavenger of ROS based on prior data with DMSO [28].

We tested 8 other promising drugs, which we found to have no significant irradiation protector or mitigator effects with CB MNCs. These included 3 other mitochondrial targeted drugs. The small molecule, MCF-201–89 [3], was designed to target the mitochondria and mitigate the effects of radiation induced nitric oxide and peroxynitrites. This nitric oxide synthase (NOS) inhibitor was shown to be an effective radiation mitigator *in vitro* and *in vivo* by inhibiting irradiation induced NOS production [3]. A second mitochondrial targeted small molecule radiation mitigation is the p53/mdm2/mdm4 inhibitor, BEB55, an effective irradiation mitigator for 32D cl 3 hematopoietic progenitor cells [3] and in C57BL/6 Nhsd mice when given after 9.5Gy TBI. [3]. BEB55 was ineffective as a protector or mitigator for CB MNCs. The mechanism of action of BEB55 may be through its direct inhibition of the interaction of Mdm2 and Mdm4 with p53 [3]. A third mitochondrial targeted small molecule, triphenylphosphonium-imidazole fatty acid (TPP-IOA) [13], was also not an effective radiation protector or mitigator for CB MNCs. The effectiveness of TPP-IOA with total body irradiated mice was hypothesized to be by stabilizing the interaction between cardiolipin and cytochrome c, thereby preventing apoptosis [13]. The lack of effectiveness of these three drugs with human CB MNCs may reflect differences in the drug uptake or biochemical pathways between species with respect to hematopoietic progenitor cells. While these three drugs may have indirect effects *in vivo* or direct effects on intestinal stem cells or in other organs, their direct effect on irradiated human hematopoietic progenitors was suboptimal compared to other drugs tested.

The PI3K pathway and its inhibitor, LY294002, was shown to be important in radiation biology in that the drug was a potent radiation mitigator for 32D cl 3 cell lines and in total body irradiated mice [14]. This drug was not an effective radiation protector or mitigator with human CB MNCs. The present negative results including those with methoxamine [29], isoproterenol, propranolol, and glyburide in CB should help avoid costly animal testing. The ATP sensitive potassium channel blocker, Glyburide, a clinically used oral antidiabetic drug, was also identified as a possible radioprotective compound using siRNA library screening [15]. It has been shown to be radioprotective in mice and in murine 32D cl 3 hematopoietic progenitor cells [15]. The radioprotective mechanism of glyburide in mice is unknown, but may act to block potassium channels, which affects apoptosis [15].

These negative drugs tested still may have radioprotective or mitigative results *in vivo* by indirect action or through other tissues and organs. Species differences in ion channel blocking or in downstream effector pathways may have explained the differences in human

cell responses. There also could be heterogeneity in drug uptake between species with respect to critical stem cell populations. Carbamazepine was ineffective with irradiated human cells *in vitro* [6–7] and was then shown in retrospective analysis of clinical data to have no protective or mitigative effects by reducing toxicity to patients during radiotherapy [8–9].

The use of the CB MNCs as targets for screening new radiation protectors and mitigators may save significant animal care costs by earlier identification of drugs, which are ineffective for human hematopoietic cells. The present data support assay of new candidate clinical radiation protectors and mitigators using human CB MNCs cells early in the drug discovery process, reducing the number and cost of required animal experiments.

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Figure 1.

Irradiation survival curves of CB MNC treated with 10 μ M JP4-039 or XJB-5-131 pre- or post-irradiation. Irradiated MNC were plated in methylcellulose containing medium as described in the methods and CFU-GM (A, D), BFU-E (B, E) and CFU-GEMM (C, F) scored on day 14. Irradiated cells only, JP4-039 10 μ M pre or post-irradiation, XJB-5-131 10 μ M pre or post irradiation.

Figure 2.

Irradiation survival curves of CB MNC treated with 50, 100 or 200 μ M MMS-350 pre- or post-irradiation. Irradiated MNC were plated in methylcellulose containing medium as described in the methods and CFU-GM (A, D), BFU-E (B, E) and CFU-GEMM (C, F) scored on day 14. Irradiated cells only, 50 μ M MM-S350 pre or post-irradiation, 100 μ M MMS-350 pre or postirradiation, 200 μ M MMS-350 pre or post-irradiation.

Table 1

JP4-039 and XJB-5-131 are effective ionizing irradiation protectors and mitigators of CB MNCs.

Experimental conditions	Pre or post irradiation	CFU-GM		BFU-E		CFU-GEMM	
		D ₀	$\bar{n}$	D ₀	$\bar{n}$	D ₀	$\bar{n}$
cells only		1.49 ± 0.23	1.87 ± 1.28	1.33 ± 0.23	2.91 ± 1.90	1.65 ± 0.05	1.23 ± 0.26
	pre	1.93 ± 0.13 p=0.0445	1.55 ± 0.49	1.71 ± 0.13	2.13 ± 1.15	2.01 ± 0.44	1.16 ± 0.28
XJB-5-131 10μM	pos	1.70 ± 0.38	1.32 ± 0.55	1.52 ± 0.10	3.35 ± 0.36 p=0.0012	1.94 ± 0.24	1.10 ± 0.18
	pre	1.99 ± 0.13 p=0.0293	1.44 ± 0.39	1.71 ± 0.34	2.49 ± 1.03	2.04 ± 0.07 p=0.0012	1.67 ± 1.15
JP4-039 10μM	post	1.80 ± 0.30	1.61 ± 0.77	1.46 ± 0.21	3.80 ± 0.95 p=0.0445	2.00 ± 0.11 p=0.0074	1.32 ± 0.28

D₀ and $\bar{n}$ for cord blood mononuclear cells in different treatment groups. Data are expressed as mean ± standard deviation. Sample size is n=3/group. In each column, comparisons were made between the cells-only group and each of the other groups. These pairwise comparisons were made with the two-sided two-sample t-test. P-values 0.05 were regarded as significant and are shown in red.

Table 2

MMS350 is an effective ionizing irradiation protector and mitigator for CB MNCs.

Experimental conditions	Pre or post irradiation	CFU-GM		BFU-E		CFU-GEMM	
		D ₀	$\bar{n}$	D ₀	$\bar{n}$	D ₀	$\bar{n}$
cells only		1.48 ± 0.14	1.48 ± 0.42	1.56 ± 0.07	1.89 ± 0.49	1.54 ± 0.23	1.11 ± 0.13
MMS-350 50 μ M	pre	1.83 ± 0.09 p=0.028	2.47 ± 0.87	1.47 ± 0.09	4.09 ± 1.48	2.20 ± 0.06 p=0.009	1.24 ± 0.21
	post	1.84 ± 0.31	2.93 ± 2.49	1.65 ± 0.20	2.33 ± 1.18	2.00 ± 0.26	1.68 ± 1.1
MMS-350 100 μ M	pre	1.78 ± 0.17	2.30 ± 0.72	1.62 ± 0.19	2.71 ± 1.37	2.13 ± 0.47	1.16 ± 0.15
	post	1.88 ± 0.12 p=0.018	1.78 ± 0.39	1.50 ± 0.17	2.57 ± 0.07	2.43 ± 0.07 p=0.003	1.16 ± 0.15
MMS-350 200 μ M	pre	2.01 ± 0.18 p=0.016	1.15 ± 0.26	1.60 ± 0.20	2.90 ± 0.98	1.9 ± 0.45	2.41 ± 0.75 p=0.042
	post	1.98 ± 0.31	1.41 ± 0.37	1.76 ± 0.11 p=0.057	1.94 ± 0.81	1.87 ± 0.26	2.50 ± 1.0

D₀ and $\bar{n}$ for cord blood mononuclear cells in different treatment groups. Data are expressed as mean ± standard deviation. Sample size is n=3/group. In each column, comparisons were made between the cells-only group and each of the other groups. These pairwise comparisons were made with the two-sided two-sample t-test. P-values 0.05 were regarded as significant and are shown in red.

Table 3

Lack of effect of the phosphoinositol kinase inhibitor LY294002 as an ionizing protector or mitigator of CB MNCs.

Experimental conditions	Pre or post irradiation	CFU-GM		BFU-E		CFU-GEMM	
		D0	$\bar{n}$	D0	$\bar{n}$	D0	$\bar{n}$
Cells only		1.58 $\pm$ 0.25	1.49 $\pm$ 0.25	1.67 $\pm$ 0.20	1.77 $\pm$ 0.49	2.05 $\pm$ 0.49	1.00 $\pm$ 0.0
		1.70 $\pm$ 0.24	2.24 $\pm$ 1.01	1.50 $\pm$ 0.39	3.36 $\pm$ 2.93	2.38 $\pm$ 0.26	1.00 $\pm$ 0.0
LY294002 0.1 μ M	pre	1.57 $\pm$ 0.45	6.48 $\pm$ 1.78	1.60 $\pm$ 0.32	2.90 $\pm$ 1.65	2.24 $\pm$ 1.61	1.00 $\pm$ 0.0
	post		p=0.014				
LY294002 1 μ M	pre	1.58 $\pm$ 0.25	1.49 $\pm$ 0.23	1.67 $\pm$ 0.21	1.77 $\pm$ 0.25	2.06 $\pm$ 0.49	1.00 $\pm$ 0.0
	post	1.53 $\pm$ 0.20	3.86 $\pm$ 2.47	1.42 $\pm$ 0.22	3.74 $\pm$ 2.88	2.09 $\pm$ 0.18	1.11 $\pm$ 0.15
LY294002 10 μ M	pre	1.74 $\pm$ 0.24	1.86 $\pm$ 0.82	1.65 $\pm$ 0.31	2.45 $\pm$ 2.06	2.01 $\pm$ 0.09	1.12 $\pm$ 0.11
	post	1.59 $\pm$ 0.46	1.00 $\pm$ 0.0	1.61 $\pm$ 0.22	1.28 $\pm$ 0.49	2.14 $\pm$ 0.0	1.00 $\pm$ 0.0

D0 and $\bar{n}$ for cord blood mononuclear cells in different treatment groups. Data are expressed as mean $\pm$ standard deviation. Sample size is n=3/group. In each column, comparisons were made between the cells-only group and each of the other groups. These pairwise comparisons were made with the two-sided two-sample t-test. P-values 0.05 were regarded as significant and are shown in red.

Table 4

Lack of effect of the mitochondrial targeted fatty acid, TPP-IOA, as an ionizing irradiation protector or mitigator of CB MNCs.

Experimental conditions	Pre or post irradiation	CFU-GM		BFU-E		CFU-GEMM	
		D ₀	$\bar{n}$	D ₀	$\bar{n}$	D ₀	$\bar{n}$
cells only		1.54 ± 0.20	1.68 ± 0.61	1.37 ± 0.23	2.32 ± 1.35	1.57 ± 0.23	1.60 ± 0.41
TPP-IOA 2.5 µM	pre	1.84 ± 0.09	1.15 ± 0.15	1.68 ± 0.12	2.09 ± 0.44	2.13 ± 0.18 p=0.030	1.25 ± 0.42
	post	1.38 ± 0.19	2.67 ± 0.34	1.35 ± 0.25	2.50 ± 0.44	1.85 ± 0.12	1.45 ± 0.49
TPP-IOA 5.0 µM	pre	2.1 ± 0.38	1.35 ± 0.6	1.98 ± 0.49	1.8 ± 1.12	2.25 ± 0.36 p=0.051	1.06 ± 0.11
	post	1.76 ± 0.39	1.48 ± 0.47	1.48 ± 0.21	3.10 ± 0.67	1.90 ± 0.18	1.27 ± 0.37
TPP-IOA 10 µM	pre	1.65 ± 0.1	1 ± 0	1.65 ± 0.25	2.52 ± 1.6	1.84	1.60
	post	1.56 ± 0.02	1.44 ± 0.35	1.35 ± 0.02	2.58 ± 0.33	1.92 ± 0.13	1.00 ± 0.0

D₀ and $\bar{n}$ for cord blood mononuclear cells in different treatment groups. Data are expressed as mean ± standard deviation. Sample size is n=3/group. In each column, comparisons were made between the cells-only group and each of the other groups. These pairwise comparisons were made with the two-sided two-sample t-test. P-values 0.05 were regarded as significant and are shown in red.

Table 5

Lack of effects of the ion channel modifying agents methoxamine, isoproterenol, propranolol, and glyburide as ionizing irradiation protectors and mitigators for CB MNCs.

Experimental conditions	Pre or post irradiation	CFU-GM		BFU-E		CFU-GEMM	
		D ₀	$\bar{n}$	D ₀	$\bar{n}$	D ₀	$\bar{n}$
cells only		1.82 ± 0.06	1.18 ± 0.17	1.64 ± 0.13	1.80 ± 0.44	1.72 ± 0.48	1.72 ± 0.74
methoxamine 10μM	pre	1.99 ± 0.17	1.29 ± 0.21	1.51 ± 0.21	3.11 ± 1.53	1.91 ± 0.13	1.44 ± 1.18
	post	1.94 ± 0.52	1.44 ± 0.52	1.68 ± 0.18	2.28 ± 0.64	1.86 ± 0.22	1.56 ± 0.79
isoproterenol 10 μM	pre	1.99 ± 0.19	1.10 ± 0.12	1.42 ± 0.18	2.77 ± 0.22 p=0.018	1.97 ± 0.32	1.06 ± 0.12
	post	1.94 ± 0.20	1.35 ± 0.23	1.62 ± 0.10	2.13 ± 0.60	2.06 ± 0.29	1.06 ± 0.06
propranolol 10 μM	pre	1.83 ± 0.21	1.54 ± 0.57	1.62 ± 0.12	2.37 ± 1.01	2.24 ± 0.35	1.28 ± 0.55
	post	1.88 ± 0.36	1.73 ± 0.90	1.56 ± 0.19	2.79 ± 0.61 p=0.040	1.93 ± 0.41	1.25 ± 0.29
cells only		2.03 ± 0.061	2.62 ± 2.06	2.31 ± 0.47	1.32 ± 0.51	2.10 ± 0.17	1.10 ± 0.07
glyburide 10 μM	pre	1.69 ± 0.20	1.00 ± 0.0	1.38 ± 0.33 p=0.047	1.56 ± 0.96	* < 3 colonies/1 x 10 ⁵ cells	
glyburide 10 μM	post	1.57 ± 0.28	1.02 ± 0.03	1.50 ± 0.18 p=0.048	1.70 ± 1.22	* < 3 colonies/1 x 10 ⁵ cells	

D₀ and $\bar{n}$ for cord blood mononuclear cells are shown for each group. Data are expressed as mean ± standard deviation. Sample size was n=3/group. In each column, comparisons were made between the cells-only group and each drug treated group. Pairwise comparisons made with the two-sided two-sample t-test that were significant are shown in red. Cells treated with glyburide either pre- or post- irradiation, had D₀ for BFU-E that was significantly lower than that for control irradiated cells indicating sensitization to irradiation not protection, and glyburide treated cultures produced no CFU-GEMM. Glyburide is shown with its own separate control cells only.

Table 6

Lack of effect of the small molecule Mdm2/Mdm4 inhibitor (BEB55) and mitochondrial/neuronal nitric oxide synthase inhibitor (MCF201-89) as ionizing irradiation mitigators for CB MNCs.

Experimental conditions	CFU-GM		BFU-E		CFU-GEMM	
	D ₀	$\bar{n}$	D ₀	$\bar{n}$	D ₀	$\bar{n}$
cells only	1.61 ± 0.61	1.82 ± 0.33	1.55 ± 0.09	1.71 ± 0.22	2.33 ± 0.42	1.23 ± 0.40
BEB-55 10μM	1.62 ± 0.04	1.12 ± 0.07 p=0.0231	1.30 ± 0.12 p=0.0386	3.60 ± 2.56	2.40 ± 0.45	1.00 ± 0.0
MCF201-89 10μM	1.41 ± 0.12	3.41 ± 2.75	1.41 ± 0.19	2.38 ± 1.88	2.34 ± 0.40	1.00 ± 0.0

D₀ and $\bar{n}$ for cord blood mononuclear cells in different treatment groups. Data are expressed as mean ± standard deviation. Sample size is n=3/group. In each column, comparisons were made between the cells-only group and each of the other groups. These pairwise comparisons were made with the two-sided two-sample t-test. P-values 0.05 were regarded as significant. Cells treated with BEB-55 showed irradiation sensitization not protection, as the $\bar{n}$ for GFU-GM and the D₀ for BFU-E were significantly less than that for control irradiated cells.