Induced Pluripotent Stem Cells and Hormesis

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Abstract

This paper represents the first assessment of agent-induced hormetic dose responses in induced pluripotent stem cells and their derived cells. The hormetic dose responses were induced by a broad range of chemicals, including pharmaceuticals (eg, metformin), dietary supplements/extracts from medicinal plants (eg, curcumin), and endogenous agents (eg, melatonin). The paper assesses the mechanistic foundations of these induced hormetic dose responses, their therapeutic implications and comparison with hormetic responses in multiple adult and embryonic stem cells.

Introduction

In 2007, Okita et al[1] reported that fully differentiated fibroblasts could be transformed into pluripotent cells with the same potential as embryonic cells of the inner mass of the blastocyst. These findings revolutionized the stem cell research domain with Yamanaka receiving the Nobel Prize in 2012 for this discovery. While the area of induced pluripotent stem cells (IPSC) research has vastly expanded since the discovery by Yamanaka and his colleagues, the present paper offers a unique assessment on IPSCs and cells derived from IPSCs with respect to the capacities of chemical and physical agents to induce hormetic dose responses in these cells. These responses will also be compared with hormetic responses from other stem cell types, principally various adult stem cells but also embryonic stem cells as well.[2-10] While the extent of the hormetic dose responses reported with IPSCs and their derived cells is modest at the present time, the findings are nonetheless important to report as a complement to extensive findings in other stem cells, especially those of adipose, bone marrow, embryonic and neural origin.

Hormesis Overview

Hormesis is a biphasic dose/concentration response. It displays a low-dose/concentration stimulation and a high-dose/concentration inhibition.[11-14] It exhibits specific quantitative characteristics with a maximum stimulatory response typically between 30% and 60% greater than the control group (Figure 1).

The hormetic stimulatory dose/concentration range is approximately 10–20-fold but may show considerable variability not uncommonly exceeding 50-fold. The hormetic response results from a direct subtoxic (hormetic) dose, a subtoxic (hormetic) preconditioning dose, and a subsequent toxic dose,[16,17] or a modest overcompensation stimulation following an initial disruption in homeostasis.[18] The hormetic dose/concentration response exhibits broad generality, being independent of biological model (eg, microbes, plants, animal models and humans), endpoint, level of biological organization (i.e., cell, organ, organism), *in vitro* and *in vivo* evaluations, inducing agent, and mechanism.[19-21] Comprehensively, integrated evaluations of hormetic dose responses for both chemicals and ionizing radiation provide historical foundations of hormesis beginning very early in the experimental literature, from the 1880s to the present.[22-28]

As a result of the general lack of linkage between hormesis as a phenomenon and stem cells in the literature, including IPSCs, it was necessary to develop a broader and more general search strategy for this review. Using principally PubMed and Web of Science databases, key words such as "stem cells,"

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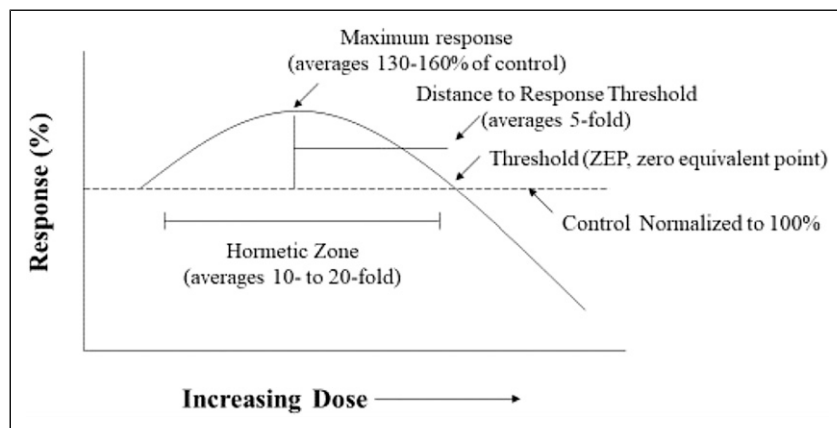Email: edwardc@schoolph.umass.edu

Figure 1. General representation of the hormetic dose response (modified from: Calabrese and Baldwin[15]).
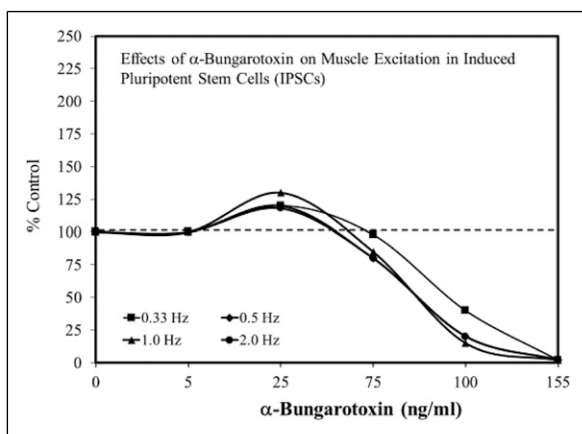

Figure 2. Effects of α-bungarotoxin on muscle excitation in IPSC-derived myotube motor neurons (modified from: Santhanam et al.[29]).

"IPSCs," "cell proliferation," "cell differentiation," "biphasic dose responses," "bimodal dose responses," "low dose stimulation," as well as "hormesis" and "hormetic" and various combinations were used in this search. In addition, all papers obtained were cross-referenced. Further, all relevant papers were assessed for each article that cited these papers (using Web of Science), and were evaluated for relevance. Finally, all active research groups in the area were followed for all their relevant publications.

Assessment of Hormesis in induced pluripotent stem cells (IPSCs) or Derivative Cells

Bungarotoxin–IPSC-Derived Myotubule-Motor Neuron System Displays Hormesis

Motoneuron diseases (MNDs) such as amyotrophic lateral sclerosis (ALS) are being assessed with progressive intensity, but significant clinical and therapeutic progress has been very limited. As part of an overall research framework for addressing MNDs, such as ALS, there is a need for high content screening of *in vitro* neuron muscular junctions. Within this context, Santhanam et al[29] used mouse IPSCs-derived myotubes and motor neurons (MNs) from functional neuromuscular junctions (NMJs) in a manner comparable to endogenous MNs and their embryonic stem cell (ESC)-MN counterpart. In light of this recognition, these researchers developed an experimental system whereby human MNs derived from IPSCs were co-cultured, becoming an integrative biological system. This system is made up of twin chambers, joined together via micro-tunnels which permit axonal outgrowth to the muscle chamber that permits the stimulation of tissue in either chamber. This system permitted muscle contraction to be induced by motoneuron activation. This system was then administered various synapse blocking drugs. Of particular interest was that the agent α-bungarotoxin (α-BTX) induced a hormetic-biphasic dose response. At low concentrations the α-BTX showed a potential excitatory effect, while at higher concentrations inhibition became evident (Figure 2). Similar experimentation with BOTOX showed only an inhibitory effect. The authors noted that this system permits the assessment of NMJs for conditions such as ALS. This is of particular interest since the use of the IPSC technology permits functional assessment of the affected patient.

Curcumin–IPSC-Derived Retinal Pigment Epithelial (RPE) Cells Show Hormesis

Following the development of human IPSC technology, efforts have been made to generate IPSCs from patients with various diseases, for evaluation of possible clinical applications. Retinal diseases are of particular interest since these tissues are not candidates for routine biopsy and use in experimental systems. However, several groups have shown that human IPSCs can differentiate into retinal progenitors, retinal pigment epithelial (RPEs), and photoreceptors. As a result of these developments, expandable experimental platforms have

been created for *in vitro* screening of candidate drugs that may reduce reactive oxygen species (ROS) production and protect RPE cells from age-related macular degeneration (AMD). Since curcumin is effective as an anti-inflammatory agent in multiple systems, Chang et al[30] employed AMD patient-specific IPSCs with a drug screening platform to assess the capacity of curcumin to enhance the viability of RPEs using reprogramed T-cells from patients with dry-type AMD. The T–cell-derived IPSCs were then differentiated to RPEs via the use of differentiation medium and targeted experimental protocols. The IPSCs that were obtained were morphologically indistinguishable from human ESCs and showed pluripotent potential. RPE is a single layer of pigmented cells. These cells form part of the blood-retina barrier (BRB) and are quite susceptible to oxidative stress due to high *in vivo* oxygen exposure. The accompanying chronic oxidative stress induces RPE damage and AMD pathogenesis. Initial screening of eight possible antioxidant treatments (e.g., β-carotene, curcumin, resveratrol, Q10) indicated that curcumin showed the most chemoprotective potential, leading to further study of that agent. Chang et al[30] showed that curcumin enhanced the cell viability and cell proliferation of AMD-RPE cells in an hormetic biphasic manner (Figure 3). In follow-up studies to assess the protective effects of curcumin on H_2O_2-induced cell death, the AMD-RPE cells were preconditioned with 10 μM curcumin at (.0, .5, 1.0, 2.0, 4.0, 8.0, 12, 24, 36, and 48 hours) prior to 200 μM H_2O_2 for six hours. Of interest is that the protective effects induced by curcumin only occurred for pretreatments of <12 hours. The mechanisms of curcumin protection were related to its blocking of H_2O_2-induced ROS formation. The protective curcumin pretreatment was associated with an increase in the expression of antioxidant genes (e.g., HO-1, SOD-2, GPX-1). In addition, the curcumin pretreatment blocked the effects of H_2O_2 on platelet-derived growth factor (PDGF) and the c-Jun N-terminal kinase (JNK) signaling pathways which also contributed to the chemoprotective effects. These findings suggest that IPSC-derived RPE-like cells have considerable chemotherapeutic potential for drug and dietary supplement screening.

Hypoxia-IPSCs Showed Hormesis for Cell Proliferation and for Differentiation to Neurons and Oligodendroglial Cells

Proliferation and differentiation of neural stem cells (NSCs) are markedly affected by aging processes. Numerous experimental approaches have been reported to blunt or reverse the decline in such NSC functions. Cultured cells of multiple types of stem cells display enhanced resilience to hypoxic stress.[31,32] Santilli et al[32] assessed the effects of hypoxic stress (1.0, 2.5, 5.0, and 20.0% O_2) on both proliferation and differentiation of a human IPSC line. Optimal concentrations ranged from 2.5% to 5.0% for cell proliferation whereas 1% and 20% O_2 adversely affected cell survival. Differentiation
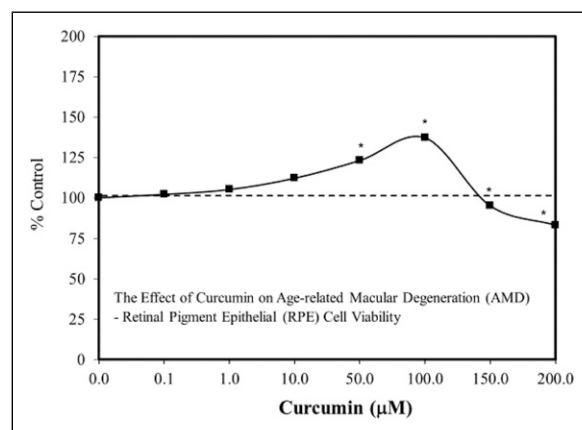

Figure 3. The effects of curcumin on IPSC-derived age-related macular degeneration (AMD)–retinal pigment epithelial (RPE) cell viability (modified from: Chang et al[30]) *= $P \leq$ .05.
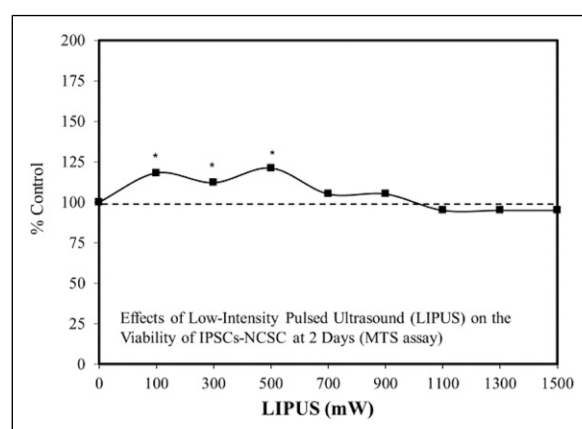

Figure 4. Effects of low-intensity pulsed ultrasound (LIPUS) on the viability of IPSC-NCSC at 2 days (MTS assay) (modified from: Lv et al[35]) *= $P \leq$ .05.

was also enhanced at low O_2 concentrations with multiple biomarkers optimized at 2.5% O_2 at 17 days *in vitro*. Since mild hypoxia can promote human IPSC proliferation and neuronal and oligodendroglial differentiation, it suggested numerous therapeutic possibilities. The findings with human IPSCs by Santilli et al[32] were consistent with a series of studies with similar findings in mouse NSCs showing hypoxia induced proliferation and astrocyte differentiation with hormetic dose responses, again with optimal responses at similar oxygen concentrations (2.0%).[31]

Low-Intensity Pulsed Ultrasound (LIPUS)–IPSC-Derived Neural Crest Stem Cells Showed Hormesis

Since the use of low-intensity pulsed ultrasound (LIPUS) is a non-invasive therapeutic modality with an excellent clinical record, it has generated considerable interest in its capacity to enhance peripheral nerve repair. The fact that several studies

showed that LIPUS enhanced cell proliferation and differentiation of mesenchymal stem cells (MSCs)[33,34] led Lv et al[35] to hypothesize that LIPUS may act on IPSC-derived neural crest stem cells (IPSCs-NCSC) to promote nerve regeneration, restoring function. In the case of NCSCs, they are multipotent undifferentiated stem cells with the capacity to differentiate into numerous cell types, including neurons, Schwann cells, smooth muscle cells, glia melanocytes, endocrine cells, chondrocytes, and axons. In their study, Lv et al[35] assessed the effects on cell viability and cell proliferation as well as neural differentiation. A hormetic-biphasic dose response was reported for cell viability (Figure 4). Subsequent experiments using only a single stimulatory and inhibitory dose showed the low dose stimulation and high dose inhibition for cell proliferation, displaying the consistent hormetic concentration response pattern. Differentiation biomarker experiments, using only doses in the low dose stimulatory zone for cell viability, showed consistent hormetic stimulatory differentiation patterns for the expression of neurons (NF-M and Tuj1), and Schwann cells (S100 B and GFAP).

Melatonin-IPSC Derived neural stem cells (NSCs) Showed Hormesis

In 2018, Shu et al[36] evaluated the capacity of melatonin (MT) to protect mouse IPSC-derived NSCs from H_2O_2-induced damage. This research was based on previous studies showing that cell proliferation and differentiation could be stimulated by several neurotrophic factors[37,38] as well as findings showing that luzindole reduced MT-induced proliferative effects of IPSC-derived NSCs that were mediated by the P13 K/Akt pathway.[39] Using mouse IPSCs, Shu et al[36] noted that MT enhanced cell viability (CCK-8 assay) in IPSC-derived NSCs at one and three days in an hormetic manner with 1 μM being the optimal concentration (Figure 5). Subsequent studies indicated that the MT (1 μM) pretreatment (3 days) of IPSC-derived NSCs prevented the occurrence of H_2O_2-induced cytotoxicity (CCK-8 assay).

The MT studies by Shu et al[36] are of particular interest since the survival of IPSCs-NSCs subjected to hyperoxic exposure is markedly compromised.[40,41] The MT findings show the potential to lessen oxidative stress while enhancing overall cellular resilience. In fact, Shu et al[36] showed that MT enhanced the formation of spheres and prevented the increase of neuronal apoptosis-induced by H_2O_2. These responses were mediated in large part by enhancing the stabilization of the mitochondrial membrane potential, reducing DNA fragmentation and enhancing the Bcl2/BAX ratio. Blockage of the MT receptors MT1 and MT2 prevented MT-induced chemoprevention. These findings therefore established that MT can promote IPSC-derived NSC survival and protect these cells from damage due to excessive ROS exposure, creating the possibility of therapeutic staging needed for unfavorable, inflammatory environments.
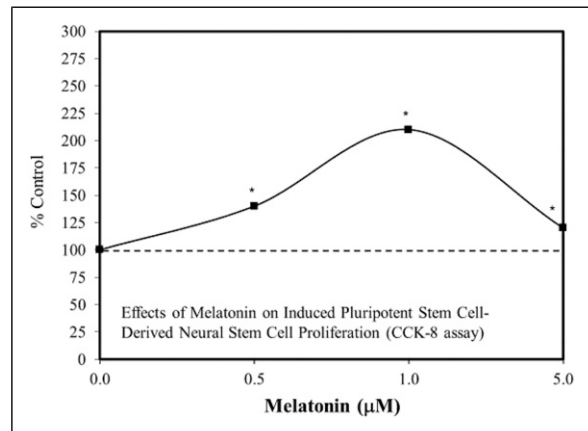

Figure 5. Effects of melatonin on IPSC-derived neural stem cell proliferation (CCK-8 assay) (modified from: Shu et al[36]) *= P ≤ .05.
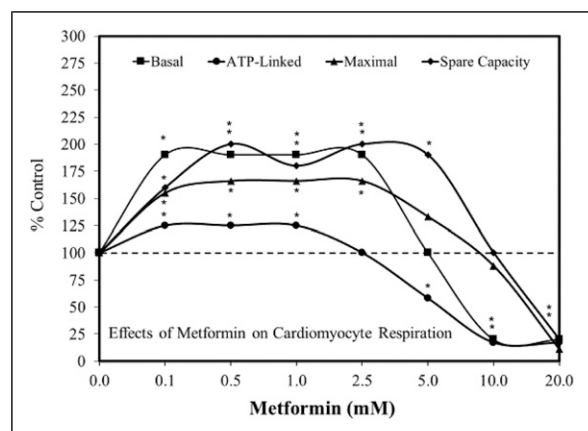

Figure 6. Effects of metformin on IPSC-derived cardiomyocyte respiration (modified from: Emelyanova et al[46]) *= P ≤ .05.

Metformin–IPSC-Derived Cardiomyocytes Showed Hormesis

Among the multiple beneficial effects of metformin (MF) are those showing a diminished risk of damage resulting from myocardial infarction. MF also prevents cardiovascular mortality while enhancing positive clinical outcomes of patients with diabetes and heart failure.[42-45] In an effort to better discern how MF is cardioprotective, Emelyanova et al[46] evaluated the effects of MF on human cardiac mitochondria *in vitro* using a broad concentration range (100 to 20 000 μM). These researchers found that low doses of MF increased cellular respiration due to AMP-activated protein kinase A (AMPK)-mediated mitochondrial biogenesis, while showing inhibitory effects at higher concentrations. MF was used to treat cardiomyocytes derived from human IPSCs for 24 hours. The MF induced consistent hormetic-biphasic concentration responses under multiple respiratory conditions (e.g., basal, ATP-linked, maximal, and spare capacity) but not within a non-mitochondrial system (Figure 6). Subsequent experiments

showed that the low-dose stimulatory responses enhanced the occurrence of mitochondrial biogenesis. Such stimulatory effects of MF were blocked by compound C, a specific inhibitor of AMPK. These observations indicate that the MF-induced cardio-protection observed in epidemiological studies may have been due to the hormetic effects of MF on mitochondria. The protective effects at low doses were mediated, at least in part, via mitochondrial biogenesis via AMPK and increases in oxygen consumption rate. Higher doses of MF inhibited mitochondrial respiration by directly affecting complex I, reducing oxidative stress, and delaying the formation of mitochondrial permeability transition pores. The high dose treatment affected a type of metabolic reprogramming by increasing glycolysis and glutaminolysis, effects that can help patients improve their endogenous cardioprotective and cardiorepair mechanisms. The upregulation of glycolysis and glutaminolysis represented an additional dose-dependent compensatory mechanism in response to the inhibition of the mitochondrial oxidative phosphorylation by MF.

Neurotoxic Agents–IPSC-Derived Sensory Neurons Showed Hormesis

Patients undergoing chemotherapy commonly may experience peripheral neuropathy side effects. These often include altered sensation, sensory loss, and neuropathic pain. In fact, nearly 70% of patients who are administered cytotoxic treatments develop the chemotherapy-induced peripheral neuropathy. Trying to find ways to reduce the occurrence and severity of this chemotherapy-induced peripheral neuropathy has been an important priority. Of interest, in this regard have been efforts to use IPSC-derived sensory neurons as a disease model of chemotherapy-induced neurotoxicity. Schinke et al[47] have addressed this issue with a focus on whether neurotoxin chemotherapy in clinically relevant steady-state concentrations affects cell viability of IPSC-derived sensory neurons. In their experimental protocol, 40 days were required for neurogenic differentiation to achieve morphologically pure neuronal cultures with ganglia and extensive axons. At this time the cultures were exposed to the different agents (i.e., bortezomib, cisplatin, doxorubicin, vincristine) over a 10^5-fold concentration range. While each agent reduced cell viability at the higher concentrations, all enhanced cell viability at the lower concentrations (Figure 7). Despite the fact that hormetic effects were observed in cultures after 40 days, hormesis was not observed when tested at Day 13, during a more immature stage of cell development.

Radiation/Gene Editing-IPSCs Showed Hormesis in Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Methodologies

This section concerns the role of hormesis in enhancing the efficiency and accuracy of gene editing and its capacity to
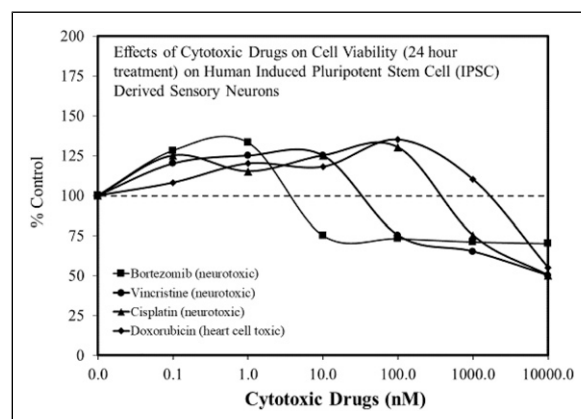

Figure 7. Effects of cytotoxic drugs on cell viability (24 hour treatment) on human IPSC-derived sensory neurons (modified from: Schinke et al.[47]).
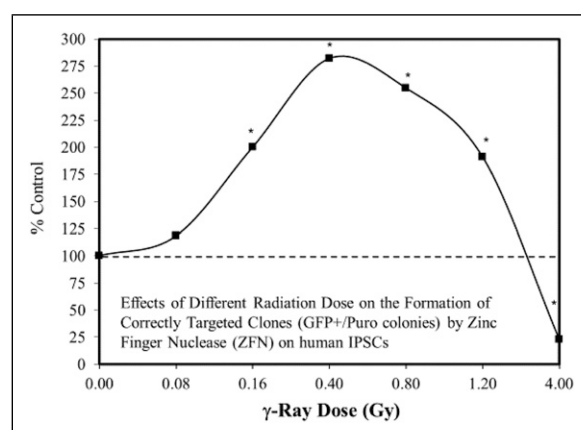

Figure 8. Effects of radiation on the formation of correctly targeted clones (GFP+/Puro colonies) by zinc finger nuclease (ZFN) on human IPSCs (modified from: Hatada et al[53]) *= $P \leq .05$.

improve technologies such as clustered regularly interspaced short palindromic repeats (CRISPR). This section assessed these developments via the use of human embryonic stem cells and with human IPSCs.

The long-standing assumption that all doses of ionizing radiation are harmful to organisms is undergoing reconsideration. While higher linear energy transfer (LET) radiation with α and neutron particles priming can induce DNA double strand breaks (DSBs), low LETγ and X-ray ionizing radiation at low dose (.1–.5 Gy) do not frequently induce DNA DSBs but, in contrast, may reduce DNA damage, eliminate cells with DNA damage, activate tumor-suppressor genes, and enhance detoxification.[48-50] Reinforcing the above findings are studies with mouse spermatogenia stem cells administered at low dose of γ- and X-rays, showing a reduced mutation frequency compared with a substantial control group.[51] Likewise, numerous studies have shown that low doses of X-rays in animals induced a therapeutic effect by reducing the occurrence of lung metastases.[52]

Within the context of the capacity of low dose radiation to enhance homologous chromosome recombination (HR) frequency, most likely due to the induction of DNA repair processes in IPSCs, Hatada et al[53] assessed whether γ- and X-rays may be employed in IPSCs for gene targeting. This research was of particular interest since it sought to enhance the efficiency/success of gene editing methods such as zinc finger nucleases, (ZFNs), transcription activator-like effector nucleases (TALENS), and CRISPR for HRs with viral donor vectors which have often had low success, being very time consuming and expensive.

Hatada et al[53] reported an enhanced HR frequency following the induction of DNA DSBs via the use of specific types of endonucleases. For example, ZFNs, TALENS, and CRISPR can significantly enhance gene targeting by generating site-specific DSB in a specific locus. Transcription activator-like effector nucleases (TALENS) and CRISPR are quite efficient for non-homologous end joining and producing insertion/deletion (indel) mutations. However, such genome engineering has been much less effective with respect to HR frequency when used to replace DNA with a donor vector, especially for human IPSCs.

Building upon these observations and insights, Hatada et al[53] reported that engineered nuclease-mediated HR was significantly increased in a hormetic fashion by low-dose ionizing radiation. For example, using ZFN along with low dose radiation yielded a 51% target frequency with ZFNs by itself yielding only 4%. The results followed the features of the hormetic dose response. The low dose radiation also enhanced the frequency of correctly targeted clones (GFF+/Pun) (Figure 8). This enhanced efficiency was shown to occur in human ESCs and human IPSCs at the same optimal radiation hormetic dose. A similar enhanced efficiency was also seen in the TALENS and CRISPR gene targeting systems. Follow-up research with the optimal IR showed that the 0.4 Gy treatment enhanced the correctly targeted recombination efficiency of gene targeting by the TALENS and CRISPR methods in a similar manner as seen with ZFNs. A follow-up assessment of the occurrence of genomic mutations in the irradiated human IPSCs at the hypoxanthine-guanine phosphoribosyltransferase (HPRT) locus revealed a hormetic dose response with mutation frequency suggesting a reduction at 0.13 and 0.4 Gy, but increasing at 1.26 and 4 Gy.

While the Nobel Prize in chemistry in 2021 was awarded for the development and application of CRISPR technology, the research of Hatada et al[53] indicated that low dose radiation can markedly enhance its efficiency. Further, the low dose radiation enhanced gene targeting therapy in a traditional gene targeting protocol with the involvement of engineered nucleases. The low dose radiation did not increase mutations in the human IPSCs, based on exome sequences. The authors suggested that correction of genetic defects by HR will profoundly improve cell replacement therapies by the use of autologous transplantation of a patient's own corrected cells. They argued that the simple approach of combining low dose radiation with current gene editing can significantly improve
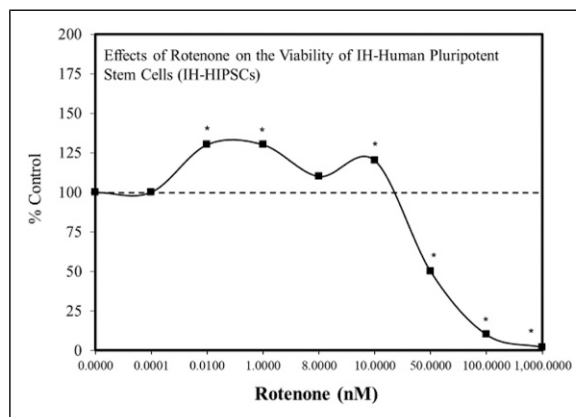

Figure 9. Effects of rotenone on the cell viability on human IPSCs (modified from: Pistollato et al[55]) *= $P \le .05$.

targeting frequency in human IPSCs, markedly enhancing the therapeutic applications.

Rotenone–IPSC-Derived NSCs Showed Hormesis

Rotenone is a crystalline isoflavone that has been widely employed as a broad spectrum pesticide. It can induce oxidative stress via inhibiting the transfer of electrons from iron-sulfur centers in mitochondrial complex 1 and ubiquinone. Prolonged exposure to rotenone has been associated with the induction of Parkinson's-like symptoms with toxicity reported in dopaminergic neurons. Of relevance to the present assessment is that Zagoura et al[54] reported that acute rotenone exposure (24 hours) can activate the Nrf2 pathway in a neuronal and astrocyte model derived from human IPSCs. These findings were extended by Pistollato et al[55] who assessed the expression and activation of Nrf2 signaling in human IPSC-derived NSCs undergoing differentiation. This research indicated that under control group settings the Nrf2 pathway activation is enhanced during the differentiation process which leads to neurons and glia. When repeated dosing with rotenone is superimposed on the differentiation process, there is progressive enhancement of Nrf2. In a toxicity study during the differentiation process, rotenone was administered after day 7 for the next 7 days at six different concentrations (.1, 1.0, 5.0, 10.0, 50.0, 100.0 and 500.0 nM). The findings indicate that rotenone displayed a striking hormetic-biphasic concentration response, with the lowest 4 concentrations enhancing viability (Figure 9). Thus, the hormetic response occurred over a 100-fold concentration range. It would have been of value explore whether these protective effects occurred at even lower concentrations.

Silicate–IPSC-Derived Pluripotent Embryoid Bodies Showed Hormesis

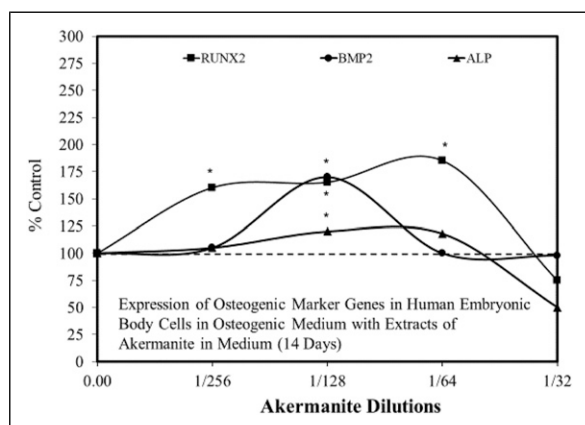Since silicate-based biomaterials, including various bioactivated glucose and calcium silicate bioceramics, have

Figure 10. Expression of osteogenic marker genes in human embryoid body cells in osteogenic medium with extracts of akermanite in medium (14 days) (modified from: Dong et al[56]) *= P ≤ .05.

displayed the capacity to stimulate osteogenesis and angiogenesis, Dong et al[56] sought to assess the capacity of akermanite bioceramics to affect osteogenic differentiation of embryoid bodies. Embryoid bodies (EBs) are aggregates of pluripotent stem cells. The pluripotent cell types that comprise embryoid bodies include ESCs that are derived from the blastocyst stage of the embryo. Akermanite ($Ca_2MgSi_2O_7$), a Ca-, Mg-, and Si-containing bioceramic, has been previously shown to stimulate the proliferation and osteogenic differentiation of multiple stem cells, including osteoblasts, human MSCs, and human adipose-derived stem cells. Of relevance to the work of Dong et al[56] is that when IPSCs are grown in a suspension without the differentiation inhibitory factor, an EB was formed. Using the EB from human IPSCs, the cells were then placed in osteogenic medium with a broad concentration range of akermanite extracts. The akermanite biphasically enhanced the expression of multiple osteogenic biomarkers including alkaline phosphatase (ALP) activity, bone morphogenic protein-2 (BMP-2), COL-1, OCN, and RUNX2 (gene member of the RUNX family of transcription factors) (Figure 10). Of further interest is that the akermanite affected an increase in osteogenic biomarkers, again showing a hormetic concentration increase with the same optimal concentration with and/or without the osteogenic media. It is also interesting to note that the hormetic biphasic concentration response was strongly associated with changes in the concentration of Si in the media but not with changes of Ca, Mg, and P.

Discussion

The present paper provides an evaluation of the hormetic dose response features of IPSCs or cells differentiated from IPSCs. With the exception of hypoxia, ionizing radiation and silicate, all agents that were assessed for hormetic dose responses in cells differentiated from IPSCs, with all but one (i.e., melatonin-mouse) being derived from human IPSCs. The derived cells included a range of cell types, including myocytes, NSCs and highly differentiated neurons, cardiomyocytes, and RPEs. Regardless of the cell type (IPSC or derived), or inducing agent, the quantitative features of the dose responses were similar with the stimulatory amplitude being 133% median (120-210% range) while the width of the stimulatory concentration was 10-fold (median). These values were within the range reported for the Hormetic Data Base[57] for a vast range of biological models, cell types, inducing agents and endpoints and consistent with hormetic responses reported in a broad range of adult and embryonic stem cells.[2-10] In studies using cells derived from IPSCs, the research that concerned hormesis had a considerably different focus than the papers published on hormesis and specific adult stem cells (adipose-derived stem cells (ADSCS), apical papilla (APSCs), bone marrow stem cells (BMSCs), dental pulp stem cells (DPSCs), embryonic stem cells (ESCs), endothelial stem cells, muscle stem cells (MSCs), neural stem cells (NSCs), and periodontal ligament stem cells (PDLSCs). In each of these adult stem cell papers, a major research theme was the evaluation of whether and to what extent each stem cell type could be induced to show cell proliferation/differentiation. In about 1/3 of the studies, the stem cells were also assessed for the capacity to upregulate adaptive mechanism to enhance resilience to oxidative stress. However, in the studies published on cells derived from IPSCs, a more diverse series of study endpoints/applications were presented. The present paper indicates that these studies do not yet follow an apparent systematic scheme. It is not uncommon for the investigations to use the IPSCs to go directly to an advanced application of interest. For example, RPE cells were derived from IPSCs to be tested for the capacity of curcumin to enhance resilience to oxidative stress.[30] Induced pluripotent stem cells were used to derive cardiomyocytes to assess the effects of metformin on various respiratory functions.[46] As a third example, highly differentiated sensory neurons over a 40-day period were then tested with a series of chemotherapeutic agents for toxicological evaluations.[47] Only in a limited number of cases were studies undertaken that assessed the capacity for cell proliferation and differentiation as seen with MT for IPSCs and NSCs, respectively. There was also a more apparent effort made to discern underlying molecular mechanism pathways in the collective individual stem cell-hormesis areas where such efforts have been substantial. However, given the extensive diversity of research questions focused in the IPSCs-derived cell area, any underlying mechanism efforts became unique to that area, with less capacity for generalization. Such area-specific mechanisms that were assessed are therefore addressed within the specific content areas of this paper rather than reintegrated for generalized discussion purposes in this section. Finally, it should be noted that even though IPSCs have been viewed as being essentially the same as embryonic inner mass, this question was not able to be addressed with respect to the issue of chemically induced cell proliferation and differentiation due to a lack of research in that area.

Conclusion

The present paper represents the first integrated assessment of the occurrence of hormetic dose responses in IPSCs and cells derived from IPSCs. The quantitative features of hormetic dose responses were commonly reported for a wide range of inducing agents and are fully consistent with hormetic responses reported with organ-specific adult stem cell types and embryonic stems cells as well as non-stem cell types. These findings further extend the generality of the hormesis concept.

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References

1. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007;448(7151):313-317.
2. Calabrese EJ. Human periodontal ligament stem cells and hormesis: Enhancing cell renewal and cell differentiation. *Pharmacol Res* 2021;173:105914.
3. Calabrese EJ. Hormesis and adult adipose-derived stem cells. *Pharmacol Res*. 2021;172:105803.
4. Calabrese EJ, Giordano J, Dhawan G, Kapoor R, Calabrese V. Hormesis and neural stem cells. *Free Radic Biol Med* 2021;178:1-16.
5. Calabrese EJ. Hormesis and apical papilla stem cells. *Chem-Biol Inter* 2022. In press.
6. Calabrese EJ. Hormesis and stem cells: Enhancing cell proliferation, differentiation and resilience to inflammatory stress in bone marrow stem cells and their therapeutic implication. *Chem-Biol Inter* 2021;351:109730.
7. Calabrese EJ. Hormesis and embryonic stem cells. *Chem-Biol Inter* 2022;352:109783. doi:10.1016/j.cbi.2021.109783. In press.
8. Calabrese EJ. Hormesis and endothelial progenitor stem cells. *Dose-Response* 2021;R1:221-229.
9. Calabrese EJ, Agathokleous E, Dhawan G, Kapoor R, Calabrese V. Human dental pulp stem cells and hormesis. *Ageing Res Rev* 2021;73:101540.
10. Calabrese EJ, Calabrese V, Dhawan G, Kapoor R, Giordano J. Hormesis and neural stem cells. *Free Radic Biol Med* 2021;178:1-16.
11. Calabrese EJ, Baldwin LA. Defining hormesis. *Hum Exp Toxicol*. 2002;21:91-97.
12. Calabrese EJ, Mattson MP. Hormesis provides a generalized quantitative estimate of biological plasticity. *J Cell Commun Signal*. 2011;5:25-38.
13. Calabrese EJ, Mattson MP. How does hormesis impact biology, toxicology, and medicine? *Npj Aging Mech Dis*. 2017;3:13.
14. Mattson MP. Hormesis defined. *Ageing Res Rev*. 2008;7:1-7.
15. Calabrese EJ, Baldwin LA. A general classification of U-shaped dose-response relationships in toxicology and their mechanistic foundations. *Hum Exp Toxicol*. 1998;17(7):353-364.
16. Calabrese EJ. Preconditioning is hormesis part I: Documentation, dose-response features and mechanistic foundations. *Pharmacol Res*. 2016;110:242-264.
17. Calabrese EJ. Preconditioning is hormesis part II: How the conditioning dose mediates protection: Dose optimization within temporal and mechanistic frameworks. *Pharmacol Res*. 2016;110:265-275.
18. Calabrese EJ. Overcompensation stimulation: A mechanism for hormetic effects. *Crit Rev Toxicol*. 2001;31:425-470.
19. Calabrese EJ. Hormesis: Why it is important to toxicology and toxicologists. *Environ Toxicol Chem*. 2008;27:1451-1474.
20. Calabrese EJ. Hormetic mechanisms. *Crit Rev Toxicol*. 2013;43:580-606.
21. Calabrese EJ, Kozumbo WJ. The hormetic dose-response mechanism: Nrf2 Activation. *Pharmacol Res*. 2021;167:105526.
22. Calabrese EJ, Baldwin LA. Chemical hormesis: Its historical foundations as a biological hypothesis. *Hum Exp Toxicol*. 2000;19:2-31.
23. Calabrese EJ, Baldwin LA. The marginalization of hormesis. *Hum Exp Toxicol*. 2000;19:32-40.
24. Calabrese EJ, Baldwin LA. Radiation hormesis: Its historical foundations as a biological hypothesis. *Hum Exp Toxicol*. 2000;19:41-75.
25. Calabrese EJ, Baldwin LA. Radiation hormesis: The demise of a legitimate hypothesis. *Hum Exp Toxicol*. 2000;19:76-84.
26. Calabrese EJ, Baldwin LA. Tales of two similar hypotheses: The rise and fall of chemical and radiation hormesis. *Hum Exp Toxicol*. 2000;19:85-97.
27. Calabrese EJ. Toxicology rewrites its history and rethinks its future: Giving equal focus to both harmful and beneficial effects. *Environ Toxicol Chem*. 2011;30:2658-2673.
28. Calabrese EJ. Dose-response: A fundamental concept in toxicology. In: Hayes AW, Kruger CL, eds. *Hayes' Principles and Methods of Toxicology*. 6th ed. Boca Raton: CRC Press; 2014:139.

29. Santhanam N, Kumanchik L, Guo X, et al. Stem cell derived phenotypic human neuromuscular junction model for dose response evaluation of therapeutics. *Biomaterials*. 2018;166:64-78.

30. Chang YC, Chang WC, Hung KH, et al. The generation of induced pluripotent stem cells for macular degeneration as a drug screening platform: Identification of curcumin as a protective agent for retinal pigment epithelial cells against oxidative stress. *Front Aging Neurosci*. 2014;6:191.Article 121

31. Horie N, So K, Moriya T, et al. Effects of oxygen concentration on the proliferation and differentiation of mouse neural stem cells in vitro. *Cell Mol Neurobiol*. 2008;28:833-845.

32. Santilli G, Lamorte G, Carlessi L, et al. Mild hypoxia enhances proliferation and multipotency of human neural stem cells. *PLoS One*. 2010;5(1):e8575.

33. Lee HJ, Choi BH, Min B-H, Park SR. Low-intensity ultrasound inhibits apoptosis and enhances viability of human mesenchymal stem cells in three-dimensional alginate culture during chondrogenic differentiation. *Tissue Eng*. 2007;13:1049-1057.

34. Lai C-H, Chen S-C, Chiu L-H, et al. Effects of low-intensity pulsed ultrasound, dexamethasone/TGF-β1 and/or BMP-2 on the transcriptional expression of genes in human mesenchymal stem cells: Chondrogenic vs. osteogenic differentiation. *Ultrasound Med Biol*. 2010;36:1022-1033.

35. Lv Y, Zhao P, Chen G, Sha Y, Yang L. Effects of low-intensity pulsed ultrasound on cell viability, proliferation and neural differentiation of induced pluripotent stem cells-derived neural crest stem cells. *Biotechnol Lett*. 2013;35:2201-2212.

36. Shu T, Wu T, Pang M, et al. Effects and mechanisms of melatonin on neural differentiation of induced pluripotent stem cells. *Biochem Biophys Res Commun*. 2016;474:566-571.

37. Huang J-Y, Ma Y-Z, Yuan Y-H, et al. Claulansine F promoted the neuronal differentiation of neural stem and progenitor cells through Akt/GSK-3β/β-catenin pathway. *Eur J Pharmacol*. 2016;786:72-84.

38. Kocsis K, Frank R, Szabó J, et al. Acetyl-l-carnitine restores synaptic transmission and enhances the inducibility of stable LTP after oxygen-glucose deprivation. *Neuroscience*. 2016;332:203-211.

39. Shu T, Fan L, Wu T, et al. Melatonin promotes neuroprotection of induced pluripotent stem cells-derived neural stem cells subjected to H2O2-induced injury in vitro. *Eur J Pharmacol*. 2018;825:143-150.

40. Salewski RP, Mitchell RA, Li L, et al. Transplantation of induced pluripotent stem cell-derived neural stem cells mediate functional recovery following thoracic spinal cord injury through remyelination of axons. *Stem Cells Transl*. 2015;4:743-754.

41. Yuan T, Liao W, Feng N-H, et al. Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion. *Stem Cell Res Ther*. 2013;4:73.

42. UKPDS (UK Prospective Diabetes Study Group). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet*. 1998;352:854-865.

43. Johnson JA, Majumdar SR, Simpson SH, Toth EL. Decreased mortality associated with the use of metformin compared with sulfonylurea monotherapy in type 2 diabetes. *Diabetes Care*. 2002;25:2244-2248.

44. Aguilar D, Chan W, Bozkurt B, Ramasubbu K, Deswal A. Metformin use and mortality in ambulatory patients with diabetes and heart failure. *Circulation: Heart Fail*. 2011;4:53-58.

45. Norwood DK, Chilipko AA, Amin SM, Macharia D, Still KL. Evaluating the potential benefits of metformin in patients with cardiovascular disease and heart failure. *Consult Pharm*. 2013;28:579-583.

46. Emelyanova L, Bai X, Yan Y, et al. Biphasic effect of metformin on human cardiac energetics. *Transl Res*. 2020;229:1-23.

47. Schinke C, Fernandez Vallone V, Ivanov A, et al. Modeling chemotherapy induced neurotoxicity with human induced pluripotent stem cell (iPSC)-derived sensory neurons. *Neurobiol Dis*. 2021;155:105391.

48. Feinendegen LE, Pollycove M. Biologic responses to low doses of ionizing radiation: detriment versus hormesis. Part 1. Dose responses of cells and tissues. *JNM (J Nucl Med)*. 2001;42:17N-27N.

49. Tubiana M, Feinendegen LE, Yang C, Kaminski JM. The linear no-threshold relationship is inconsistent with radiation biologic and experimental data. *Radiology*. 2009;251:13-22.

50. Pollycove M, Feinendegen LE. Biologic responses to low doses of ionizing radiation: Detriment versus hormesis. part 2. Dose responses of organisms. *JNM (J Nucl Med)*. 2001;42:26N-37N.

51. Russell WL, Kelly EM. Mutation frequencies in male mice and the estimation of genetic hazards of radiation in men. *Proc Natl Acad Sci Unit States Am*. 1982;79:542-544.

52. Hosoi Y, Sakamoto K. Suppressive effect of low dose total body irradiation on lung metastasis: Dose dependency and effective period. *Radiother Oncol*. 1993;26:177-179.

53. Hatada S, Subramanian A, Mandefro B, et al. Low-dose irradiation enhances gene targeting in human pluripotent stem cells. *Stem Cells Transl*. 2015;4:998-1010.

54. Zagoura D, Canovas-Jorda D, Pistollato F, Bremer-Hoffmann S, Bal-Price A. Evaluation of the rotenone-induced activation of the Nrf2 pathway in a neuronal model derived from human induced pluripotent stem cells. *Neurochem Int*. 2017;106:62-73.

55. Pistollato F, Canovas-Jorda D, Zagoura D, Bal-Price A. Nrf2 pathway activation upon rotenone treatment in human IPSC-derived neural stem cells undergoing differentiation towards neurons and astrocytes. *Neurochem Int*. 2017;108:457-471.

56. Dong X, Li H, Zhou Y, Ou L, Cao J, Chang J. The stimulation of osteogenic differentiation of embryoid bodies from human induced pluripotent stem cells by akermanite bioceramics. *J Mater Chem B*. 2016;4:2369-2376.

57. Calabrese EJ, Blain RB. The hormesis database: The occurrence of hormetic dose responses in the toxicological literature. *Regul Toxicol Pharmacol*. 2011;61(1):73-81.