From:	Carol Marcus
To:	vietti-cook annette NRC
Cc:	CMRBARAN Resource; Chairman Resource; CMRCaputo Resource; CMRWright Resource
Subject:	[External_Sender] Addendum 12 to my petition of 02-09-15
Date:	Friday, April 09, 2021 2:45:38 PM
Attachments:	Calabrese-Nrf2 and hormesis 03-10-2021.pdf

April 9, 2021

Dear Ms. Vietti-Cook:

Attached please find an article that I wish to add as Addendum 12 to my petition of 02-09-15 concerning the false LNT theory of radiation damage. The importance of this added article is that we now ***have a biochemical mechanism for hormesis.***

Sincerely,

Carol S. Marcus, Ph.D., M.D.

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Review

The hormetic dose-response mechanism: Nrf2 activation

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ABSTRACT

A generalized mechanism for hormetic dose responses is proposed that is based on the redox-activated transcription factor (TF), Nrf2, and its upregulation of an integrative system of endogenous anti-oxidant and anti-inflammatory adaptive responses. Nrf2 can be activated by numerous oxidative stressors (e.g., exercise, caloric restriction/intermittent fasting) and by exposures to synthetic, naturally occurring and endogenous chemicals, to non-ionizing (e.g., low-level light) and ionizing radiation, and to low-to-moderate stress from aging processes, among others. Nrf2 conducts crosstalk with other TFs to further integrate and enhance the effectiveness of adaptive metabolic strategies that produce acquired resilience. This adaptive mechanism of Nrf2 accounts for the generality and ubiquity of hormetic dose responses and supports the fundamental hormetic characteristic of protecting biological systems. At the same time, Nrf2 is highly evolutionarily conserved and quantitatively constrained in response (i.e., modest stimulatory response), further conserving biological resources and enhancing metabolic efficiencies. The notion that Nrf2 may serve as an hormetic mediator not only provides a regulatory-based evolutionary understanding of temporal acquired resilience and adaptive homeostasis but also causally integrates toxicological and pharmacological detoxification processes that are central to ecological and human risk assessments as well as to the development of drugs and therapeutics. These findings can also account for considerable inter-individual variation in susceptibility to toxic substances, the differential effectiveness of numerous therapeutic agents, and the variation in onset and severity of numerous age-related illnesses, such as type II diabetes.

1. Introduction

Over the past several decades, there has been a resurgence of scientific interest in hormesis, a biphasic dose response characterized by a low-dose stimulation and a high-dose inhibition [13,16,8]. Hormetic responses are classically defined as overcompensation reactions to the direct and immediate disruptions in cellular homeostasis by subtoxic or subthreshold doses of various stressor agents. Such hormesis-induced overcompensations often enhance cellular resiliencies without generating any observable phenotypic alterations. Hormesis may be phenotypically observed, however, when hormetic resiliency is induced and used to prevent the occurrence of various toxic phenotypes, such as death. For example, administering a high toxic dose of a stressor to cells either after (in preconditioning protocols) or before (in postconditioning protocols) inducing hormesis with a low subtoxic dose of the stressor results in the protection of cells from damage induced by the high toxic dose. Pre- and post-conditioning protocols highlight the adaptive resiliency or hormesis and have been studied extensively in medical research [10,11].

Studies on hormetic mechanisms with highly diverse biological models [9] have provided little evidence of a common, unifying, integrative. and evolution-based mechanism to account for the diversity and multitude of hormetic dose responses compiled over the past several decades. However, over the past several years, a general pattern has emerged suggesting a common mechanistic framework that may account for the generality of hormetic responses. It is proposed here that hormetic dose responses that are cytoprotective for chemicals and radiation are largely mediated following the redox activation of the transcription factor (TF) Nrf2. Nrf2 mediates a network of antioxidant defenses and initiates productive crosstalk with other TFs that not only act together with Nrf2 via dose-dependent hierarchal processes to enhance biological resilience but also conform to the quantitative features of the hormetic dose response. This paper demonstrates that activation of Nrf2 is the general and dominate underlying mechanistic basis of hormetic dose responses. It accounts for the striking “generality” of hormetic dose responses, that is, they are independent of biological

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models, levels of biological organization (cell to whole organism), endpoints measured, and inducing agents as well as the occurrence of inter-individual variations in susceptibility to toxic substances, pharmaceuticals, and aging processes [14]. Support for this conclusion is derived from the integration of findings from diverse areas of biological and biomedical research (e.g., aging, multi-organ system diseases, toxicology, radiation biology), affecting all organ systems and cell types.

Although the biomedical literature concerning Nrf2 is vast and expanding rapidly, the capacity of Nrf2 to affect adaptive responses is also extensive. When experiments incorporate rigorous study design features, including adequate numbers of doses, and their proper dose spacing within a repeat measure of temporal framework for Nrf2-mediated effects, hormetic dose responses predominate [15]. In presenting the case for a common hormetic mechanism, a convergent experimental approach is documented that demonstrates the occurrence, generality, and requirement of Nrf2 activation for a broad spectrum of hormetic responses.

As mentioned, hormesis is a biphasic, dose/concentration response that is characterized by a low-dose stimulation and a high-dose inhibition [13,14,8]. The hormetic/biphasic dose response has specific quantitative features such that the maximum stimulation is modest, being in the 130–160% range as compared to controls (100%) (Fig. 1). A recent detailed assessment indicates that the maximum hormetic response is affected by study-design features such as, for example, when the maximum response increases by about 5–7% for each additional dose that is evaluated below the threshold dose. Under such circumstances it appears that the maximum hormetic response appears to approach the 180–200% range [17,18]. The dose range of the hormetic response can be highly variable but is typically < 50-fold when starting immediately below the threshold dose [14].

2. Neurological diseases: hormesis and Nrf2

2.1. Stroke

2.1.1. Hormetic dose responses and neuroprotection

This section documents 7 agents and 8 studies that displayed hormetic dose responses in both their activation of Nrf2 and their neuroprotection against stroke-induced damage. These neuroprotective and Nrf2-activating agents included the following: anthraquinone-BME [66], Z-ligustilide [82], curcumin [109], ginsenoside [29], 2,7,2′-trihydroxy-4,4′7′-trimethoxy-1,1′-bephenanthrene (TTB) [67], protodioscin [91], and sulforaphane (SFN) [1,108]. Among these 8 studies, three experimental models were employed: rat pheochromocytoma PC 12 cells [29,66,82,91], one-day-old Sprague-Dawley rats [108,109,67] and adult male Sprague-Dawley rats [1]. The initial study by Qi et al. [82] using PC 12 cells was soon followed and supported by Wu et al. [108] using one-day-old Sprague-Dawley rat primary cortical neurons. Later that year Alfieri et al. [1] extended these *in vitro* efforts with an *in vivo* study in adult male Sprague-Dawley rats (Table 1).

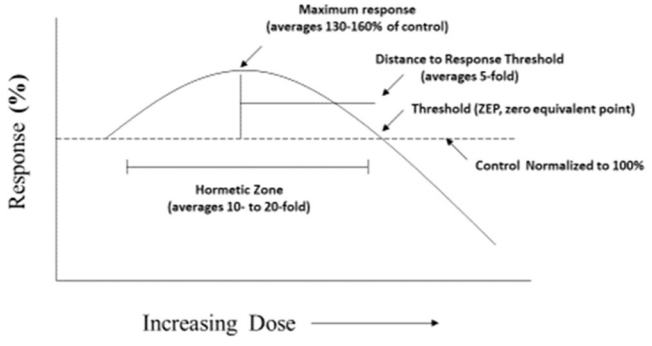

Fig. 1. General representation of the hormetic dose response.
(Modified from [12]).

Table 1
Hormetic activation of Nrf2 in stroke.

Citation	Biological model	Nrf2-activating agent	Stroke-inducing agent
[1,108]	Sprague-Dawley rats: one day old, and adult male	SFN	OGD/R, and Hemo restriction/R
[82]	PC 12 Cells	Z-ligustilide	OGD
[67]	Sprague-Dawley rats, one day old	TTB	OGD
[29]	PC 12	Ginsenoside Rg1	OGD/R
[66]	PC 12	BME	OGD
[109]	Sprague-Dawley rats, one day old	Curcumin	OGD/R
[91]	PC 12 Cells	Protodioscin	OGD/R

OGD/R - Oxygen Glucose Deprivation/Reperfusion

2.1.2. Hormesis: independent of model and experimental protocol

Five *in vitro* experimental studies used 3 different experimental protocols: (1) Six-hour preconditioning with BME prior to oxygen glucose deprivation (OGD) [66]; (2) concurrent treatment with curcumin along with OGD/Reperfusion (R) for 24 h [108,109]; and (3) a postconditioning protocol with ginsenoside given one hour after the OGD/R [29,91]. Regardless of the protocol or chemoprotective agent or of the diverse experimental combinations, hormetic-concentration responses occurred in each case (Figs. 2–7) (Table 1). Although both one-day-old Sprague-Dawley models displayed hormesis with different Nrf2-activating agents, the respective optimal concentrations differed with SFN being 0.5–1.0 μM while curcumin was 2.5–25 μM. Z-ligustilide displayed a hormetic concentration response in the range of 1–25 μM, with the optimum concentration at 5 μM [82].

2.1.3. Nrf2 and hormesis: convergent mechanism

Despite these differing experimental models, protocols, and activating agents the hormetic response in each case was blocked by the P13/AKT pathway inhibitor LY294002, except for the study by Liu et al. [67], which did not use this pathway inhibitor. This inhibitor also blocked the upregulation of Nrf2 [109]. These findings were supported by Qi et al. [82] who showed that LY294002 and L-N-acetyl cysteine (L-NAC), a reactive oxygen species (ROS) suppressor, blocked Nrf2 activation and its hormetic effects. These data indicate that Nrf2 activation requires a modest increase in ROS and involvement of the P13K/AKT pathway to mediate its hormetic/biphasic neuroprotective effects.

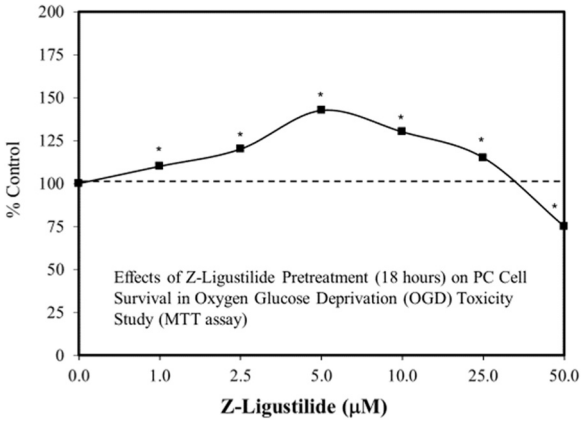

Fig. 2. Effects of Z-ligustilide pretreatment (18 h) on PC cell survival in oxygen glucose deprivation (OGD) toxicity study (MTT assay).
(Modified from: [82]).

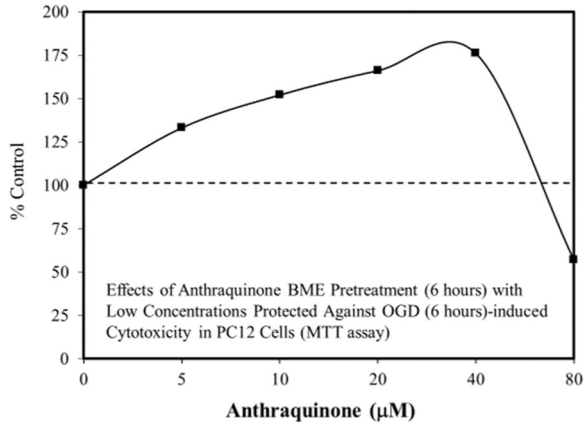

Fig. 3. Effects of anthraquinone BME pretreatment (6 h) with low concentrations protected against OGD (6 h)-induced cytotoxicity in PC12 cells (MTT assay).
(Modified from [66]).

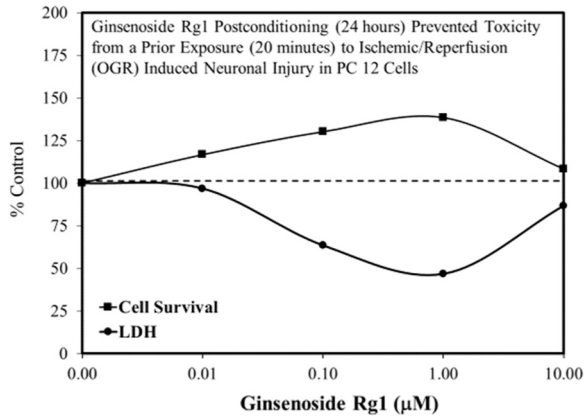

Fig. 4. Ginsenoside Rg1 postconditioning (24 h) prevented toxicity from a prior exposure (20 min) to ischemic/reperfusion (OGR) induced neuronal injury in PC12 cells.
(Modified from: [29]).

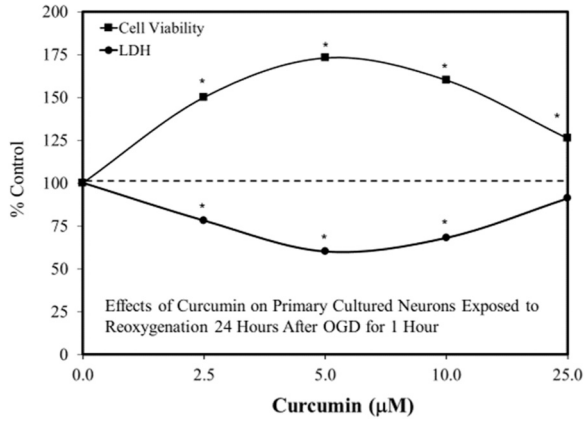

Fig. 5. Effects of curcumin on primary cultured neurons exposed to reoxygenation 24 h after OGD for 1 h.
(Modified from: [109]).

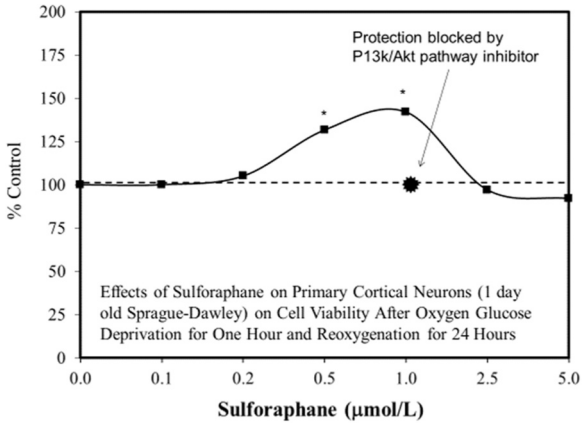

Fig. 6. Effects of sulforaphane on primary cortical neurons (1-day old Sprague-Dawley) on cell viability after oxygen glucose deprivation for one hour and reoxygenation for 24 h.
(Modified from: [108]).

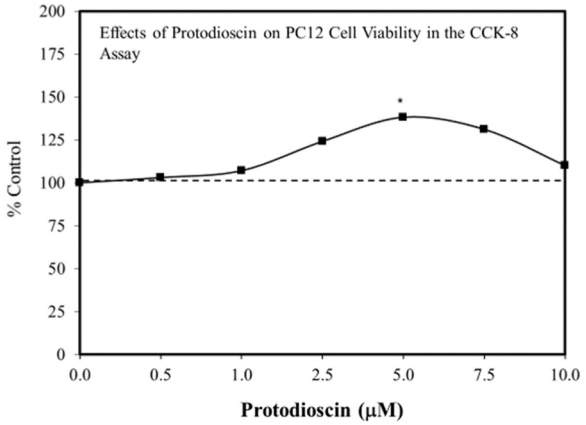

Fig. 7. Effects of protodioscin on PC23 cell viability in the CCK-8 assay.
(Modified from [91]).

2.2. Parkinson's disease

2.2.1. Multiple research strategies lead to Nrf2 and hormesis

Substantial research has assessed Nrf2 activation as a chemopreventive approach to slow the onset and progression of Parkinson's Disease (PD) using a range of *in vitro* and *in vivo* models [119]. The research employed a diverse array of methods including complimentary experimental models, a spectrum of Nrf2-activating agents, chemical libraries on dopaminergic neurons to yield synthetic molecules with improved therapeutic ratios [40], and novel siRNAs targeting Keap1 [106]. The Nrf2-based research on PD began with a series of experiments examining the effects of SFN on experimental stroke models (see [15], and the previous section). These positive findings on SFN-induced neuroprotection encouraged Vauzour et al. [101] to evaluate the specific neuroprotective capacity of SFN in a PD model using primary mouse cortical neurons exposed to the toxic dopamine metabolite, S-S-cysteinyl-dopamine (CysDa). SFN was administered for 24 h as a preconditioning agent, then removed and replaced with CysDa for an additional 24-hour period. Although the CysDa treatment reduced cortical neuronal survival by about 50%, the SFN pre-treatment significantly prevented much of the damage in an hormetic/biphasic dose-response manner (Fig. 8). The protection required activation of Nrf2/ARE (Anti-Oxidant Response Element) pathways and their subsequent mediation of downstream enzymatic detoxification products. Nrf2 activation was upregulated by approximately 10-fold at the optimal

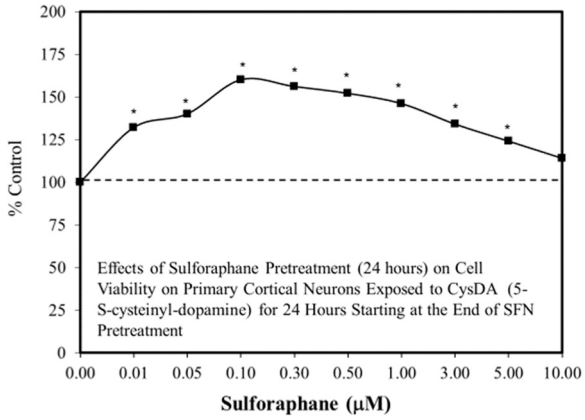

Fig. 8. Effects of sulforaphane pretreatment (24 h) on cell viability on primary cortical neurons exposed by CysDA (5-S-cysteinyl-dopamine) for 24 h starting at the end of SFN pretreatment
(Modified from: [101]).

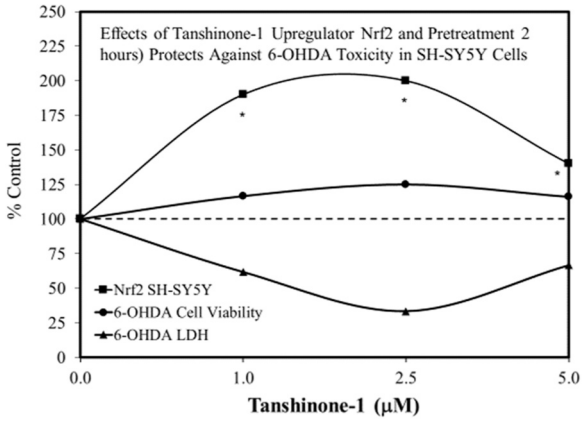

Fig. 9. Effects of tanshinone-1 upregulator Nrf2 and pretreatment (2 h) protects against 6-OHDA toxicity in SH-SY5Y cells
(Modified from: [47]).

protective concentration of SFN. Further investigation with specific pathway inhibitors revealed that the protective effect required involvement of the ERK1/2 pathway.

2.2.2. *Parkinson's disease: hormesis dose-response study features*

Further studies were performed under conditions of concurrent and preconditioning exposures to determine the dose-response relationships between multiple Nrf2-activating agents and their chemoprotective effects on PD. Studies have generally demonstrated that both the neuroprotection induced by known chemotherapeutic agents of PD and their capacity to activate Nrf2 display familiar hormetic dose-response relationships. Furthermore, the independent relationship of hormesis to the type of experimental model or to the type of toxic or Nrf2-activating agent was illustrated in six independent studies that employed in aggregate seven different biological models, seven different Nrf2-activating agents, and four different neurotoxic agents (that mimic the symptoms and toxicity of PD) (Table 2) (Figs. 9–14). The quantitative features of these hormetic dose responses displayed chemotherapeutic stimulatory responses with a maximum between 130% and 160% of control (148% median) and with a stimulatory concentration range between 5 and 1000-fold—starting immediately below the threshold concentration (Table 3). Two studies displayed hormetic responses for Nrf2 activation as measured by RNA/protein profiles and PD-endpoint responses [32,47].

Consistent with this perspective are findings from Gureev et al. [36] that methylene blue (MB) prevented rotenone-induced neurotoxicity as well as lipid peroxidation in the ventral midbrain. Detailed dose-response studies of intact brain mitochondria revealed a J-shaped hormetic dose-response profile for MB-induced neuroprotection of the rotenone-induced genetic lesions in mtDNA that was significantly less than that of background. Furthermore, the low protective doses of MB were shown to generate small increases in hydrogen peroxide that

Table 2
Hormetic activation of Nrf2 in Parkinson's disease.

Citation	Biological model	Nrf2-activating agent	PD-inducing agent
[72]	Cb Glioma cells	CORM-2	6-OHDA
[32]	BV2 cells	Luteolin	Rotenone
[117]	PC 12	Berberine	6-OHDA
[117]	Zebrafish	Berberine	6-OHDA
[59]	Male C57B/6 mice	DNP	6-OHDA
[47]	Sh-SY5Y	Tanshinone I	6-OHDA
[101]	Primary cortical neurons	SFN	5-S-cysteinyl-dopamine

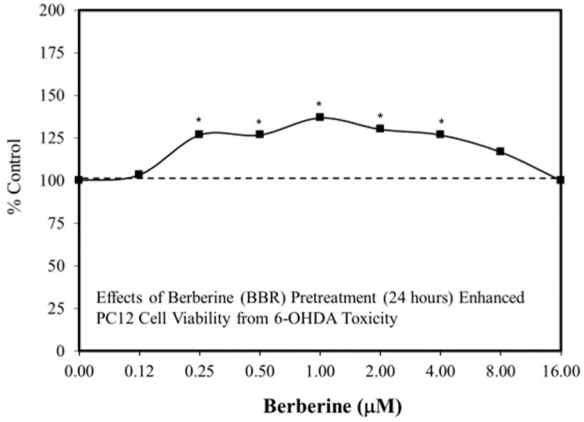

Fig. 10. Effects of berberine (BBR) pretreatment (24 h) enhanced PC12 cell viability from 6-OHDA toxicity.
(Modified from: [117]).

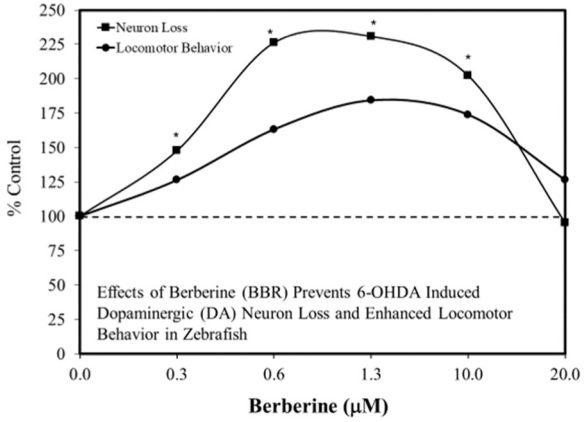

Fig. 11. Effects of berberine (BBR) prevents 6-OHDA induced dopaminergic (DA) neuron loss and enhanced locomotor behavior in Zebrafish.
(Modified from: [117]).

mediated the neuroprotective responses via the activation of Nrf2, whereas higher doses of MB enhanced rotenone-induced mutagenicity.

Other PD studies using Wistar rats demonstrated similar neuroprotection with the implementation of a modest four-week exercise program prior to the administration of MPP+, a neurotoxic agent that

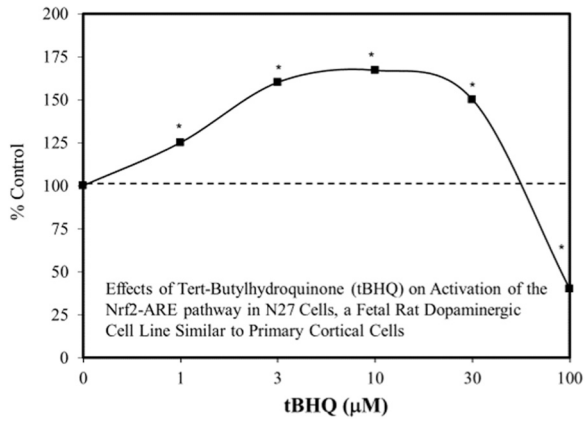

Fig. 12. Effects of tert-butylhydroquinone (tBHQ) on Activation of the Nrf2-ARE pathway in N27 cells, a fetal rat dopaminergic cell line similar to primary cortical cells.
(Modified from: [41]).

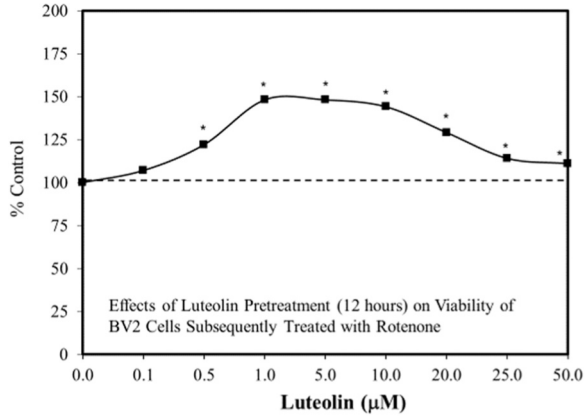

Fig. 13. Effects of luteolin pretreatment (12 h) on viability of BV2 cells subsequently treated with rotenone.
(Modified from: [32]).

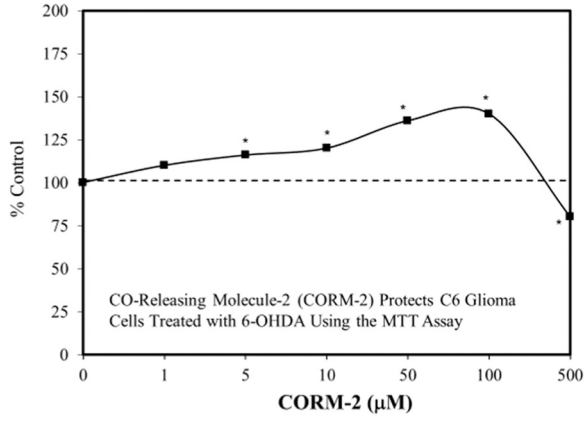

Fig. 14. CO-releasing molecule-2 (CORM-2) protects C6 glioma cells treated with 6-OHDA using the MTT assay.
(Modified from: [72]).

produces lesions resembling PD [99]. The exercise-induced neuroprotection was, however, blocked with a virus-mediated knockdown system targeting Nrf2. The authors noted that both exercise and MPP+ enhanced ROS, but whereas exercise upregulated Nrf2, MPP+ downregulated it. The opposing responses are likely due to a difference in the magnitudes of the ROS responses induced by exercise versus MPP+.

Table 3
Hormetic chemoprotection via Nrf2 in Parkinson's disease.

Citation	# Doses	Max response (%)	Hormetic concentration range (Fold)	Max Nrf2 response (%)	Hormetic concentration range (Fold)
[41]	5	149	NA	167	30
[32]	8	148	5	300	10
[47]	3	125	5	200	5
	3	33-J-shaped	5	200	5
[117]	8	136.7	33	180	Not Available (N/A)
	5	230.9	33	180	N/A
	5	184.2	66.6	180	N/A
[72]	6	140	100	280	N/A
[59]	5	150	100	146	N/A
[101]	9	126	> 1000	1000	N/A

2.2.3. *A mechanistic link between Nrf2 and hormesis*

Several PD studies have addressed the hormetic relationship between Nrf2 and the neuroprotection it mediates. Jakel et al. [41] used mice with Nrf2+/− genotypes to demonstrate dose-response relationships between Nrf2 and its downstream products, but no studies have yet targeted the upstream pathways that activate Nrf2, as in the case of the stroke studies mentioned above. Recently, however, Elmazoglu et al. [32] highlighted the hormetic relationship of luteolin-induced neuroprotection to the molecular association between Nrf2 and TRX-1 and the induction of the Park 2 gene that encodes for the Parkin protein, which can enhance the nuclear activation of Nrf2.

Although loss of the activation of Nrf2 enhances the susceptibility of dopaminergic neurons to oxidatively induced damage [5,86], the converse is also true—the activation of Nrf2 mediates neuroprotection. Support for the above is when Keap1 was silenced in astrocytes and the subsequent upregulation of Nrf2 was associated with protection against oxidative stress [24,43,48,68]. These findings indicate that activation of Nrf2 may mediate short-term neuroprotection of early adverse events in the etiology of PD. In fact, continual low-dose hormetic dosing over the course of a lifetime may alter outcomes of other age-related chronic diseases, a concept consistent with the observation that low-dose radiation exposures attenuated late-in-life chronic diseases, i.e., cancer [87].

3. Alzheimer's disease

In 2018, Zhao et al. suggested that SFN may retard the onset and progression of Alzheimer's disease (AD) via enhancing the expression of Nrf2 and thereby inducing anti-oxidative and anti-inflammatory conditions in the brain. This hypothesis was based, in part, on studies [116, 30,57,98] suggesting that the early stages of AD display adaptive responses involving modest increases in Nrf2 expression. However, Nrf2 is progressively downregulated in hippocampal neurons and astrocytes as the disease advances [84]. As a model for AD, Zhao et al. [120] therefore combined human neuroblastoma N2a cells with a human mutant amyloid processor protein to assess whether SFN could enhance cell viability by sustaining the expression of Nrf2. As it turned out, SFN treatment enhanced the nuclear translocation of Nrf2 and decreased DNA methylation of the Nrf2 promoter, resulting in the increased expression of Nrf2 itself (Fig. 15). Low concentrations of SFN also diminished the cellular levels of amyloid beta$_{1–40}$ (AB$_{1–40}$) by 20–30%. Associated with the enhancement of cell viability by low concentrations of SFN was the upregulation of Nrf2 and the concomitant reduction in multiple biomarkers of neural inflammation. These findings led to the conclusion that "low concentrations of SFN have a protective effect and reduced the production of AB$_{1–40}$ and AB$_{1–42}$ in N2a/APPSWE cells." These data further supported the findings of Kim et al. [53] that SFN reduced the AB-induced cognitive decline in mice tested for both passive avoidance

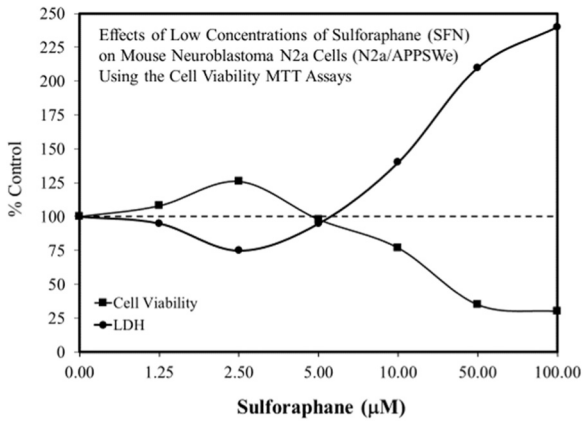

Fig. 15. Effects of low concentrations of sulforaphane (SFN) on mouse neuroblastoma N2a cells (N2a/APPSWE) using the cell viability MTT assays. (Modified from: [120]).

and spontaneous alternation in the Y-maze. Such behavioral findings, which were supported by *in vitro* findings of SFN-induced inhibition of AB aggregation, indicate that activation of Nrf2 is an hormetic response to SFN and mediates neuroprotection against AD.

The ketogenic diet is being widely explored as a treatment to slow the progression of AD [71,80]. The principal mechanism by which a ketogenic diet blocks the progression of AD is by enhancing mitochondrial function and diminishing oxidative stress. The most extensively studied ketone body, beta-hydroxybutyrate, was found to activate Nrf2, increase the NAD+/NADH ratio, and improve overall mitochondrial functioning, thereby providing a mechanistic basis for this therapeutic diet [71,80].

4. Methylene blue: neuroprotection and Nrf2

Methylthioninium chloride or, more commonly, methylene blue (MB) is a phenothiazine agent well known for its capacity to cross the blood-brain barrier and to affect a broad spectrum of neuroprotective effects. MB has a long history of diverse therapeutic applications with use in the treatment of depression, cancer, methemoglobinemia, and AD. MB is a redox cycler and an electron donor that can minimize the aggregation of Tau protein due to its capacity to oxidize cysteine residues. These findings suggest that MB has the capacity to upregulate Nrf2 and that some of its neuroprotective effects are due to this mechanism. Follow-up research using the P301S mouse model of tauopathy revealed that MB not only displays an hormetic dose response in its upregulation of Nrf2/ARE but also prevents tau-related neurotoxicity [94]. The MB-induced upregulation of Nrf2/ARE resulted in an increase in the downstream expression of antioxidant genes (*Nrf2, HO-1, NQO1*, *Gclc*, and *Gclm*) and a decrease in the downstream expression of a pro-inflammatory gene (*iNOS)*. Increased expression of the *Gclc* and *Gclm* genes via Nrf2 signaling enhanced the cellular levels of the antioxidant GSH. Likewise, the genes encoding for *Trx* and *Prxs* are ARE-activated. Follow-up experimentation with Nrf2 knockout mice confirmed that the neuroprotective effects of MB were dependent on transcriptional signaling by Nrf2. These findings are similar to those of other studies investigating the hormetic induction of Nrf2 by MB and the subsequent hormetic mediation of neuroprotective effects [33,36,85, 88].

5. Natural product activation

5.1. Nrf2 activation and phytochemicals

A broad spectrum of natural products is commonly used as dietary supplements, including curcumin, quercetin, ginseng, green tea, and sulforaphane. Detailed dose-response assessments of a number of these dietary supplements have demonstrated the occurrence of hormetic dose-response relationships and their dependence on Nrf2 activation [15,19,20]. A series of studies have assessed the potential of natural products, such as flavonoids, lignans, and terpenoids, to activate Nrf2. A flavonoid library was established using SAR (structure-activity relationship) analysis to identify agents effective in inducing quinone reductase activity in Hepa 1c1c7 cells (i.e., murine hepatoma cells). Based on an assessment of 74 flavonoids, 24 showed Nrf2-activating potential with an increase of $\geq$ 30% over controls, which was the criterion for an inductive effect. Of the 24 flavonoids identified, the most active was 7-MB, with an inductive value of 208%. This agent was studied further in human breast carcinoma (i.e., MDA-MB-231) and human lung epithelial cells (i.e., BEAS-2B). 7-MBA activated Nrf2 over a concentration range of 6.25–50 μM in BEAS-2B cells. Further experimentation was performed with BEAS-2B cells to assess the role of trivalent arsenic (III) in inducing oxidative injury [63].

5.2. Nrf2 activation prevents arsenic III toxicity

In an initial study, Li et al. [63] assessed the effects of nine concentrations (0.30–100 μM) of 7-MB and showed hormetic dose responses were generated, in each case, for Nrf2 activation and several of its downstream antioxidant enzyme products (Fig. 16). In a follow-up preconditioning experiment, they showed that 7-MBA also induced hormetic chemoprotection against arsenic III toxicity with an optimal concentration range similar to that exhibited by the downstream enzymatic endpoints (Fig. 17) in their initial study. Furthermore, specifically blocking Nrf2 activation resulted in the blocking of 7-MB-induced protection against arsenic III toxicity. Other studies showed that Nrf2 activation was blocked by specific inhibitors of the protein kinases, PKC, PERK, and PI3K. Protein kinases catalyze the phosphorylation reactions of serine, threonine, and tyrosine residues in Nrf2 and, therefore, have important roles in the regulation of Nrf2 signaling pathways. These pathway findings are similar for smaller stimulatory molecules of Nfr2 activation, such as the effects of SFN on the AKT/ERK1/2 pathways [42] and of tBHQ on the P13K/AKT and ERK2 pathways [114]. Similar pathway involvements were also observed when using Hepa 1c1c7 cell lines with quinone reductase as an endpoint.

Following the initial studies with 7-MBA, subsequent research indicated that other Nrf2-activating agents [i.e., Artocarmintin B, [110]; (25)-5,6,7, 3″,4″-pentamethoxyflavanone (PMT), [123]; 3,3′,4,4′-tetrahydroxydiphenyl (THD), [122]; Sphaeropsidin A (SA), [65]; and Sphaeropsidin C (SC), [61]] displayed the same hormetic dose-response patterns as 7-MBA. In studies of these Nrf2-activating agents, inhibitors and preconditioning protocols were used to document the involvement

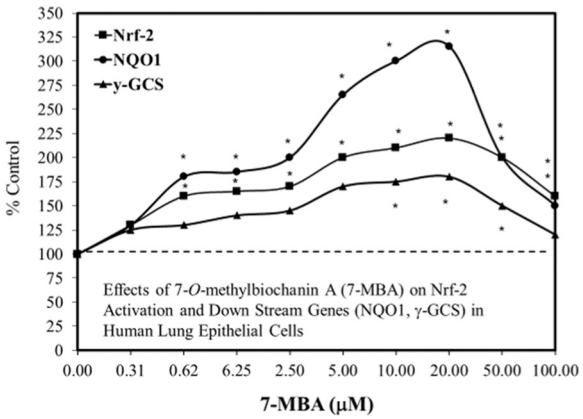

Fig. 16. Effects of 7-O-methylbiochanin A (7-MBA) on Nrf-2 activation and downstream genes (NQO1, γ-GCS) in human lung epithelial cells. (Modified from: [63]).

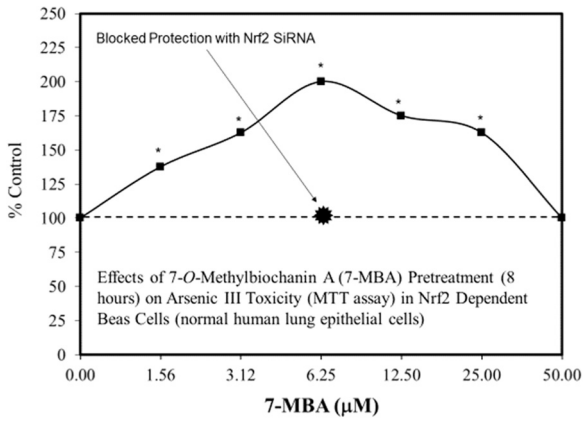

Fig. 17. Effects of 7-O-methylbiochanin A (7-MBA) pretreatment (8 h) on arsenic III toxicity (MTT assay) in Nrf2 dependent Beas cells (normal human lung epithelial cells).
(Modified from: [63]).

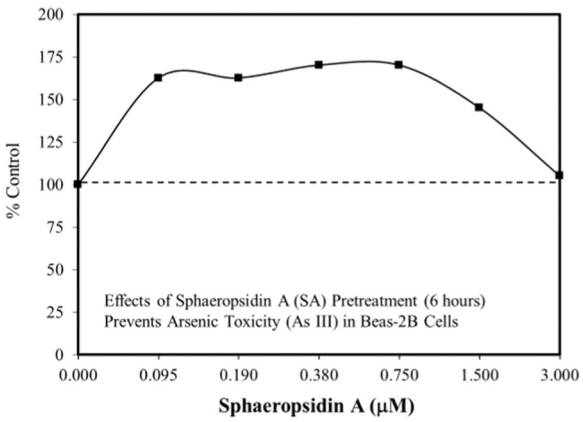

Fig. 19. Effects of sphaeropsidin A (SA) pretreatment (6 h) prevents arsenic toxicity (As III) in Beas-2B cells
(Modified from: [65]).

of specific pathways and to assess the capacity of Nrf2 activation to blunt the toxicity of arsenic III, respectively. All agents exhibited similar protective effects and induced activation of Nrf2 and its downstream products while consistently displaying hormetic dose-response relationships (Figs. 18 and 19). In general, the pathways of PI3K, PKC, p38 MAPK, MEK1/2, and PERK were shown to be involved in Nrf2 activation (with the PI3K and PKC pathways most dominant), indicating that phosphorylation is needed for Nrf2 activation. That these Nrf2-activating agents also blocked the ubiquitination—and thus degradation—of Nrf2 indicated further that Nrf2-mediated protection may be prolonged and enhanced by these agents.

Testing with curcumin revealed an hormetic dose response for Nrf2 transcriptional activity [90] (Fig. 20), which was also the case for the more bioavailable curcumin analog, Bis (2-hydroxybenzylidene) (BHBA). In a preconditioning protocol with BEAS-2B cells, BHBA induced an hormetic viability response and blocked the toxic effects of arsenic. In AJ mice, BHBA also inhibited vinyl carbamate-induced lung cancer via the activation of Nrf2.

Some polyphenolic phytochemicals, such as curcumin, EGCG, and resveratrol, were shown to activate Nrf2 at low doses in BEAS-2B cells and led to the hormetic dose-response expression of ubiquitin-activating enzyme E1-like (UBE1L), which is the E1-activation enzyme for the interferon-stimulated gene product, ISG15. Hormetic activation of Nrf2 and the subsequent expression of UBE1L, a candidate tumor suppressor gene, were shown to be regulated by the ROS redox state, suppressed the

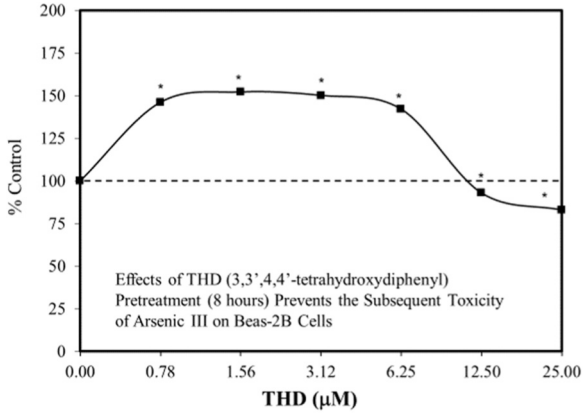

Fig. 18. Effects of THD (3,3′4,4′-tetrahydroxydiphenyl) pretreatment (8 h) prevents the subsequent toxicity of arsenic III on Beas-2B cells.
(Modified from: [122]).

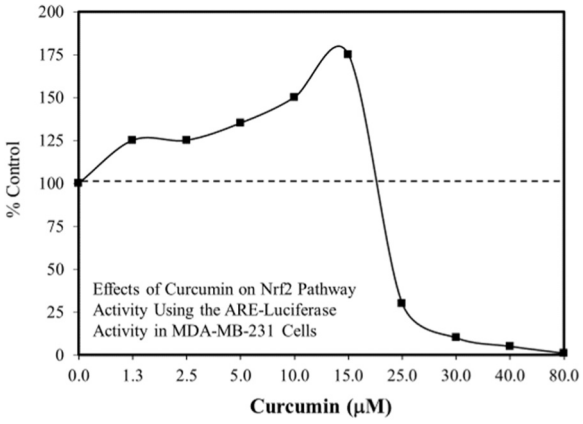

Fig. 20. Effects of curcumin on Nrf2 pathway activity using the ARE-luciferase activity in MDA-MB-231 cells.
(Modified from: [90]).

growth of numerous tumor cell types [46], and had potent activity against a range of viral infections (e.g., influenza B virus) [55].

6. Toxicology

6.1. Nrf2: hormetic stress, redox signaling, antioxidant response, and inflammation

All cells are continually exposed to ROS, including superoxide, hydrogen peroxide and hydroxyl radicals. For example, ample amounts of ROS are continuously produced by the electron transport system (ETS) of mitochondria during aerobic respiration as well as by other biochemical reactions and in other organelles. Environmental stressors, such as xenobiotics, UV light and ionizing radiation, also contribute to and enhance ROS formation. Together, the sum of oxidative stresses may upset the delicate redox balance and drive cells toward pathologically pro-oxidant as well as pro-inflammatory states. To maintain redox homeostasis and prevent deleterious effects due to excessive oxidative stress, cells utilize slight increases in ROS as signals to upregulate the production of antioxidant enzymes and molecules that may then scavenge oxidants, restore redox balance, and prevent oxidative damage. In mammalian cells, the Keap1 protein is a key redox sensor of intracellular ROS [73]. At normal redox homeostasis, Keap1 sustains the ubiquitination and degradation of Nrf2 to which it is bound, keeping Nrf2 at low levels. However, with increases in exposure to subtoxic hormetic levels of oxidative stressors, several oxidatively labile cysteine residues in

Keap1 are oxidized and Keap1 loses its capacity to mediate Nrf2 ubiquitination, stabilizing Nrf2. Nrf2 then accumulates and trans-locates to the nucleus, a process that is enhanced via the phosphorylation of Nrf2 by several protein kinases. The activated Nrf2 then binds to a region of antioxidant response elements (ARE) of numerous antioxidant genes, enhancing their expression. Thus, the Nrf2/ARE pathway is important because it downregulates oxidative stress to restore redox balance (e.g., HO-1, GCLM and GLLC), suppresses inflammation (TGF-B and NF-kB), and enhances xenobiotic metabolism and excretion (NQO1, AKRIC1, and MRPI), apoptosis (BCL2 and BclxL), and autophagy (p62). Moreover, as redox homeostasis is re-established by a Nrf2-induced surge in antioxidants and as xenobiotics are metabolized and excreted, ROS signaling terminates. Keap1 then enters the nucleus, binds Nrf2, and transports it back to the cytosol for degradation by proteosomes [102].

The adaptive functions of Nrf2 signaling have been established in numerous organs with a principal focus on tissues highly involved with antioxidation and detoxification. The cytoprotective roles of Nrf2 have been widely studied in Nrf2-knockout mice, including three different strains (i.e., ICR, C57BL/6, and Balb/c) and with tissue-targeted, Nrf2-knockout models (e.g., lung). In these studies, Nrf2 deficiency was consistently associated with enhancements in the following: organ stress, susceptibility to stressor-induced damage, magnitude of injury, and recovery time [121,38,39,44,45].

Prolonged exposures to oxidative stressors, however, may overwhelm the hormetically regulated Nrf2 mechanisms that maintain redox homeostasis and, as such, induce pro-oxidant states that promote pro-inflammatory reactions. For example, the process of polarizing macrophages from anti-inflammatory (M2) to proinflammatory (M1) states with the treatment of various polarization agents was recently shown to be dependent on the hormetic dose response of the specific polarization agent [17,18]. Macrophage polarization is thus linked to hormesis and furthermore is regulated, at least in part, by increasing levels of mitochondria-generated ROS, which also activate the MAPK and NF-kB downstream pathways to affect more broadly an inflammatory state, including the subsequent induction of TNFα [97]. Thus, in elevating antioxidant levels (e.g., GSH) to regulate redox balance, Nrf2 indirectly affects whether the immune system is predisposing toward anti-inflammatory or pro-inflammatory states [113]. Both anti-inflammatory and pro-inflammatory states are, however, double-edged swords capable of preventing and promoting diseases. Pro-inflammatory states activate the immune system to destroy infectious agents and cancer cells while anti-inflammatory states restrain the immune system to eliminate key etiological factors of chronic inflammation that are contributory to many chronic diseases, such as Alzheimer's and Parkinson's diseases, heart disease, diabetes, and cancer. [97]. Nrf2 is a redox-activated transcription factor (TF) that functions not in isolation but in concert with other TFs, including p53, NF-kB, and Keap1. If Nrf2-mediated antioxidant responses are insufficient to reduce oxidative stress then one TF in particular, NF-kB, is activated and upregulates pro-inflammatory responses, such as TNFα, which may be pathological when chronically sustained or salutary when acutely directed at infectious microbes or cancer cells. It is the redox signaling and crosstalk between Nrf2 and NF-kB, as well as the interactions of other TFs, that work in concert and are necessary to assure that the salutary and not the pathological edges of the anti- and pro-inflammatory swords are appropriately applied [2,113].

Numerous pathology studies have associated elevated levels of TNFα with inflammatory processes. Blockage of TNFα with antagonists is generally effective in the treatment of inflammatory conditions. Despite this effective use of TNFα antagonists, however, they are often problematic when chronically used, enhancing cancer risks as well as demyelination and cardiovascular disorders. These findings suggest the possibility that TNFα acts in a biphasic dose-response manner, displaying pro- and anti-inflammatory responses as a function of dose [89]. Follow up investigations with cardiomyocytes showed that TNFα acts biphasically over a 50-fold concentration range. At the low concentration range of 2–5 ng/ml, TNFα enhanced nuclear translocation of Nrf2 along with enhanced binding to the DNA promoter region and the transactivation of the Nrf2 gene (Fig. 21). These actions were associated with a 50% increase in intracellular GSH. Higher concentrations of TNFα, however, suppressed the GSH response. Similar biphasic dose-response findings were reported for NQO1, HO-1 and G-6-PD. These findings demonstrate that exposure of TNFα to cardiomyocytes, at concentrations far below those that induced inflammation, affects an increase in Nrf2 and in the subsequent anti-inflammatory response [89]. Such observations are particularly significant since they reveal a bimodal effect of TNFα in the regulation of the redox-sensitive Keap1/Nrf2 antioxidant pathway as well as the involvement of substantial crosstalk between Nrf2 and NF-kB in the process of regulating the anti- and pro-inflammatory states.

6.2. *An overcompensated Nrf2 response and hormesis*

Using hypochlorous acid (HOCl) as an oxidant stressor, Woods et al. [107] demonstrated that HOCl induced an antioxidant response in mouse macrophages that was preceded by the accumulation of Nrf2 in the nucleus. While HOCl initially affected a decrease in intracellular GSH, *de novo* GSH synthesis increased following Nrf2 activation, with the initial decreases in GSH being reversed and even exceeding control values. With respect to GSH, HOCl displayed a biphasic dose response with low doses of HOCl affecting a 50–100% greater increase in GSH over control rates and high doses of HOCl affecting a significant decrease in GSH. This hormetic dose response also occurred with multiple other downstream endpoints (e.g., NQO1) [78].

At the higher concentration of HOCl, the repression of gene expression was not the result of cytotoxicity since, at this higher concentration, cell viability remained high at 90% and other genes were upregulated. Furthermore, the cell viability response was hormetic (as it displayed a 20% increase above controls), the hormetic response profiles (e.g., for GSH, GLLC, NQO1, and cell viability) were overlapping, and the hormetic optima were coincidental—all features of an adaptive response that is biphasic and hormetic. Hormetic findings were also reported for several preconditioning experiments in which cells pretreated with a low/subtoxic dose of HOCl were protected against damage from a second high/toxic dose of HOCl [107,115].

These observations lead to the general perspective that during exposure to increasingly high levels of oxidant stresses, a hierarchical activation of cellular pathway responses occurs. That is, at very low levels of oxidant exposure, cell markers of normal function occur [115]. However, as oxidant levels modestly increase, activation of Nrf2 takes place to mediate adaptive responses by inducing phase II antioxidant

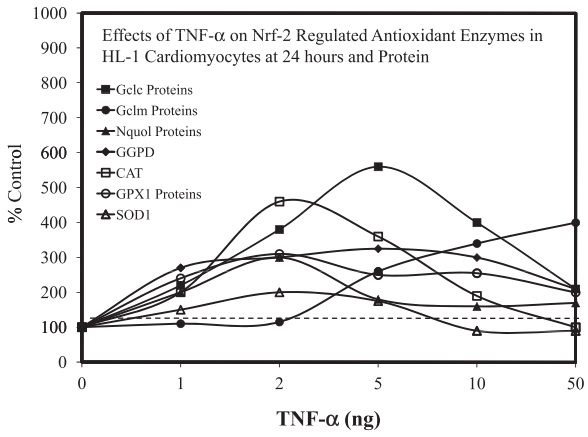

Fig. 21. Effects of TNF-alpha on Nrf-2 regulated antioxidant enzymes in HL-1 cardiomyocytes at 24 h and protein.
(Modified from: [89]).

enzymes that ensure ROS levels are kept low. Finally, at progressively higher levels of oxidant exposure, stress overwhelms the Nrf2 control systems, activating the NF-kB-mediated inflammatory response, leading to cell damage and/or death. The alterations in cell activities seen at low levels of oxidant stress are due in part to the antioxidant responses and indicate that hormesis is an expression of adaptive mechanisms to achieve redox homeostasis.

The HOCl-induced hormetic activation of Nrf2 represents an overcompensated stimulation of Nrf2 and its downstream antioxidant products that are needed to counteract a sudden and dramatic treatment-related, oxidant-driven decrease in cellular GSH and, ultimately, to restore redox balance. In effect, this overcompensated response is the hormetic response that occurs at near-threshold subtoxic doses and is a common feature in toxicology [6,7]. To observe it, however, requires rigorous study designs that include adequate numbers of doses, proper dose spacing, and repeat measures overtime to discern the initial toxic response followed by the overcompensated hormetic response. Conventional toxicology studies identify doses large enough to elicit overt inhibitory (toxic) responses that are greater than the no-observed adverse effects level (NOAEL). By contrast, hormetic studies must identify doses small enough to elicit subtle stimulatory (hormetic) responses that are less than the NOAEL. As a result, the stimulatory response of the hormetic dose response is more difficult to define as compared to the inhibitory response; this is because many smaller doses are required to define the extent of the stimulatory response, which is typically also smaller in magnitude and, therefore, more difficult-to-measure.

6.3. *Many toxicants and drugs activate Nrf2*

Many examples have been reported of low doses of toxic substances that activate Nrf2, enhance cellular resiliency, diminish damage, and elevate thresholds. In addition, Nrf2 knockout mice have consistently demonstrated markedly enhanced susceptibility to and lower thresholds for a broad range of toxic substances. A generally representative listing of toxic agents activating Nrf2 include acetaminophen [51], acrolein [95], sodium arsenite (Fig. 22) [75], benzene [64], bisphenol A [28], boron (Fig. 23) [50], cadmium [23], sodium fluoride (Fig. 24) [124], iodoacetic acid [104], methylmercury (Fig. 25) [103], ozone [70], paraquat [79], PFAS [96], silica nanoparticles [74], zinc sulphate [62], and multiple chemical carcinogens [3]. Table 4 provides a brief description of the Nrf2 activation by various toxic agents and their toxicological significance.

Within this toxicological perspective, recent studies show that the induction of Nrf2 by a low dose of metformin blocks lead toxicity [112]

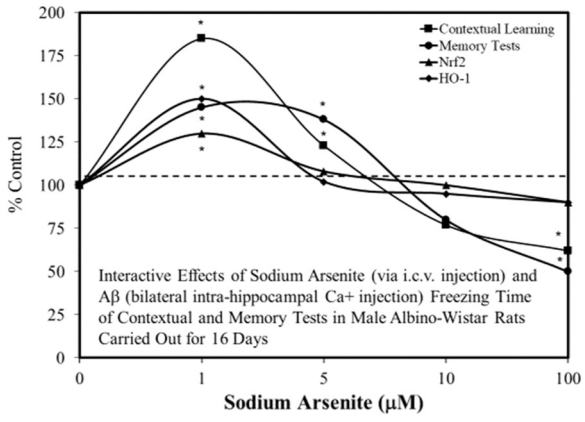

Fig. 22. Interactive effects of sodium arsenite (via i.c.v. injection) and AB (bilateral intra-hippocampal Ca+ injection) freezing time of contextual and memory tests in male Albino-Wistar rats carried out for 16 days.
(Modified from: [75]).

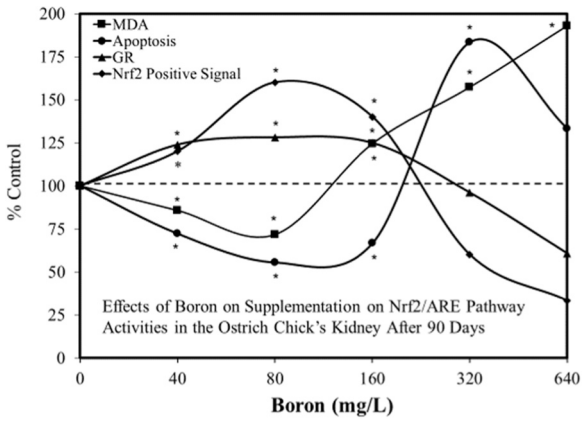

Fig. 23. Effects of boron on supplementation on Nrf/ARE pathway activities in the Ostrich chick's kidney after 90 days.
(Modified from: [50]).

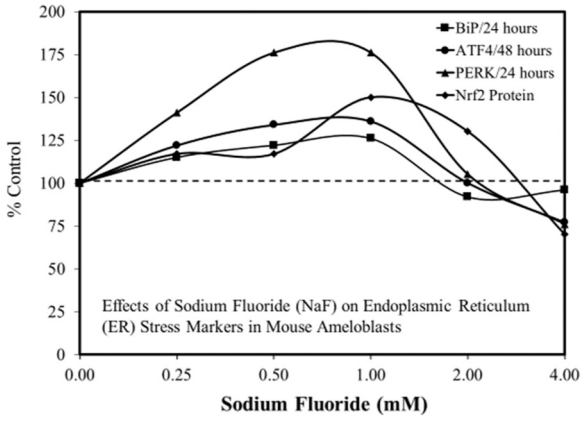

Fig. 24. Effects of sodium fluoride (NaF) on endoplasmic reticulum (ER) stress markers in mouse ameloblasts.
(Modified from: [124]).

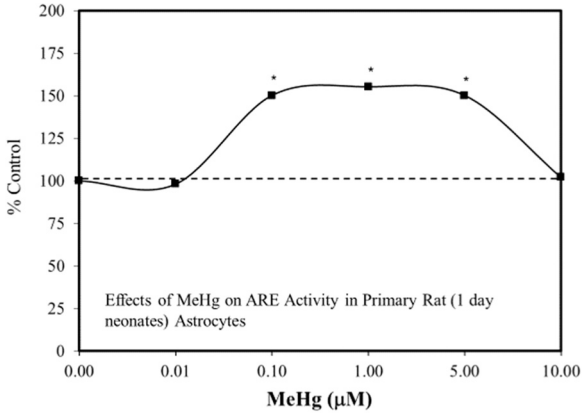

Fig. 25. Effects of MeHg on ARE activity in primary rat (1 day neonates) astrocytes.
(Modified from: [103]).

as well as protects the blood brain barrier from harmful constituents of cigarette smoke [81]. The induction of Nrf2 via metformin is also protective in a wide range of biological systems, such as protecting against age-related ocular damage [83] and stress-induced periodontal damage. The latter protection suggests a role for metformin in bone regeneration

Table 4
Nrf2 activation: adaptive response induced by toxic substances.

Toxic agents	Description of activity	Reference
Acetaminophen (AAP)	In the liver of NMRI mice, APP activated Nrf2 and led to a reduction in liver toxicity.	[51]
Acrolein	Pretreatment with a low dose of acrolein protected human bronchial cells from a second higher dose of acrolein. Adaption was induced by activation of Nrf2, which mediated GSH gene expression.	[95]
Benzene	Accumulation of excessive oxidative stress was detected in Nrf2-knockout mice following benzene exposure, indicating that Nrf2 has a critical role in resisting oxidative stress.	[64]
Benzopyrene	Nrf2 activation prevented both BAP-induced forestomach neoplasia and BBN-induced urinary bladder cancer in wild type mice. In Nrf2 deficient mice, however, DMBA-induced mammary tumors were enhanced and MAP-induced lung metastases were twice as frequent.	[3]
Iodoacetic Acid (IAA)	IAA activated Nrf2 in vitro (HepG2) and in vivo (SD rats), while IAA-induced toxicity was enhanced in Nrf2-deficient cells, leading to an increase in micronuclei. Pretreatment of the Nrf2-deficient cells with curcumin significantly activated Nrf2 and reduced IAA cytotoxicity.	[104]
Methylmercury (MetHg)	In primary rat astrocytes, MetHg activated Nrf2 and resulted in increased GSH. Blocking PI3 kinase suppressed the activation of Nrf2 and enhanced neurotoxicity.	[103]
Ozone (0_3)	O_3 is both a pollutant and a therapeutic agent. Pre-exposures of adult male SD rats to low concentrations of O_3 upregulated Nrf2 and prevented injuries to their mitochondria and heart from subsequent exposures to high-concentrations of O_3.	[70]
Sodium arsenate (NaAs)	NaAs enhanced memory performance in Wistar rats via activation of the hippocampal Nrf2.	[75]
Sodium fluoride (NaF)	NaF induced ER stress and oxidative stress in mouse ameloblasts; Nrf2 was induced in a biphasic dose-dependent manner via PERK-activation.	[124]

[44,45] based on its hormetic dose response.

Neuroprotection is also induced by atypical antipsychotic drugs that display hormetic dose responses for the activation of Nrf2 [20]. This is also true for riluzone as the standard ALS treatment [21]. The activation of Nrf2 is also a key mechanism for multiple applications in ischemia reperfusion, such as with remote ischemic preconditioning for cardiac damage, stroke, and shock [60,93].

6.4. Hormesis-induced Nrf2, thresholds and response latency

How Nrf2 activation may affect cancer risk assessment is an ongoing area of discussion. Indeed, Nrf2 activation has been proposed as a mechanism by which thresholds may be experimentally identified for carcinogens [15,3,49,76]. Since the hormetic activation of Nrf2 can lead to both diminished genetic damage and a reduced inflammatory state [36,105], a framework for assessing cancer risk within an hormetic context is provided [22] via a fast-forward feedback mechanism that is predictive of a J-shaped dose response [4]. Not only does activation of Nrf2 provide a mechanistic basis and rationale for the existence of thresholds in the case of toxicants and carcinogens [3], it may also be beneficial in reducing tumor incidence below background via an hormesis-mediated response [15] as, for example, seen with DDT-induced liver cancer in the F344 rat [34], a process that now may be linked to the activation of Nrf2 by DDT [69].

These recent mechanistic developments can also affect the latency period of the toxic/carcinogenic response. The mechanistic features of Nrf2 are important in mediating hormetic dose responses that enable organisms to adapt to environmental stressors by blocking and reversing stressor-induced pathological processes, including those involved in carcinogenesis. Thus, both the hormetic activation of Nrf2 by ubiquitous environmental stressors and the associated interruption of early pathological processes together predict that low hormetic doses not only will significantly prolong the latency period of toxicants/carcinogens but may even—in the case of carcinogens—extend it beyond that of the control group, a truly protective response. Essentially, hormesis can either enhance the resiliency of cells exposed to subtoxic (subthreshold) levels of stressors, as in preconditioning experiments, or reverse the pathology of cells exposed to toxic (suprathreshold) levels of stressors, as in post-conditioning experiments. In the former preconditioning circumstance, subtoxic hormetic doses are effectively protecting cells by elevating the threshold for both carcinogens and toxicants, while in the latter post-conditioning circumstance, ongoing carcinogenic or toxic responses are being interrupted and/or reversed by subtoxic hormetic doses used as treatments. Whether hormesis is used for the prevention (altering the threshold) or treatment of diseases, however, the toxic or carcinogenic response will be delayed or effectively eliminated (i.e., delayed beyond the normal lifespan) due to an enhancement of the latency period.

7. Radiation

The role of Nrf2 in toxicity assessment and adaptive responses has an extensive literature in chemical toxicology. However, its first linkage to ionizing radiation was established by Tsukimoto et al. [100] who had previously shown [54] that a low dose of ionizing radiation (0.5 Gy) enhanced the production of GSH in RAW 264.7 mouse macrophages. They used the same cellular model and observed that the same radiation dose of 0.5 Gy enhanced the cytosolic accumulation of Nrf2 and its translocation into the nucleus, thereby evidencing the activation of Nrf2. Further experimentation by Tsukimoto et al. [100] revealed that the radiation-induced activation of Nrf2 was blocked by pretreatment with an inhibitor of the ERK1/2 pathway. The minimum induction dose that activated the translocation of Nrf2 was 0.1 Gy, a dose similar to that which increased HO-1 expression. These results indicate that low doses of radiation induce antioxidant responses via the transactivation of downstream effects following the accumulation of Nrf2 in the nucleus.

These findings were extended by Lee et al. [58] who confirmed the upregulation of Nrf2 and the enhanced expression of SOD and HO-1 in human skin fibroblast HS 27 cells following X-ray irradiation at 0.5 Gy (Fig. 26). In other studies, diabetic mice [111] and rats [118] were protected by low doses of X-rays (75 mGy) or by the same dose administered in three spaced doses of 25 mGy each. These protective

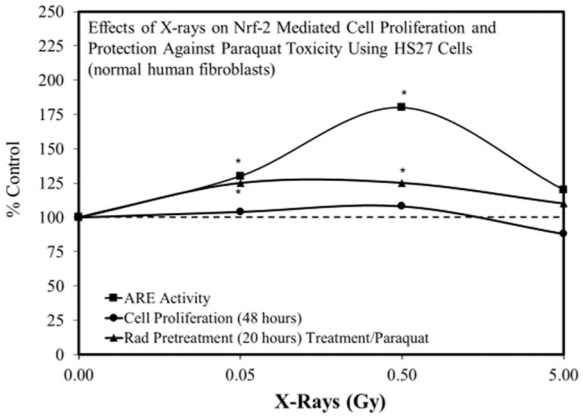

Fig. 26. Effects of X-rays on Nrf2 mediated cell proliferation and protection against paraquat toxicity using HS27 cells (normal human fibroblasts). (Modified from: [57]).

responses involved the activation of Nrf2 and the upregulation of its downstream antioxidant enzyme products. Nrf2 also upregulated BCl-2, leading to the prevention of apoptotic cell death and the reduction of diabetes-induced renal cell death. The optimized dose range was the same as that reported for numerous other radiation-induced anti-inflammatory responses in animal models and humans [17,18].

Radiation-induced adaptive responses have been reported that incorporate a fundamental role for Nrf2. Besides the above noted Nrf2-mediated upregulation of antioxidant systems, Nrf2 also has a role in upregulating DNA repair systems, as has been demonstrated in irradiated human colonic epithelia cells [52]. Nrf2 also promoted enzymatic repair of oxidative DNA damage in breast cancer [92]. Further, Nrf2 activation in irradiated U-2 OS cells enhanced cell survival along with decreasing micronucleus formation [27]. Cytoplasmic irradiation induced activation of Nrf2 and the radio-adaptive response in human lung fibroblast WI-38 cells, a response blocked by the Nrf2-selective inhibitor ML385. In that study the activation of Nrf2 diminished double-strand breaks, a protective response that was reversed also by the administration of ML385. These data extended the findings of Chen et al. [26] and Paraswani et al. [77] who demonstrated that the activation of Nrf2 was central to the radiation-induced adaptive response.

As shown by Tsukimoto et al. [100], low-dose radiation activates the radio-adaptive response via the upregulation of ERK1/2, a well-established upstream regulator of Nrf2. The increased phosphorylation of ERK1/2 was largely due to the enhanced production of superoxide by mitochondria. Suppressing the phosphorylation of ERK1/2 with the specific inhibitor U0126 blocked the adaptive response. Of note is that the study by Wang et al. [105] also revealed a similar threshold dose dependency of proton radiation for induction of the radio-adaptive effect, with no effect occurring at a low dose of 100 protons and an activation effect occurring at a higher dose of 500 protons.

8. General concepts: Nfr2 and hormesis

As has been discussed and to summarize, subthreshold (hormetic) doses of oxidative stressors activate Nrf2-mediated cytoprotective mechanisms that are powerful enough to overcompensate for the immediate oxidative threats and, in the process, engender cells with enhanced resiliency to fend off future and bigger threats. This is the hormetic framework; it is adaptive and intrinsic to all cells and organisms and protects them from environmental threats, ensuring their survival and enabling them to reproduce and participate in the natural selection process of evolution. The Nrf2-activating stressors are manifold and ubiquitous. They include endogenous ROS, electrophilic stress, caloric restriction, exercise, and ischemia-reperfusion. The Nrf2-activating stressors also include a broad spectrum of various physical, chemical and biological agents, such as toxic metals, gaseous pollutants, inorganic and organic contaminants, numerous drugs, routine dietary supplements and phytochemicals, and both ionizing and non-ionizing forms of radiation. Furthermore, Nrf2 has been studied across an array of medical disciplines (e.g., radiation biology, toxicology, pharmacology, neuroscience, immunology, and cardiology) as well as from the perspective of understanding its role in causing, treating and/or curing various diseases (e.g., neurodegenerative diseases, cardiovascular diseases, diabetes, and cancer). In this respect, a major focus of Nrf2 research has been to mobilize innate cellular defenses against the pathological effects of free radicals and pro-oxidants, with an eye toward advancing the preventive and curative options for disease treatments and for enhancing the biological performances, resiliencies, and adaptive capacities of cells and organisms, including humans. Thus, the hormetic dose-response relationship is important in defining the hormetic doses that are required for any of these stressors to activate Nrf2 and mediate the powerful cytoprotective mechanisms needed to prevent tissue damage, treat diseases, and/or enhance human performance.

Crosstalk between Nrf2 and the tumor suppressor p53 has both positive and negative feedback loops that enhance the integrity of p53 and use the p53 interaction to minimize the degradation of Nrf2 by proteosomes via Keap1 activation (Fig. 27). Under mildly stressful (hormetic) conditions, an activated Nrf2 not only induces ARE-mediated antioxidant responses but also suppresses p53 by upregulating MDM2, a protein inhibitor of p53. At such low levels, p53 then signals the upregulation of p21, a protein that stabilizes and activates Nrf2, preventing it from being degraded by proteosomes [15]. Thus, within the context of low oxidative stress, p53 and apoptosis are suppressed and Nrf2 is activated, thereby diminishing cellular stress and enhancing cell repair and survival. However, at the higher doses of oxidant stress, p53 is upregulated to levels that suppress both p21 and Nrf2, blocking the Nrf2-mediated antioxidant and pro-survival responses while activating pro-apoptotic pathways toward death.

Crosstalk also occurs between Nrf2 and Hsfl that enhances cellular adaptions and cytoprotection. For example, while activation of Nrf2 blunts oxidative and electrophilic stresses, Hsfl activation prevents protein misfolding [35]. Key to the successful mediation of adaptive responses by cells and organisms are the integrative actions of these TFs that are largely responsible for sustaining and maintaining cytoprotective and constitutive functions under a range of stress-related conditions. For example, Nrf2 and Hsfl regulate different pathways during stress conditions, such as the heat shock response by Hsfl and the Keap1/Nrf2/ARE pathways by Nrf2. However, they also share targets such as HO-1 (i.e., HSP 32), Hsp 70, activating transcript (ATF-2), and the autophagy cargo protein p62. Both also significantly impact the redox balance of cells and affect their GSH levels, with Nrf2 affecting GSH biosynthesis and its regeneration from GSSG via multiple enzymes [e.g., GPX, GR, NADPH generating enzymes, and G6PD and 6-phosphogluconate dehydrogenase (PGD)] while Hsfl affects GSH maintenance via the upregulation of G6PD. In addition, Nrf2 activation by paraquat and hydrogen peroxide also causes increased expression of proteasome activity designed to rapidly degrade mildly-oxidized proteins before they can aggregate and cross-link to form insoluble cell inclusion bodies.

Although oxidant stressor agents activate these TFs, some important functional differences among these TF activations have emerged, despite their complementarities and similarities. For example, lower levels of oxidant stress oxidize cysteine residues of Keap1 to activate Nfr2, while higher oxidant levels transform HSP 90 and HSP 72 into negative regulators of Hsfl, leading to the subsequent activation of HSP. Thus, Nrf2/Keap1 appears to be a sensitive detector of redox disequilibria that initiates early actions to defend the cell and, on the other hand, HSP is activated later if (or when) Nrf2 activation fails to reestablish rapid redox homeostasis. Interestingly, the gene coding for a later HSP70 response is integrated into the part of the ARE target of Nrf2 that would enable expression of the HSP70 gene to occur later in the temporal sequencing of the ARE target genes by Nrf2, suggesting that HSP70

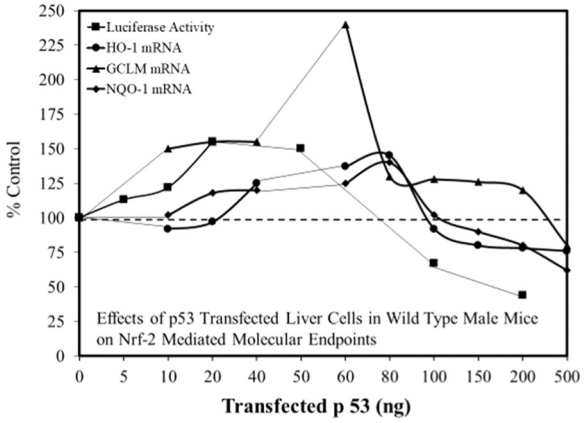

Fig. 27. Effects of p53 transfected liver cells in wild type male mice on Nrf2 mediated molecular endpoints.
(Modified from: [25]).

would indeed be a later ARE response and, presumably, not needed if redox homeostasis is quickly reestablished after initial Nrf2 activation [35].

9. Discussion

Several decades ago, hormesis was considered an anomalous and obscure event that occurred only rarely when a subthreshold dose of either a noxious chemical or ionizing radiation surprisingly elicited a stimulatory rather than an inhibitory toxic response. Since then, however, hormesis has expanded into and been recognized as a fundamental, ubiquitous, and unifying biological mechanism that can rapidly enhance resiliency and thus enable organisms to adapt quickly to a plethora of potentially lethal and non-lethal exogenous and endogenous stressors. These hormetic stressors are more than toxic chemicals and ionizing radiation, however, and include non-ionizing radiation (e.g., light and radiofrequency radiation), pressure, mechanical agitation, heat, and biological agents such as plant and microbiological irritants and toxins, (e.g., capsaicin, anthrax, botulinum, and tetanus). Stress is also produced when normal receptors and pathways are overstimulated by naturally occurring endogenous molecules (e.g., glutamate and histamine) due to pathological dysregulation or by foreign substances that mimic endogenous agonists of receptor-mediated responses, such as plant opioids. As such, the enhanced resiliency and adaptive potential of hormesis protects the individual cell or organism and helps ensure its survival, reproductive capacity, and, ultimately, participation in the evolutionary process.

Protection, however, comes at a price; it requires extra energy to ramp up metabolic pathways that defend cells against stressor agents and mitigate and repair any stress-related damage. In response to a stressor, the cell employs oxidative phosphorylation and increases the flow of electrons through mitochondrial ETS to generate a proton motive force and the extra ATP energy needed to defend and repair the cell. As more electrons leak from the ETS and are scavenged by molecular oxygen to form more ROS, the redox homeostasis begins to shift toward oxidative, which only further depletes the endogenous antioxidant protections and threatens a positive feedback loop that may lead to a vicious downward death spiral. It is the initial early rise in ROS that serves as a hormetic signal to activate Nrf2, translocate it to the nucleus, bind it to ARE, and trigger the expressions of a cascade of defensive and reparative genes, resulting in the restoration of redox homeostasis and protection of the cell from stressor agents. Although hormesis clearly requires an energy investment, low-dose radiation data indicate that if such energy investments are made continually (at regular intervals) throughout life then the activation of Nrf2 and its associated downstream events are not costly to lifespan and, on the contrary, are likely to extend lifespan by reducing the occurrence of life-shortening chronic diseases [87]. Perhaps this point is best illustrated by the life-extending benefits of exercise, a widely recognized hormetic stimulus that functions optimally when performed continually (at intervals) rather than continuously (never-ending) throughout life. Turning hormesis on-and-off in cycles may enable cells to expend energy in periodic bursts and then recharge without completely running down—much like a battery [56]. Mitochondria, redox balance, and the regulation of cellular bioenergetics are clearly fundamental to hormesis, Nrf2 activation, and, ultimately, cytoprotection and survival.

This analysis has revealed not only the hormetic activation of Nrf2 and its mediation of adaptive and cytoprotective mechanisms but also the important linkage of Nrf2 to the hormetic activation of several other TFs that also mediate cytoprotective mechanisms with adaptive consequences. For Nrf2, these mechanisms include numerous Nrf2-activated genes that encode for specific enzymes to detoxify a vast range of xenobiotics, that increase GSH synthesis, GSH conjugation, sulfate conjugation and glucuronidation, and that facilitate phase 3 processes to enhance toxicant excretion. The multifaceted aspects to Nrf2-mediated detoxification are amplified by observations that Mt1 and Mt2 genes for metallothionein expression are induced by Nrf2 [76]. The integration of Nrf2 with metallothionein affects the chelating, transporting, and excreting of toxic metals, such as arsenic, cadmium, and mercury. As discussed above, Nrf2 also conducts crosstalk with other transcription factors, such as HSF1, which mediates HSP in a dose-response manner that enhances detoxification and resiliency. Nrf2 has a similar role in framing adaptive responses to exogenously and endogenously induced endoplasmic reticulum stress [37]. For example, Nrf2 affects an adaptive response to damage induced by both age and environmental stress, as exemplified by multi-system degenerative diseases such as cataracts [31]. In numerous inflammatory conditions, Nrf2 displays an inverse regulatory relationship with the NF-kB pathway [89] that mirrors crosstalk between p53 and Nrf2 GSK-3B and leads to the activation of apoptosis to eliminate genetically damaged cells [2]. Further extending the metabolic reach of Nrf2 is the numerous plant-based and health-enhancing food supplements that activate Nrf2 to increase autophagy, a process whereby damaged organelles and proteins are processed. Nrf2-mediated autophagy enhances adaptive success by removing damaged organelles, including mitochondria. Regulatory features of Nrf2 and autophagy are also suggested as autophagic activities are inversely related to Nrf2 levels. Finally, the activation of Nrf2 provides a mechanism-based rationale for the establishment of toxicology-based thresholds for both carcinogenic and non-carcinogenic agents and stressors [3].

10. Conclusion

Stressors shift the redox homeostasis of cells toward the oxidative state and, in the process, activate Nrf2—a TF and redox sensor—to mediate a host of integrated and cytoprotective responses, including antioxidation, detoxification, anti-inflammation, autophagy, and the facilitation of mitochondrial biogenesis. The fact that hormetic (subthreshold) doses of cellular stressors generate the ROS needed to create the oxidative state and signal the activation of Nrf2 indicates that Nrf2-mediated cytoprotection and resiliency is regulated by a hormetic dose-response relationship and integrates well into the hormetic framework. Crosstalk between Nrf2 and other cytoprotective TFs further amplify the significance of Nrf2 activation in mediating a hormetic response. These actions provide the first unifying, biologically based, and integrated mechanism for explaining the generality of the hormetic dose response. That is, hormesis applies across phyla, and is independent of biological model, level of biological organization (cell to organism), endpoint measured, and inducing agent. Thus, Nrf2 activation provides a sound mechanism to explain the observed hormesis-induced enhancement in metabolic resiliency that protects cells and repairs their damage from the harmful effects of physical, chemical, and biological stressors. In looking to the future, a deeper understanding of the hormesis-mediated activation of Nrf2 and its cytoprotective mechanisms should enable the exploitation and development of hormetic dose-response strategies to apply in maintaining health, enhancing performance, delaying aging, as well as preventing and combating disease, including most chronic degenerative diseases.

Declaration of Competing Interest

The authors declare no conflict of interest.

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