ECRONICON OPEN ACCESS

EC PHARMACOLOGY AND TOXICOLOGY
Research Article

3H-1,2-Dithiole-3-Thione Reduces Lipopolysaccharide-Induced Liver and Kidney Inflammation in C57BL/6 Mice

Gabriella Gaje[1], Jimaree Legins[1], Jaziah Rispress[1], Y Robert Li[2], Hong Zhu[2]* and Zhenquan Jia[1]*

[1]*Department of Biology, University of North Carolina at Greensboro, NC, USA*

[2]*Campbell University, School of Osteopathic Medicine, Buies Creek, NC, USA*

***Corresponding Author**: Zhenquan Jia, Professor, Department of Biology, The University of North Carolina at Greensboro, Greensboro, NC, USA and Hong Zhu, Professor, Campbell University School of Osteopathic Medicine, Buies Creek, NC, USA.

Received: October 06, 2021; **Published:** December 30, 2021

Abstract

Lipopolysaccharide (LPS) is a bacterial endotoxin known for being critical in triggering septic shock. It is widely used for studies to understand systemic inflammation that mimics the inflammatory storm of human sepsis patients. 3H-1,2-dithiole-3-thione (D3T) is a common dithiolethione that works as an chemotherapeutic agent in cancers. However, it is unknown whether D3T can reduce the LPS-induced expression of inflammatory and oxidative stress responses in liver and kidney tissues. This study aimed to examine the effect of D3T on LPS-induced expressions of oxidative and inflammatory genes *in vivo*. C57BL/6 mice trials demonstrated that the administration of LPS showed a significant increase in expression of two key pro-inflammatory genes interleukin-6 (IL-6) and monocyte chemoattractant protein (MCP-1) in in both liver and kidney tissues. D3T significantly reduced the LPS-induced gene expression of MCP-1 and IL-6, in mouse kidney tissue. Similarly, D3T mitigated the LPS-induced gene expression of MCP-1 in mouse liver tissue. Mouse liver tissue treated with D3T showed significant increases in the antioxidant gene expression of glutamate cysteine ligase modifier subunit (GCLM) and heme oxygenase-1 (HO-1). The results indicate that D3T has an anti-inflammatory effect in vivo. In conclusion, the results of the current study could contribute to D3T as a potential therapeutic agent to reducing the inflammatory responses caused by the LPS endotoxin *in vivo*.

Keywords: *Lipopolysaccharide (LPS); Endotoxin; Liver; Kidney Tissues; Inflammation; 3H-1,2-Dithiole-3-Thione*

Introduction

Sepsis is defined as being a storm of various abnormalities induced in an organism by infection. Sepsis has many physiological, pathological, and biochemical effects on the body [1,2]. In sepsis patients, it appears that the failure of one organ will often lead to the failure of other subsequent organs. This can occur through a multitude of processes, including inter-organ crosstalk, where one organ's dysfunction influences the function of another [3].

Lipopolysaccharides (LPS, also termed endotoxin), is found in the walls of gram-negative bacteria and has an established role in pro-inflammatory pathways. It is known that inflammatory cytokines and chemokines such as tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) can contribute to the underlying pathologies of sepsis [4]. Larger doses of LPS are necessary to induce septic shock and pro-inflammatory cytokines in the rodent model. LPS can induce hypothermia and cause decreased cardiac output with increased peripheral vascular resistance. Additionally, the effects of increased cytokine levels can happen relatively quickly, with high doses of LPS administration. In general, LPS is a reliable model for its convenience and ease of reproducibility. It is able to be measured reliably and can be easily standardized. Overall, the LPS induced rodent model has more long-standing physiological responses, which makes it ideal to study [5].

3H-1,2-dithiole-3-thione, also known as D3T, is a common dithiolethione [6]. This compound is known for its chemoprotective property that also codes for phase II and antioxidant enzymes. The cytoprotective effects of D3T include the upregulation of phase II enzymes during the Keap-1/Nrf2/ARE pathway as seen in a study with zebrafish embryos [6]. D3T has been heavily indicated in protecting cortical neurons *in vitro* from the formation of hyperoxidized peroxiredoxins after oxidative trauma occurs. A study of Alzheimer's disease in a mouse model demonstrated that the administration of D3T led to the amelioration of oxidative stress and improved hippocampal neurogenesis [7]. As previously mentioned, LPS, a bacterial endotoxin, is known for triggering septic shock. However, it is unknown if D3T can reduce LPS-induced inflammatory and oxidative responses.

Aim of the Study

This study aimed to o determine whether or not D3T can be used to mitigate the effects of LPS-induced liver and kidney inflammation in C57BL/6 mice.

Materials and Methods

Materials

Trizol RNA extraction agents were purchased from MP Biomedicals (Solon, OH). Chloroform used for RNA isolation, ethanol and lipopolysaccharide from *Escherichia coli* were purchased from Sigma-Aldrich (Sheboygan Falls, WI). Isopropanol that was used in RNA isolation, was bought from Alfa Aesar (Ward Hill, MA). cDNA synthesis materials including nuclease-free water, MMLV-RT, 5X buffer and random primer were purchased from Invitrogen (Waltham, MA). Deoxynucleoside triphosphate used for cDNA synthesis was purchased from New England Biolabs (Ipswich, MA). Mouse forward and reverse primers used for qRT-PCR were bought from Eurofins Genomics (Louisville, KY). SYBR Green fluorescent dye and MicroAmp® Fast 96-well reaction plates were purchased from Applied Biosystems by Thermo Fisher Scientific (Waltham, MA).

Animal study

The *in vivo* study was used to analyze LPS-induced inflammatory and oxidative stress in mice and whether D3T treatment could ameliorate this oxidative and inflammatory response. Animal protocol No. 010517-ZHU-1 was used for this experiment. 8 to 10-week male C57BL/6 mice from Charles River Laboratories (Wilmington, MA) were utilized in this experiment. This strain is known to be the most widely used strain in biomedical and toxicology research [8]. This strain is known to be the most widely used strain in biomedical and toxicology research [8]. Mice were maintained in a humane environment at the Campbell University vivarium compliant with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. This *in vivo* study was approved by the Campbell University Institutional Animal Care and Use Committee (IACUC). Mice were fed an AIN-93G diet (BioSey, NJ) as a control for confounding variables. 4 groups of mice were generated (control, LPS, D3T, and LPS + D3T) with 4 mice randomly placed into each group. The diet was given to every mouse for one week before the treatments. D3T was prepared and administered by gastric gavage at 0.3 mmol/kg body weight for 24 hours followed by the administration of LPS via intraperitoneal injection at 7.5 mg/kg body weight for 15 hours. Oral gavage has been proven to be an accurate method of dosing mice by delivering a substance directly to the stomach, which can then be absorbed almost immediately [9].

Following treatments, Mouse euthanasia was performed to obtain organ tissue and plasma samples. Organs were stored at -80°C and transferred to UNCG. Mouse liver and kidney organ tissue samples were obtained to analyze the anti-oxidative/anti-inflammatory gene expression as well as pro-inflammatory cytokine expression levels. The pro-inflammatory cytokine gene expression of IL-6 and MCP-1 was measured via qRT-PCR following RNA extraction of liver and kidney samples. RNA was extracted from liver and kidney samples to measure gene expression of glutamate-cysteine ligase catalytic subunit (GCLC), glutamate cysteine ligase modifier subunit (GCLM), nuclear factor-erythroid factor 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1).

RNA isolation

In order to prepare for RNA isolation of the tissues, frozen liver and kidney samples were processed and weighed on ice to avoid thaw-

ing. Tissue samples weighing 50 - 75 mg were homogenized in 1 mL of Trizol reagent using a PRO Scientific PRO250 homogenizer (Oxford, CT). The volume of the sample could not exceed 10% of the Trizol reagent volume that was used for homogenization. The solution was pipetted into 1 mL Eppendorf tubes. 200 µL of chloroform was added, followed by agitation and then centrifuged at 12,000 rcf for 15 minutes. The top aqueous phase was transferred to another set of 1 mL Eppendorf tubes and combined with 500 µL isopropanol before centrifuging again at 12,000 rcf for 10 minutes. The resulting pellet (RNA) was washed with 1 mL 75% ethanol and centrifuged at 7400 rcf for two intervals of 5 minutes each. The pellet was air-dried for 8 - 10 minutes then resuspended in 10 - 20 µL of DEPC H_2O.

cDNA synthesis

RNA concentrations were measured by taking 1 µL of the sample and measuring it in the Nanodrop UV-Vis spectrophotometer. The RNA concentrations were diluted to 500 ng/µL. A reverse transcriptase was used to synthesize cDNA from the 500 ng/mLRNA. cDNA reagents include DEPC-treated water, 5x first strand buffer, deoxynucleotide triphosphate (dNTP) solution, Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) and random primers in addition to 2 µL of RNA. Using the Applied Biosystems™ Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA), the 25 µL solution was converted to cDNA.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Once the cDNA was synthesized, target genes such as IL-6, GCLM, GCLC, HO-1, NRF2 and TNF-α were used compared to the expression levels of the constitutive gene of GAPDH for cell samples and the housekeeping gene of beta-actin for tissue samples. These target genes were created using forward and reverse primers. cDNA and target gene mixtures were placed in a clear 96-well plate prior to being synthesized. The StepOnePlus Real-Time PCR system ran for 40 cycles at 95°C for 15 seconds, 58°C for 1 minute and 60°C for 15 seconds. Gene expression was quantified by using comparative threshold values.

Statistical analysis

GraphPad Prism® software was used for statistical analyses and the results are expressed as mean ± S.E.M. Student t-tests were performed to obtain statistical significance (P value). Changes were considered significant when $P < 0.05$.

Mouse primer sequence

Results

D3T has previously been shown to provide protection against inflammation in various cancers, but it is unclear if it shares similar

Target Gene	Forward Primer	Reverse Primer
B-Actin	5'- CTT CCT TCC TGG GTA TGG AAT C-3'	5'- CCA GGA TAG AGC CAC CAA TC-3'
IL-6	5'- CTGCAAGAGACTTCCATCCAGTT- 3'	5'- AGGGAAGGCCGTGGTTGT- 3'
MCP-1	5'- TTCCTCCACCACCATGCAG- 3'	5'- CCAGCCGGCAACTGTGA- 3'
GCLC	5'- TGGAGCAGCTGTATCAGTGG- 3'	5'- TGGCACATTGATGACAACCT- 3'
GCLM	5'- AACACAGACCCAACCCAGAG- 3'	5'- ATCCTGGGCTTCAATGTCAG- 3'
HO-1	5'- CACGCATATACCCGCTACCT- 3'	5'- CCAGAGTGTTCATTCGAGCA- 3'
CCL2	5'- GTC TCA GCC AGA TGC AGT TAA T-3'	5'- GCT GAA GTC CTT AGG GTT GAT G-3'

properties when used with the endotoxin LPS *in vivo*. The role of D3T was examined in C57/BL6 mice that were treated with LPS, D3T, or a cotreatment of LPS and D3T. Both liver and kidney tissue were analyzed via qRT-PCR following RNA isolation and cDNA synthesis of the tissue. There was 185-fold change of MCP-1 pro-inflammatory gene expression in LPS treated mice compared to control ($p < 0.05$) but only a 1-fold change of LPS+D3T treated mice (Figure 1A), suggesting that there was an anti-inflammatory effect of D3T in the *in vivo* treatment. As shown in figure 1B, the kidney tissue of D3T treated mice showed a statistically significant decrease in LPS-induced IL-6 pro-inflammatory gene expression compared to LPS group ($p < 0.05$).

Antioxidant genes GCLM, GCLC, HO-1 and NRF2 were measured in the mice kidney tissues with no statistically significant changes

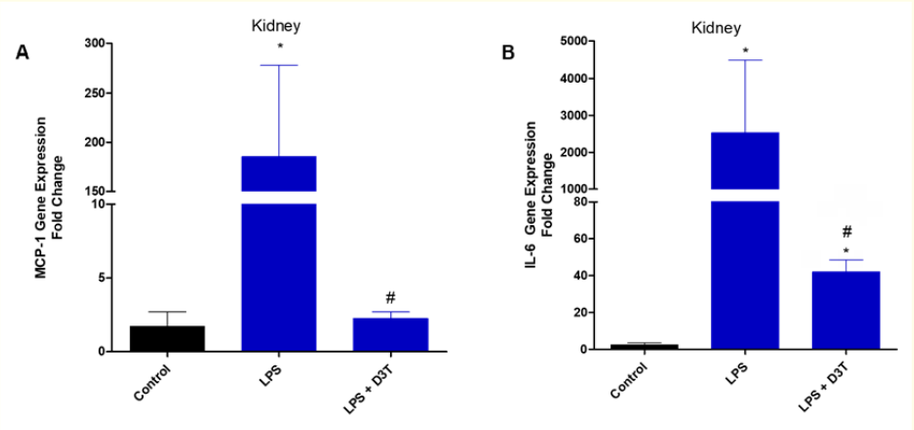


Figure 1: D3T reduced expressions of pro-inflammatory genes (MCP-1 and IL-6) in kidney tissue in LPS treated mice. Exposure to LPS in the presence and absence of D3T to the C57BL/6 mice was described in Methods. The relative mRNA of each above gene was evaluated by real-time PCR and data were normalized using beta -Actin. Values are mean ± SEM, n = 3-4. *, $p < 0.05$ vs. control; #, $p < 0.05$ vs. LPS alone-treated mice.

between control and D3T treated mice ($p < 0.05$) although all gene expressions were shown to have a decrease in D3T treated mice compared to control (Figure 2).

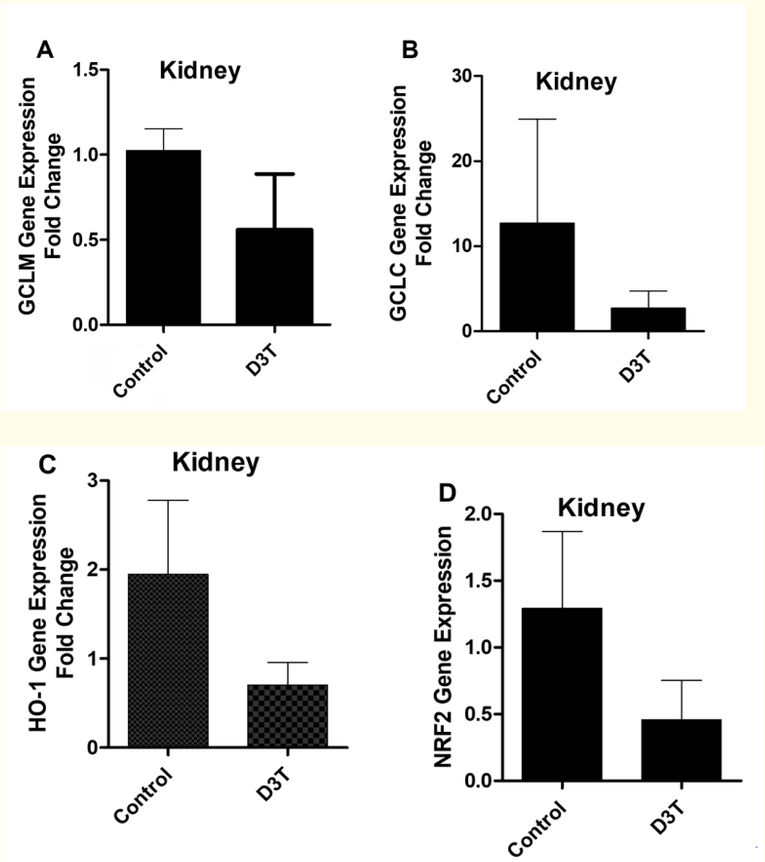


Figure 2: Effect of D3T on expressions of anti-inflammatory/antioxidant genes (GCLM, HO-1, GCLC, NRF2) in kidney tissues. The relative mRNA of each above gene was evaluated by real-time PCR and data were normalized using beta-Actin. The values are shown mean ± SEM, n = 4.

As shown in figure 3A, the liver tissue showed an approximate statistically significant 300-fold change between control and LPS for pro-inflammatory gene expression of MCP-1 ($p < 0.05$). The treatment of D3T caused a significant inhibition of LPS-induced MCP-1 gene expression in liver tissues ($P < 0.05$). This is consistent with the inhibitory effects seen on MCP-1 in kidney tissues. However, the addition of D3T did not affect the LPS-induced gene expression of IL-6 in liver tissue (Figure 3B).

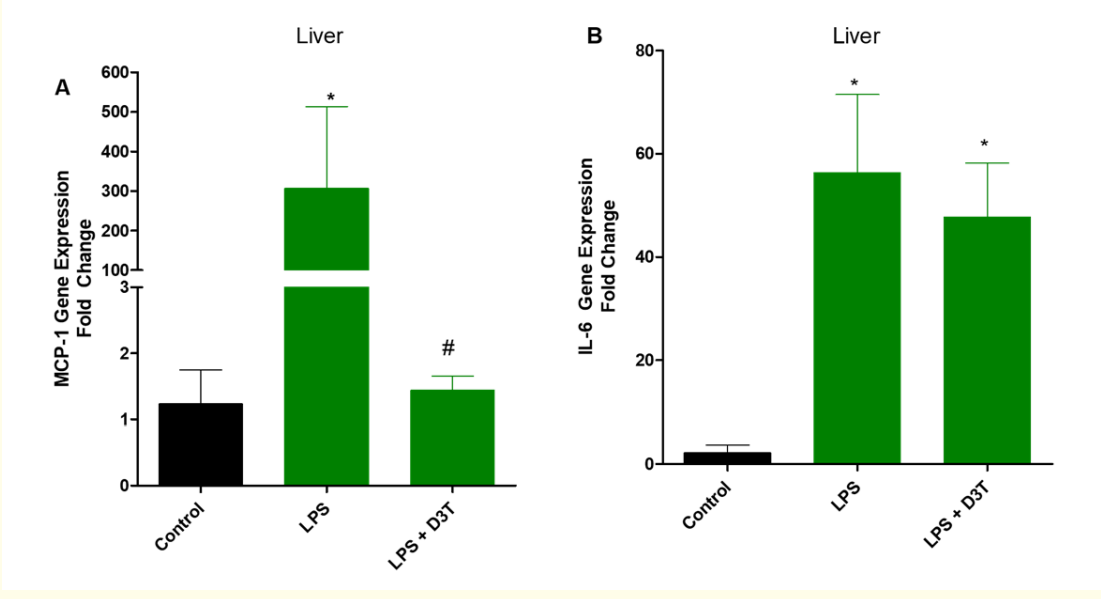
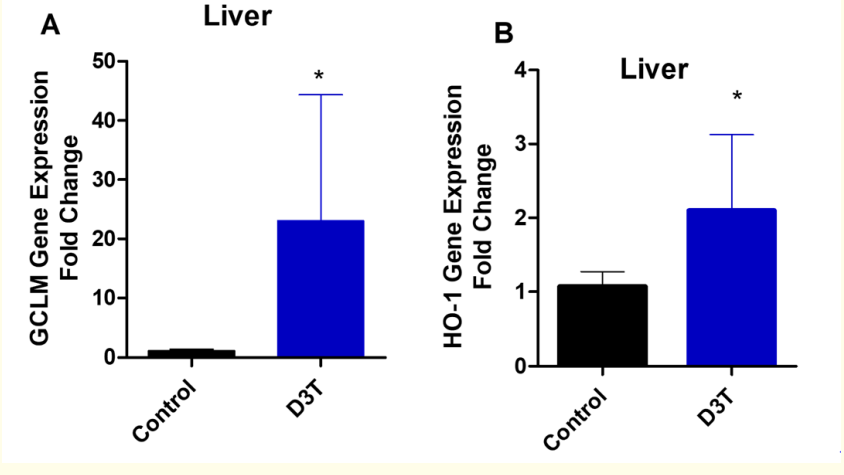


Figure 3: Effect of D3T on expressions of pro-inflammatory genes (MCP-1 and IL-6) in liver tissues of mice treated with LPS. The relative mRNA of each above gene was evaluated by real-time PCR and data were normalized using beta -Actin. Values are mean ± SEM, n = 4. *, $p < 0.05$ vs. control; #, $p < 0.05$ vs. LPS alone-treated mice.

Antioxidant genes GCLM, GCLC, HO-1 and NRF2 were measured in mice liver tissue. The expression of GCLM and HO-1 show a significant increase when treated with D3T and compared to control (Figure 4A and 4B). While there was an increase in the gene expression of GCLC (Figure 4C) and a decrease in Nrf2 gene expression (Figure 4D), these changes were not statistically significant ($p < 0.05$) when compared to control.



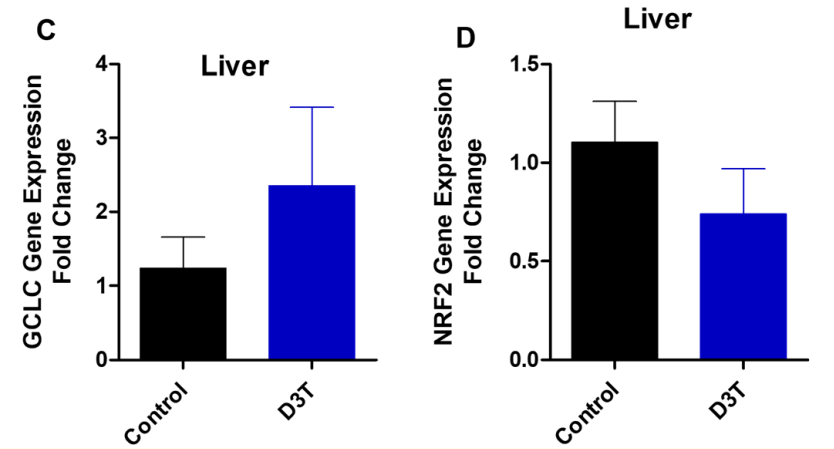


Figure 4: Effect of D3T on expressions of anti-inflammatory/antioxidant genes (GCLM, HO-1, GCLC, NRF2) in liver tissues. The relative mRNA of each above gene was evaluated by real-time PCR and data were normalized using beta-Actin. Values are mean ± SEM, n = 4.

Discussion

Lipopolysaccharide (LPS) is an endotoxin found in the walls of gram-negative bacteria. The compound has previously been associated with its capabilities of inducing inflammation by stimulating cells to produce pro-inflammatory cytokines [10,11]. For this reason, LPS has been a long-standing model to simulate inflammation-mediated sepsis both *in vivo* and *in vitro* [10,11]. However, there have been no specific studies exploring the effect of D3T on LPS-induced inflammatory injuries. This study aimed to examine the effect of D3T on LPS-induced expressions of anti-oxidative and anti-inflammatory genes *in vivo.* The *in vivo* mice trials indicated that D3T mitigated LPS-induced gene expressions of two notable pro-inflammatory cytokines, MCP-1 and IL-6, in the kidney tissues. D3T-treated mice showed a decrease in the expression of MCP-1 in the liver tissue, which correlates to the inhibitory effects of the MCP-1 gene expression in kidney tissue. The results are indicative that D3T has an anti-inflammatory effect *in vivo*. Liver tissue showed significant increases in the antioxidant gene expression of GCLM and HO-1 by D3T, but kidney tissue did not.

Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemokine known for its potency and recruitment of monocytes. MCP-1 is produced following the induction of oxidative stress and has been seen in the pathologies of many inflammatory diseases such as sepsis, cancer and HIV. MCP-1/CCL2 has long been studied as it was one of the first discovered chemokines [12-14]. The gene for MCP-1 is located on chromosome 17 and can be induced by a variety of factors such as TNF-α. The binding of MCP-1 to coupled G-protein receptors on leukocytes activates cellular reactions, including intracellular calcium release. MCP-1 recruits monocytes during infection when bound to its receptor CCR2. Infection can cause MCP-1 to promote the migration of inflammatory monocytes from bone marrow to blood in circulation [15]. The present studies show an over 300-fold-increase in MCP-1 gene expression in mice liver tissue treated with LPS and a 185-fold-increase in the mice kidney tissues when compared to control. The treatment of D3T in kidney tissue eradicated the LPS-induced gene expression of MCP-1 by 185 times to the same levels as control. Similarly seen in the liver tissue was a 300 times decrease to the LPS-induced gene expression of MCP-1 with the administration of D3T. Such results indicate that D3T can potentially reduce inflammation by targeting and inhibiting MCP-1 gene expression.

Interleukin-6 (IL-6) is known as a regulator to transit from neutrophil to monocyte recruitment during inflammation [16-19]. This cytokine travels through the bloodstream to the liver following inflammation. This movement induces rapid gene expression of acute phase

proteins such as serum amyloid A (SAA). The rapid proliferation of the phase proteins can lead to chronic inflammatory pathologies due to the generation of amyloid A amyloidosis, eventually causing organ failure [16-19]. IL-6 is also known to promote specified differentiation for naïve CD4 T-cells. This works to link the innate to acquired immune response. There is a variety of responses to IL-6 that are found in other cell types besides hepatocytes and lymphocytes that can contribute to chronic inflammatory diseases. Its ultimate function is to work as a mediator of notification when an emergent event occurs, such as infection. The results of this study displayed that IL-6 gene expression in mice liver tissue was increased by more than 50 times with the administration of LPS compared to control mice. IL-6 gene expression in mice kidney tissue increased by more than 2500 times when compared to control mice. The treatment of D3T inhibited IL-6 gene expression induced by LPS in kidney tissue from 2500 times to 40 times. Despite these findings, there was no effect from D3T on the IL-6 gene expression in liver tissue of mice co-treated with LPS. Such results indicate that inhibition of IL-6 gene expression by D3T may be specific to each organ.

Nuclear factor erythroid 2-related factor 2 (Nrf2) has been shown in previous studies to have downstream upregulations of anti-inflammatory and anti-oxidative genes such as heme oxygenase-1 (HO-1) and GCL that are pivotal to the downregulation of NF- κB [20,21]. It has been shown that Nrf2 can interfere with NF κB for p-50 translocation to the nucleus and the inhibition of Nrf2 activity can be a target in inflammation therapy [20,21]. HO-1 contains cytoprotective qualities, such as antioxidant and anti-inflammatory characteristics, which can reduce inflammation, apoptosis and cellular distress [22,23]. Glutathione (GSH) is a protective antioxidant in various organs and possesses anti-inflammatory properties. GSH synthesis from constituent amino acids requires an ATP-dependent enzyme, γ-glutamylcysteine ligase (GCL) [24,25]. The heterodimer GCL is a rate limiting enzyme in the pathway and contains a catalytic (GCLC) and modulatory (GCLM) subunit. The previously mentioned Nrf2 acts as a transcription factor in the cell's cytosol and is isolated by keap1. Nrf2 works with the antioxidant response element (ARE) to activate the expression of anti-oxidative and anti-inflammatory genes such as GCLM and GCLC [26,27]. The results of this study showed that D3T treatment leads to a significant increase in the gene expression of GCLM and HO-1 in liver tissue indicating that activation of Nrf2 maybe be a potential pathway for D3T to protect against LPS-induced inflammation *in vivo*. Future studies are needed to examine the relationship between NF- κB and Nrf2 to determine whether the activated Nrf2 pathway occurs *in vivo* with the administration of D3T. Such a study could further explain how D3T contributes to the decrease of LPS-induced pro-inflammatory gene expression.

Conclusion

In summary, the results of this study demonstrated that D3T mitigated the LPS-induced gene expression of key pro-inflammatory cytokines, MCP-1 and IL-6, in mouse kidney tissue. D3T treated mice also showed a decrease in the LPS-induced gene expression of MCP-1 in mouse liver tissue. The mouse liver tissue from D3T-treated mice showed significant increases in GCLC and HO-1 antioxidant gene expression. These results are indicative that D3T has an anti-inflammatory effect *in vivo.* The results of this study may contribute to the use of D3T being a potential alternative to reduce inflammatory responses caused by endotoxin LPS.

Funding Support

The work was supported in part by grants from the U.S. National Institutes of Health (GM124652-01A1).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Bibliography

1. Mervyn Singer M., *et al*. "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)" (2016).

2. Faix. "Established and novel biomarkers of sepsis". *Biomarkers in Medicine* (2011): 117-130.

3. Prauchner CA. "Oxidative stress in sepsis: Pathophysiological implications justifying antioxidant co-therapy". *Burns* 43.3 (2017): 471-485.

4. Devi Ramnath R., *et al*. "Inflammatory mediators in sepsis: Cytokines, chemokines, adhesion molecules and gases". *Journal of Organ Dysfunction* 2.2 (2006): 80-92.

5. Nemzek JA., *et al*. "Modeling Sepsis in the Laboratory: Merging Sound Science with Animal Well-Being". *Comparative Medicine* 58.2 (2008): 120-128.

6. Wible RS., *et al*. "Pharmacogenomics of Chemically Distinct Classes of Keap1-Nrf2 Activators Identify Common and Unique Gene, Protein, and Pathway Responses In Vivo". *Molecular Pharmacology* 93.4 (2018): 297-308.

7. Cui Y., *et al*. "Pharmacological activation of the Nrf2 pathway by 3H-1, 2-dithiole-3-thione is neuroprotective in a mouse model of Alzheimer disease". *Behavioural Brain Research* 336 (2018): 219-226.

8. Åhlgren J and V Voikar. "Experiments done in Black-6 mice: what does it mean?" *Lab Animal* 48.6 (2019): 171-180.

9. Larcombe AN., *et al*. "Confounding Effects of Gavage in Mice: Impaired Respiratory Structure and Function". *American Journal of Respiratory Cell and Molecular Biology* 61.6 (2019): 791-794.

10. Brooks D., *et al*. "Human lipopolysaccharide models provide mechanistic and therapeutic insights into systemic and pulmonary inflammation". *European Respiratory Journal* 56.1 (2020).

11. Brinkworth JF and N Valizadegan. "Sepsis and the evolution of human increased sensitivity to lipopolysaccharide". *Evolutionary Anthropology* 30.2 (2021): 141-157.

12. Deshmane SL., *et al*. "Monocyte chemoattractant protein-1 (MCP-1): an overview". *Journal of Interferon and Cytokine Research* 29.6 (2009): 313-326.

13. Zhu S., *et al*. "The molecular structure and role of CCL2 (MCP-1) and C-C chemokine receptor CCR2 in skeletal biology and diseases". *Journal of Cellular Physiology* (2021).

14. Dommel S and M Blüher. "Does C-C Motif Chemokine Ligand 2 (CCL2) Link Obesity to a Pro-Inflammatory State?" *International Journal of Molecular Sciences* 22.3 (2021).

15. Yadav A., *et al*. "MCP-1: chemoattractant with a role beyond immunity: a review". *Clinica Chimica Acta* 411.21-22 (2010): 1570-1579.

16. Tanaka T., *et al*. "IL-6 in inflammation, immunity, and disease". *Cold Spring Harbor Perspectives in Biology* 6.10 (2014): a016295.

17. Li L., *et al*. "Effects of immune cells and cytokines on inflammation and immunosuppression in the tumor microenvironment". *International Immunopharmacology* 88 (2020): 106939.

18. Koschmieder S and N Chatain. "Role of inflammation in the biology of myeloproliferative neoplasms". *Blood Reviews* 42 (2020): 100711.

19. Jahandideh B., *et al*. "The pro-Inflammatory cytokines effects on mobilization, self-renewal and differentiation of hematopoietic stem cells". *Human Immunology* 81.5 (2020): 206-217.

20. El-Shitany NA and BG Eid. "Icariin modulates carrageenan-induced acute inflammation through HO-1/Nrf2 and NF-kB signaling pathways". *Biomedicine and Pharmacotherapy* 120 (2019): 109567.

21. Rubio V., *et al*. "Different roles of Nrf2 and NFKB in the antioxidant imbalance produced by esculetin or quercetin on NB4 leukemia cells". *Chemico-Biological Interactions* 294 (2018): 158-166.

22. Araujo JA., *et al*. "Heme oxygenase-1, oxidation, inflammation, and atherosclerosis". *Frontiers in Pharmacology* 3 (2012): 119.

23. Choi AM and J Alam. "Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury". *American Journal of Respiratory Cell and Molecular Biology* 15.1 (1996): 9-19.

24. Franklin CC., *et al*. "Structure, function, and post-translational regulation of the catalytic and modifier subunits of glutamate cysteine ligase". *Molecular Aspects of Medicine* 30.1-2 (2009): 86-98.

25. Chen Y., *et al*. "Glutathione defense mechanism in liver injury: insights from animal models". *Food and Chemical Toxicology* 60 (2013): 38-44.

26. Syed AM., *et al*. "A review on herbal Nrf2 activators with preclinical evidence in cardiovascular diseases". *Phytotherapy Research* (2021).

27. Song MY., *et al*. "The Role of NRF2/KEAP1 Signaling Pathway in Cancer Metabolism". *International Journal of Molecular Sciences* 22.9 (2021).

Volume 10 Issue 1 January 2022