ROS

RESEARCH HIGHLIGHTS

3*H*-1,2-Dithiole-3-Thione as a Potentially Novel Therapeutic Compound for Sepsis Intervention

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ABSTRACT | Through the history of modern medicine, bioactive components in natural products have been either employed directly as medicines or used as prototypes for synthetic drug development. This brief Research Highlights paper considers 3*H*-1,2-dithiole-3-thione (D3T), a member of the 1,2-dithiole-3-thiones—compounds which may naturally occur in cruciferous vegetables. Among 1,2-dithiole-3-thiones, D3T is the most potent member with regard to the capacity of inducing tissue defenses against oxidative and inflammatory stress. Oxidative and inflammatory stress is a major pathophysiological process involved in numerous human disorders, including cancer, cardiovascular diseases, neurodegeneration, and sepsis, to name just a few. This article surveys recent major research findings on D3T as an inducer of tissue antioxidative and anti-inflammatory defenses and as a potential therapeutic modality for sepsis intervention.

KEYWORDS | Anti-inflammation; Antioxidants; 3*H*-1,2-Dithiole-3-thione; NF-κB; Nrf2; Sepsis

ABBREVIATIONS | AO/AI, antioxidative and anti-inflammatory; CAT, catalase; D3T, 3*H*-1,2-dithiole-3-thione; γGCL, gamma-glutamylcysteine ligase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced form of glutathione; GST, glutathione *S*-transferase; HO-1, heme oxygenase-1; NQO1, NAD(P)H:quinone oxidoreductase 1; SOD, superoxide dismutase

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1. OVERVIEW

1,2-Dithiole-3-thiones refer to a group of chemicals that contain a five-membered cyclic sulfur structure [1]. Cruciferous vegetables, such as broccoli, and cabbage, contain sulfur compounds that render the unique smell associated with their dietary use. Dithiole-3-thiones have been suggested to occur naturally in certain cruciferous vegetables; however, it remains controversial regarding their exact natural existence and abundance in dietary cruciferous vegetables. In this context, Marks et al. proposed that dithiole-3-thiones could be formed during the processing of the cruciferous vegetables via reactions between the chemical ingredients, rather than occur naturally [2]. Since 1,2-dithiole-3-thiones possess unique biological activities as summarized below, studying the presence of those compounds in natural products via using modern sensitive analytical techniques would seem to be imperative.

Despite the controversies on their natural occurrence, a number of 1,2-dithiole-3-thione compounds have been synthesized over the past decades for studying their potential efficacy in disease intervention [1, 3]. The simplest member of this chemical class is 3*H*-1,2-dithiole-3-thione, which is conventionally abbreviated as D3T (the structure is shown in **Figure 1**). The other members may be considered as derivatives of D3T as they possess additional chemical groups. Among the available 1,2-dithiole-3-thiones, oltipraz is the only United States Food and Drug Administration (FDA)-approved drug, which is indicated for treating Schistosomiasis. Early work by Kensler and others demonstrated cancer chemoprotective properties of oltipraz in experimental animals [4]. However, the clinical trial on oltipraz in cancer chemoprotection was not successful, due, partly, to its unacceptable adverse effects [5]. Except for oltipraz, none of the other 1,2-dithiole-3-thione compounds has entered clinical trials. Their biological activities have been solely investigated in experimental animals and in vitro systems over the past two decades, and D3T has recently emerged as a novel member that potently induces cellular antioxidative and anti-inflammatory (AO/AI) defenses and affords protection in a variety of disease models. In fact, with regard to induction of AO/AI enzymes, such as glutathione *S*-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NOQ1), D3T was shown to be the most potent chemical inducer among the available 1,2-dithiole-3-thione compounds [4, 6–9].

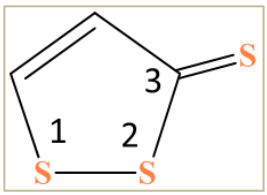

FIGURE 1. Chemical structure of 3*H*-1,2-dithiole-3-thione (D3T). The italic letter *H* indicates the locant of the added hydrogen before substitution. As shown, before substitution by S, there are 2 hydrogens (with one being called the added hydrogen) at position 3. Hence, 3*H* denotes the locant of that initial "added hydrogen" before S substitution—i.e., being at the position 3.

2. REGULATION OF AO/AI GENES BY D3T IN MULTIPLE ORGANS

Studies in our laboratories and others over the past decades have shown that D3T, among diverse 1,2-dithiole-3-thiones, is not only the most potent inducer of AO/AI defenses in cells and tissues, but also an effective inducer of such defenses in multiple organs/tissues. These include cardiovascular tissues and cells, bone marrow and immune cells, neuronal tissues and cells, renal tissue and cells, hepatic tissue and cells, and gastrointestinal tissues and cells. Among the most notable AO/AI defenses (including genes, enzymes/proteins, and non-protein molecules) induced by D3T are superoxide dismutase (SOD), catalase (CAT), reduced form of glutathione (GSH), gamma-glutamylcysteine ligase (γGCL), glutathione

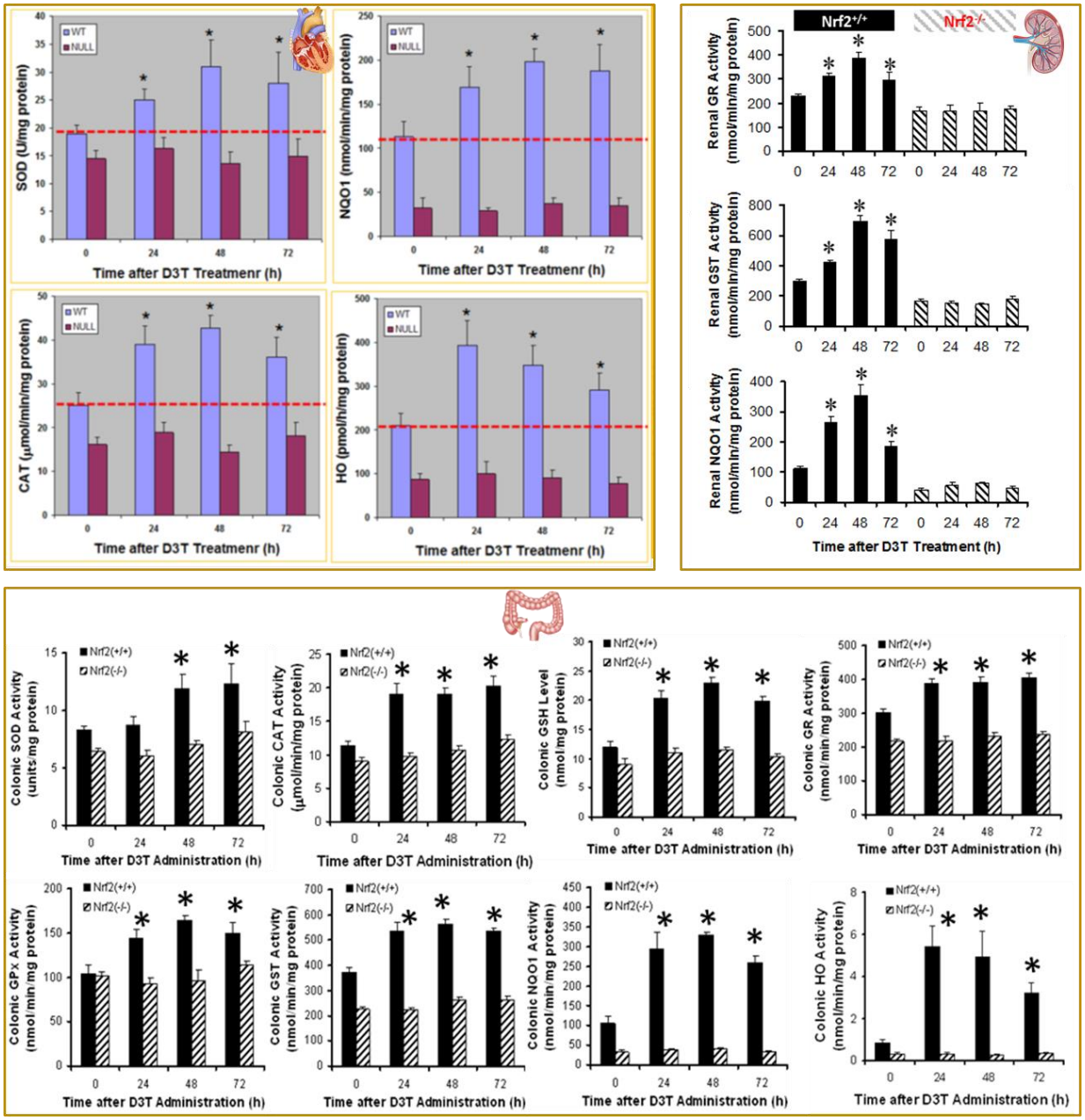

FIGURE 2. Sustained induction of tissue antioxidative and ant-inflammatory (AO/AI) defenses by a single oral dose of D3T (0.3 mmol/kg) in male C57BL/6 mice. As shown, the induction of the AO/AI defenses lasts for at least 3 days following the single oral dosage of D3T in wild-type mice, and no significant induction is seen with Nrf2-null mice. Data represent mean ± SE (n = 8–10); *, $p < 0.05$ vs. no D3T treatment.

peroxidase (GPx), glutathione reductase (GR), GST, NQO1, and heme oxygenase-1 (HO-1) [4, 6–9] (some in vivo data are shown in **Figure 2**). While the induction of the above AO/AI defenses by D3T oc-

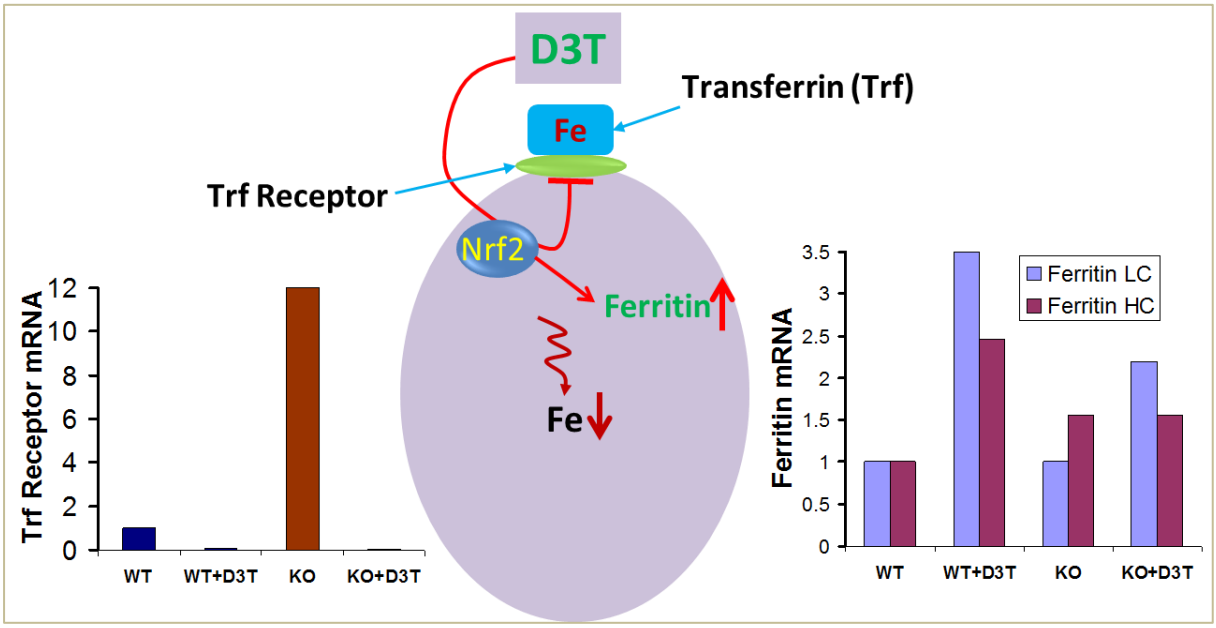

FIGURE 3. Modulation of myocardial ferritin and transferrin receptor mRNA by a single oral dose of D3T (0.3 mmol/kg). Gene expression profiling was carried out on RNA samples isolated from 3 male mice per group 24 h after D3T administration. Data represent the average values from 4–6 separate probes. WT and KO denote wide-type and Nrf2-null C57BL/6 mice, respectively.

curs primarily via an Nrf2-dependent mechanism, D3T also increases the expression of many other AO/AI genes independent of Nrf2 signaling [10–12]. Moreover, D3T treatment also results in decreased expression of a number of genes encoding proteins involved in cell immunity and inflammation (unpublished gene profiling data).

Most notably, Nrf2 status controls the basal expression of transferrin receptor, and D3T, via upregulating ferritin and downregulating transferrin receptor, appears to reduce tissue load of redox-active iron, a key factor in sepsis (unpublished data) This D3T effect is largely uninfluenced by the Nrf2 status (**Figure 3**). The exact signaling mechanisms by which D3T modulates cellular iron homeostasis remain to be elucidated. Moreover, Nrf2 may not function solely as a regulator of AO/AI genes. Indeed, studies show that Nrf2 signaling plays a critical role in tissue homeostasis (e.g., tissue repair and immune modulation) independent of its well-known function—as a transcriptional activator of cellular antioxidant genes [13, 14].

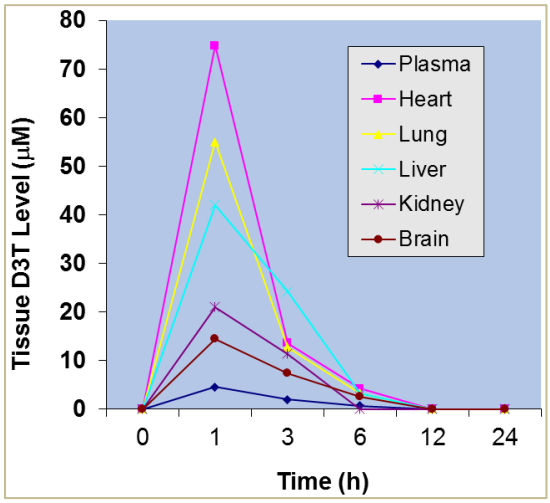

FIGURE 4. Plasma and tissue levels of D3T following a single oral dose of D3T (0.3 mmol) in C57BL/6 mice. The D3T levels were measured by GC-MASS spectrometry and the data represent the average from 6 mice per group.

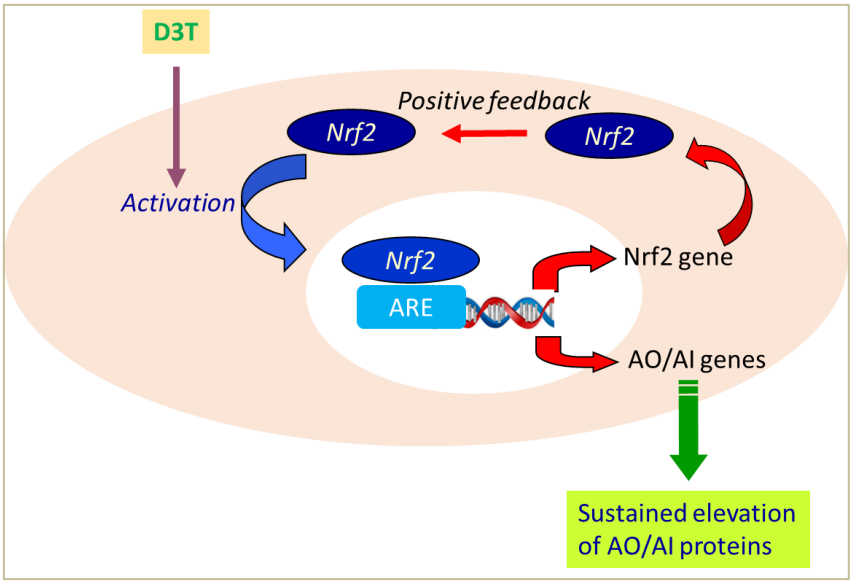

FIGURE 5. D3T-induced Nrf2-mediated Nrf2 gene expression. As illustrated, D3T causes activation of Nrf2 (nuclear translocation of Nrf2 and binding to the antioxidant response element [ARE] in the promoter region of the Nrf2 gene itself as well as many AO/AI genes). This leads to increased expression of AO/AI genes as well as Nrf2 gene itself. Increased Nrf2 gene expression results in increased production of Nrf2 protein, which can in turn amplify the Nrf2 signaling, leading to sustained elevation of tissue AO/AI defenses.

2.1. Sustained Induction of AO/AI Defenses and the Positive Feedback Loop of Nrf2 Signaling

A single oral dose of D3T results in induction of diverse AO/AI defenses that last for at least 3 days following D3T administration (**Figure 2**). This also occurs in cultured cells [15]. Pharmacokinetic studies show a rapid absorption of D3T after oral administration and also a rapid distribution to organs, including the heart, lungs, brain, liver, and kidneys. The peak concentrations in the above organs occur at 1 h after oral D3T administration and can reach from 15 to 75 μM depending on the types of the tissues. Notably, the levels of D3T in the above organs decrease rapidly and become undetectable after 12 h (**Figure 4**). Hence, the sustained induction of tissue AO/AI defenses is unlikely due to the direct effects of D3T in the organs. In cultured cells, D3T is found to not only activate Nrf2 but also increase its mRNA and protein expression [16]. This increased Nrf2 level would form a positive feedback loop, leading to prolonged activation of Nrf2-regulated AO/AI genes. Indeed, the promoter region of Nrf2 gene contains an ARE-like element, which may mediate Nrf2-induced Nrf2 gene expression [17] (**Figure 5**).

2.2. Sustained Induction of AO/AI Defenses and D3T Pharmacokinetics

As noted above, D3T levels in multiple organs are much higher than those in the plasma after oral administration (**Figure 4**), suggesting its selective tissue accumulation. This is particularly obvious in the heart, lungs, and liver. Although the exact molecular basis for tissue accumulation of D3T remains unclear, the relatively high levels of D3T in various organs apparently account for the induction of AO/AI defenses in these organs. This induction of multiorgan AO/AI defenses has important implications in the intervention of diseases that affect multiorgan functions, such as sepsis. Although direct measurement of D3T levels in the colon tissue was not done, a single dose of oral D3T also leads to remarkable sustained induction of colonic AO/AI defenses (**Figure 2**). As gut failure is a crucial pathophysiological component of sepsis [18], induction of the colonic

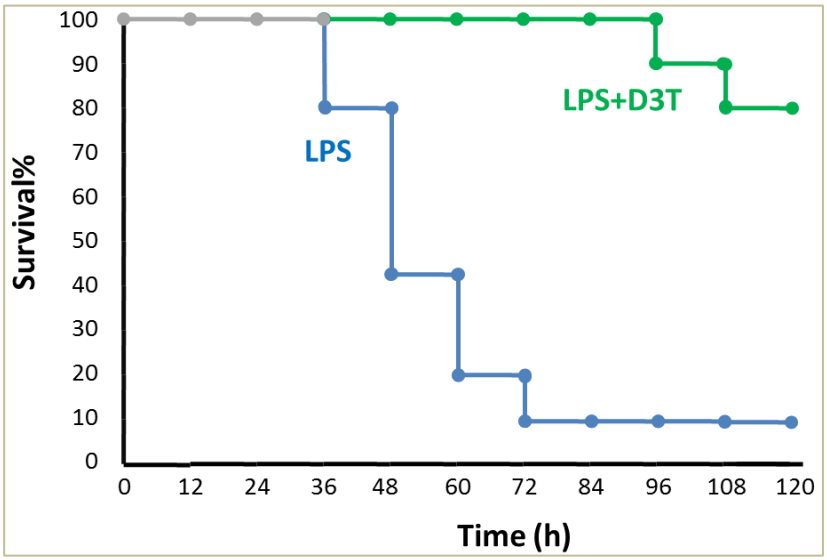

FIGURE 6. Effects of D3T on LPS-induced mortality in male C57BL/6 mice. A single oral dose (0.3 mmol/kg) of D3T was given 10 min after intraperitoneal injection of LPS (7.5 mg/kg) to the mice (10 mice per group).

AO/AI defenses by D3T may in turn play a critical role in protecting against sepsis.

3. D3T AS A POTENTIALLY NOVEL THERAPEUTIC MODALITY IN SEPSIS

3.1. Therapeutic Activity in Endotoxin-Induced Sepsis

The coordinated sustained induction of a number of AO/AI defenses in multiple organs by oral D3T has prompted us to determine if D3T can protect against experimental sepsis. As shown in **Figure 6**, a single oral administration of D3T 10 min after intraperitoneal injection of lipopolysaccharide (LPS) dramatically protected against LPS-induced mortality in C57BL/6 mice. To our surprise, treatment with sulforaphane or oltipraz, two commonly studied chemoprotective compounds, did not provide any protection against LPS-induced mortality in mice under our experimental conditions (unpublished data). The importance of the above observations lies in the ability of a single dose of D3T given after LPS exposure to nearly completely block LPS-induced mortality up to 4 days.

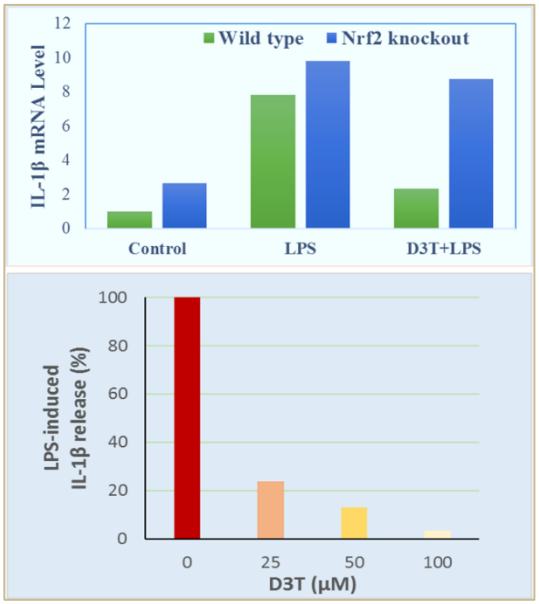

FIGURE 7. Effects of D3T on LPS-induced IL-1β expression in splenocytes (top) and RAW 264.7 macrophages (bottom). The cells were pretreated with D3T (100 μM [top] and 25–100 μM [bottom] for 24 h) followed by exposure to LPS (5 ng/ml for 1 h [top] and 10 ng/ml for 24 h [bottom]).

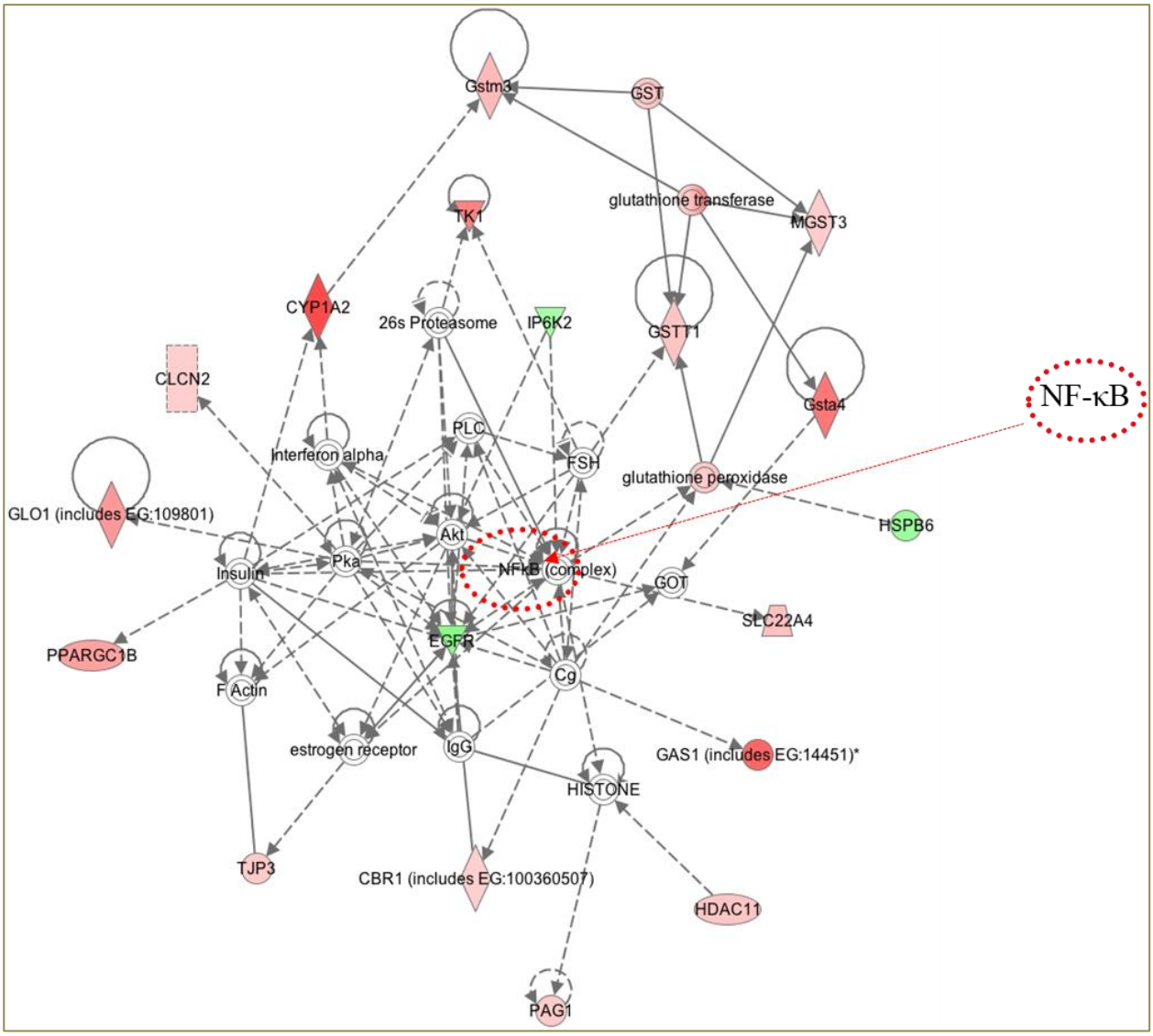

FIGURE 8. Effects of D3T on gene profiling in mice. Ingenuity pathway analysis shows impact of D3T on NF-κB pathway in the liver of male C57BL/6 mice 24 h after a single oral dose of D3T (0.3 mmol/kg).

It is imperative to study the maximal time delay in D3T administration following LPS exposure for D3T to still provide protection.

3.2. Modulation by D3T of Novel Genes Involved in Sepsis: Involvement of the NF-κB Pathway

Our studies also showed a dramatic inhibition by D3T of LPS-induced expression of pro-inflammatory cytokines (e.g., IL-1β) in inflammatory cells, including macrophages and splenocytes (**Figure 7**). In line with this finding, the Ingenuity Pathway Analysis of multiorgan (including the liver and heart) gene profiling following D3T treatment of Nrf2-null and wide-type mice revealed a drastic indirect suppression of NF-κB pathway by D3T/Nrf2 signaling (**Figure 8**), a chief machinery for pro-inflammatory gene activation and a critical player in sepsis [19–21]. This,

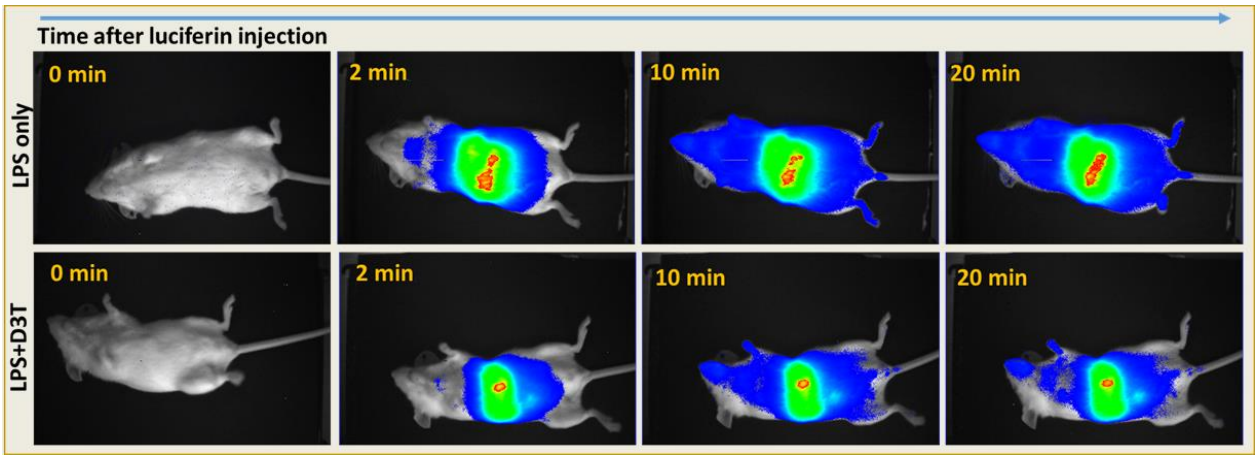

FIGURE 9. Representative in vivo bioluminescence imaging of LPS-induced NF-κB activation in male NFκB-RE-luc BALB/c mice and the effects of D3T treatment. Imaging was performed 6 h after treating the mice with LPS (0.25 mg/30 g) and oral D3T (0.3 mmol/kg). Reproduced from Ref. [22], with permission of the Cell Med Press, AIMSCI Inc.

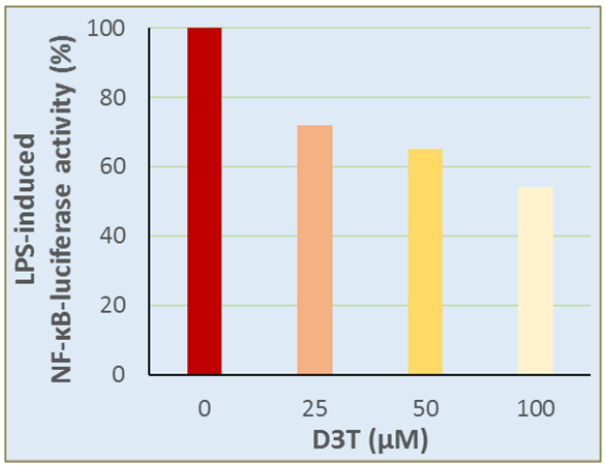

FIGURE 10. Effects of D3T treatment on LPS-induced NF-κB-luciferase activity in mouse RAW 264.7 macrophages constitutively expressing an NF-κB response element-luciferase reporter gene construct. The cells were pretreated with D3T at the indicated concentrations for 24 h followed by exposure to LPS (10 ng/ml) for an additional 6 h. Data represent averages from two separate experiments.

for the first time, suggested a potential interaction between Nrf2 activation and NF-κB suppression in vivo. Using NF-κB-response element-luciferase transgenic mice, our studies further showed a significant (> 65%) attenuation, by oral D3T treatment, of LPS-induced NF-κB activation in live mice [22] (**Figure 9**). In cultured macrophages, D3T attenuated LPS-induced NF-κB activation in a concentration-dependent manner (**Figure 10**).

While how exactly D3T suppresses NF-κB pathway remains to be determined, several possibilities exist. First, this may occur via D3T-mediated increased expression of cellular antioxidants. In this context, redox-sensitive NF-κB can be suppressed by antioxidants [23]. Second, D3T may suppress NF-κB pathway via activation of Nrf2, a factor that has been recently discovered to downregulate NF-κB [24]. Third, D3T may directly interact with NF-κB, rendering its inactivation. Indeed, two analogs of 5-[p-methoxyphenyl]-1,2-dithiole-3-thione were shown to inhibit NF-κB via direct covalent modification [25]. In this regard, D3T was also found to directly react with cellular thiols [26].

4. CONCLUSION AND PERSPECTIVES

D3T appears to act as a novel activator of multiorgan AO/AI defenses primarily via Nrf2 signaling. D3T not only activates Nrf2, but also upregulates its ex-

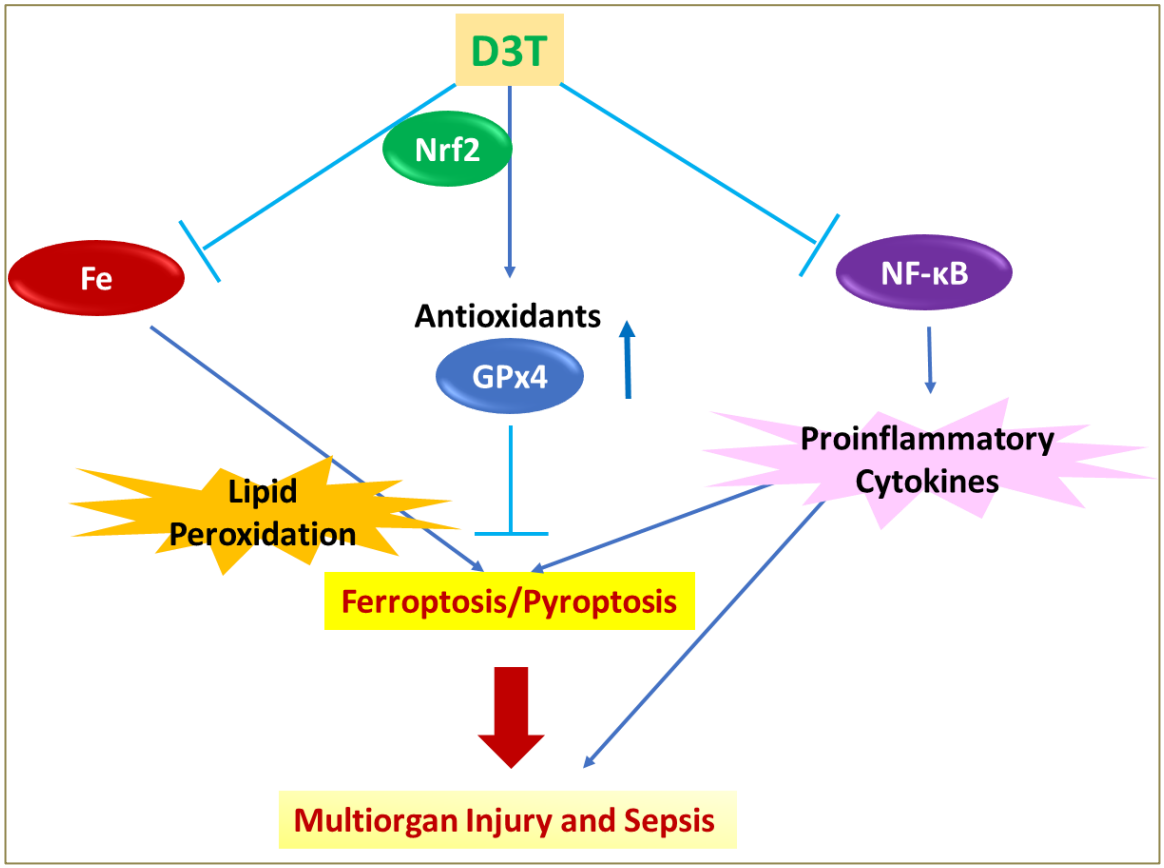

FIGURE 11. Proposed mechanisms by which D3T protects against sepsis. As illustrated, D3T is a multi-tasking compound that counteracts sepsis via modulating multiple pathophysiological pathways of sepsis.

pression. The sustained induction of tissue AO/AI defenses by D3T likely results from its rapid absorption and accumulation in multiple organs as well as the upregulation of Nrf2 expression and the subsequent positive feedback activation of Nrf2 signaling. A single oral D3T shortly after LPS injection nearly completely blocks LPS-induced mortality in mice. Mechanistically, D3T suppresses LPS-induced proinflammatory cytokine gene expression in an Nrf2-dependent manner in inflammatory cells and such suppression stems, at least partly, from D3T-mediated inhibition of the NF-κB pathway. In addition, D3T-mediated upregulation of cellular antioxidants, particularly GPx4 [9], and downregulation of cellular iron may also lead to suppression of ferroptosis and pyroptosis, two critical pathophysiological events in sepsis [27] (**Figure 11**).

ACKNOWLEDGMENTS

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