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Novel fluorinated derivative of curcumin negatively regulates thioredoxin-interacting protein expression in retinal pigment epithelial and macrophage cells

Undral Buyandelger, Douglas Gordon Walker*, Hiroyasu Taguchi, Daijiro Yanagisawa, Ikuo Tooyama**

Molecular Neuroscience Research Center, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu, Shiga, 520-2192, Japan

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ABSTRACT

Thioredoxin-interacting protein (TXNIP) has multiple disease-associated functions including inducing oxidative stress by inhibiting the anti-oxidant and thiol reducing activity of thioredoxin (TRX), reducing cellular glucose transport, and is a component of the activated inflammasome complex. Increased expression of TXNIP is encountered in diabetic conditions of high glucose. Curcumin and chemical derivatives have multiple therapeutic properties as anti-inflammatories, anti-oxidants, amyloid aggregation inhibitors and modulate a number of cellular signaling pathways. Using a fluorinated-derivative of curcumin (designated Shiga-Y6), we showed significant inhibition of TXNIP mRNA and protein expression, and induction of TRX mRNA and protein in ARPE-19 retinal pigment epithelial cells and THP-1-derived macrophages, while the non-fluorinated structural equivalent (Shiga-Y52) and native curcumin did not show these same effects. Shiga-Y6 was effective in reducing high glucose, endoplasmic reticulum stress-induced TXNIP in ARPE-19 cells, and reducing lipopolysaccharide and endoplasmic stress-induced proinflammatory gene expression in THP-1 macrophages. Moreover, TXNIP-knockdown experiments showed that the anti-inflammatory effect of Shiga-Y6 in LPS-stimulated THP-1 macrophages was TXNIP-independent.

1. Introduction

Oxidative stress is a major feature of many different degenerative diseases and results from an imbalance in production of reactive oxygen species (ROS) and deficits in cellular anti-oxidant systems. Thioredoxin-interacting protein (TXNIP), also designated as thioredoxin-binding protein-2 (TBP-2), an α-arrestin family member, was identified as a vitamin D3-induced gene but recent findings have indicated it is a central regulator of glucose homeostasis and diabetes, and involved in vascular endothelial dysfunctions associated with diabetes [1–3]. Increased levels of TXNIP promote cellular oxidative stress by binding to the catalytic center of reduced thioredoxin (TRX), a significant cellular anti-oxidant protein, and inhibiting its oxidant scavenging and thiol reducing activity [4]. Oxidative stress, inflammation and endoplasmic reticulum (ER) stress are involved in the development of diabetes-related pathologies [5]. Increased expression of TXNIP occurs in conditions of high glucose, which can result in pancreatic β cell apoptosis, the primary cause of type-1 diabetes. TXNIP also functions as a tumor suppressor and inhibits proliferation via activation of apoptosis signal regulating kinase 1 (ASK1) [6]. Loss of TXNIP expression occurs in a number of different cancers and correlates with poor outcome [7,8]. TXNIP is also directly involved in inflammatory activation through its interaction with the nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3) inflammasome complex resulting in increased procaspase-1 activity and interleukin-1 release [9]. Modulation of TXNIP expression and activity is being investigated as a therapeutic strategy for diseases, particularly those related to diabetes [2].

Curcumin is a polyphenolic compound derived from the curry spice turmeric. The native compound and chemically-modified derivatives have properties as anti-oxidants, anti-inflammatories, anti-diabetes, anti-tumor agents and amyloid and tau aggregation

* Corresponding author.
** Corresponding author.
E-mail addresses: walker@belle.shiga-med.ac.jp (D.G. Walker), kinchan@belle.shiga-med.ac.jp (I. Tooyama).

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inhibitors, and been widely tested for disease treatments. The properties of curcumin and derivatives for treatment of diabetes and Alzheimer's disease are considered in recent reviews [10,11]. Multiple cellular signaling pathways in different cell types can be affected by curcumin and related compounds, but at present due to its low bioavailability in humans, native curcumin has shown limited efficacy *in vivo*, and this has highlighted the need for development of modified derivatives. We have produced a number of curcumin derivatives based on their binding and inhibition of aggregation of amyloid β peptides [12]. In addition, curcumin with fluorine derivatives on the phenolic groups have proven useful as ligands for magnetic resonance imaging (MRI) of Aβ deposition in animal models [13]. In this study, we investigated other biological properties of curcumin and our novel derivatives. We report that a fluorinated modified curcumin (designated Shiga-Y6) showed significantly greater activity in inhibiting TXNIP expression and enhancing TRX expression in cellular models of diabetes, endoplasmic reticulum stress and inflammation, while possessing the anti-inflammatory properties of native curcumin. This agent may have utility for treating oxidative stress and inflammation −related pathologies occurring in diabetes.

2. Materials and methods

2.1. Cell culture and reagents

The ARPE-19 retinal pigment epithelial (RPE) and THP-1 monocytic leukemia cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). ARPE-19 cells were cultured in DMEM/F12 (Nacalai-Tesque, Kyoto, Japan) supplemented with 10% fetal bovine serum (FBS) and 50 μg/mL gentamicin. For experimental purposes, cells were plated at 2.5×10^5 cells/well in 12-well plates. Cells were pretreated in media with 1% FBS with or without curcumin (Wako Chemicals, Japan), Shiga-Y52 (SY52) or Shiga-Y6 (SY6) for 2 h followed by these treatments: (1) 5 mM glucose containing unmodified DMEM/F12 media (designated low glucose − LG), or media supplemented with glucose to final concentrations of 25–100 mM (high glucose − HG); or (2) ER stress inducer thapsigargin (TG) for 24 h (500 nM − ARPE-19 cells; 300 nM − THP-1 macrophages) treatments. Curcumin and derivatives were maintained in media for the duration of all experiments. Shiga-Y52 (1,7-bis(4′-hydroxy-3′methoxy)phenyl −1,6- heptadiene-3,5-dione) and Shiga-Y6 (1,7-bis(4′-hydroxy-3′trifluoromethoxy) phenyl −1,6- heptadiene-3,5-dione) curcumin derivatives were synthesized and purified in-house according to modifications of our published protocols [14]. Each reagent was dissolved in dimethyl sulfoxide (DMSO) prior to dilution for use in experiments.

THP-1 monocytic cells were cultured in suspension using RPMI 1640 media supplemented with 10% FBS and gentamicin. For experimental purposes, the cells were plated at 5×10^5 cells/well in 12-well plates in RPMI media with 1% FBS and exposed to 50 ng/mL phorbol myristate acetate (PMA) for 2 days to induce differentiation to adherent macrophage—like cells and then incubated with RPMI media with 1% FBS for 24 h to allow functional recovery. THP-1-derived macrophages were pretreated as described above with curcumin and derivatives and then with lipopolysaccharide (LPS from E. Coli 0157) for 6 h and 24 h, or TG (300 nM) for 24 h. Doses were selected based on cell viability/toxicity results. After treatments, cells were extracted for RNA or protein analysis, and culture media collected for ELISA analysis.

2.2. Antibodies and immunoblot analyses

Antibodies to TXNIP, TRX and β-actin were obtained from Abcam. Immunoblot analyses were carried out as previously described [15]. Due to the large separation in molecular weights, most immunoblots for TXNIP and TRX were analyzed by combining antibodies and detecting simultaneously but with different optimal exposures for quantification.

2.3. TXNIP gene silencing by small interfering RNA (siRNA)

Differentiated THP-1 macrophages at 5×10^5 cells/well in 12-well plates were transfected with 10 nmol/L TXNIP siRNA, sense -5′- GAAGAUCACCGAUUGGAGATT-3'; anti-sense -5′- UCUCCAAUCGGUGAUCUUCAG-3' (s20880; Ambion, MA, USA) or Stealth RNAi negative control (Invitrogen, Medium GC, sequence not available) using Lipofectamine RNAiMAX reagent (Invitrogen) according to the manufacturer's instruction. After transfection, some cells were treated with TG (300 nM) or LPS (100 ng/mL) as described above. TXNIP knockdown efficiency was evaluated by real-time PCR and immunoblots.

2.4. Gene expression analysis

ARPE-19 and THP-1-derived macrophages were processed for total RNA extraction using RNeasy-Plus Mini kit (Qiagen, Germany). Complementary DNAs were prepared from total cellular RNA using the PrimeScript RT kit with genomic DNA eraser (Takara Bio, Japan). Quantitative PCR (qPCR) analysis of gene expression was carried out using THUNDERBIRD SYBR Green qPCR Mix (Toyobo, Japan) as previously described [15]. Gene specific primers used are listed in Supplemental Table 1.

2.5. Enzyme-linked immunosorbent assay (ELISA)

The concentrations of chemokine ligand 2 (CCL2) and tumor necrosis factor-α (TNF-α) were measured in supernatants of control or treated cells using Human CCL2/MCP-1 (R&D Systems DY279) and TNF-α (DY210) ELISA development kits.

2.6. Cell viability assay

Indices of cell viability were determined using a mitochondrial activity WST-1 reagent (Cell Count Reagent; Nacalai-Tesque). Target cells (ARPE-19 or THP-1) were plated at 5×10^4 cells/well in 96-well microtiter plates. Cells were treated for 24 h with curcumin and curcumin derivatives. Cell viability reagent (10 μL) was added to each well for 4 h, and then the resulting color change measured at 450 nm wavelength. Cell viability was calculated as percent changes relative to DMSO-treated or control media-treated control cells.

2.7. Measurement of intracellular reactive oxygen species (ROS)

Intracellular ROS was measured using the Cellular ROS Assay Kit (ab113851, Abcam) according to the manufacturer's protocol. Briefly, ARPE-19 cells plated in 96 well-plates and treated with or without 5 μM curcumin, Shiga-Y52 or Shiga-Y6 compounds in medium with glucose (5 mM) or high glucose (25 mM) followed by incubation with 25 μM 2′,7' −dichlorofluorescin diacetate (DCFDA) reagent for 45 min at 37 °C prior to reading absorbance.

2.8. Statistical analysis

All experiments were performed in triplicate. All results are expressed as mean ± S.E.M. Statistical analyses were performed using GraphPad Prism 7 software. Experimental data were analyzed by one-way ANOVA followed by Dunnet and Sidak's test of multiple comparisons between groups. $p < 0.05$ was considered

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to be significant. The following designations are used on graphs to indicate p values: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

3. Results

3.1. Fluorinated curcumin derivative Shiga-Y6 reduced expression of TXNIP and increased expression of TRX

Two experimental cellular models (ARPE-19 and THP-1) were employed to investigate the effects of curcumin and two derivatives on TXNIP and TRX expression, oxidative stress and inflammation markers. We sought to determine if TXNIP was a key coordinating factor for cellular responses and how this system was affected by curcumin and our novel derivatives. The only differences between SY6 and SY52 is the presence of fluorine atoms on the 3′- methoxy group in the phenol ring. Both compounds have an alkyl side chain with carboxyl group at the C-4 position of curcumin (Fig. 1A). Optimal doses of each agent were identified using a cell viability assay. It was determined that a 5 μM dose for subsequent experiments would allow comparison between compounds. The responses differed between cell types but at low dose a stimulating effect was observed for the curcumin derivatives (ARPE-19 Fig. 1B; THP-1 Fig. 1C). SY6 strongly inhibited expression of TXNIP mRNA (Fig. 1D) and protein (Fig. 1E), while enhancing transcription of TRX mRNA (Fig. 1F) and protein levels (Fig. 1G). Similar responses were observed in both cell lines. Curcumin and SY52 did not significantly affect TXNIP expression, but had a stimulating effect on TRX expression (Fig. 1E, mRNA; Fig. 1G, protein), but with noticeably reduced effects compared to SY6. Representative western blots are shown for each cell type (ARPE-19 - Fig. 1H: THP-1 – Fig. 1I).

3.2. Different effects of Shiga-Y6 on RPE cells between high glucose and ER stress conditions

Using high glucose in media (25 mM, 50 mM and 100 mM compared to 5 mM normal media glucose concentration (LG)), a stimulating effect on mitochondrial activity measured in the cell viability assay was observed in SY6 compared to curcumin-treated ARPE-19 cells (Fig. 2A). High glucose treatments resulted in reduced cell viability but there were no significant dose-dependent differences between 25 mM, 50 mM and 100 mM doses. In subsequent experiments, 25 mM dose was used as the high glucose condition. As expected, high glucose resulted in significantly increased levels of ROS, which was inhibited by curcumin but not by SY6 treatment (Fig. 2B). Representative western blot panels for TXNIP and TRX for both treatments are shown (Fig. 2C–D). SY6 was effective at reducing high glucose mediated induction of TXNIP mRNA (Fig. 2E) and protein (Fig. 2F), and inducing TRX mRNA (Fig. 2G) and protein (Fig. 2H), while curcumin and SY52 were not. Using a strong cellular ER stressor (TG – 500 nM), which induces ER stress by blocking sarco/endoplasmic reticulum Ca2+ ATPase (SERCA) activity resulting in higher intracellular calcium, increased TXNIP mRNA (Fig. 2I) and protein (Fig. 2J), and increased TRX mRNA (Fig. 2K) and protein (Fig. 2L) were measured. It was noticeable that TG treatment alone strongly induced TXNIP and TRX expression, while SY6 treatment strongly reversed TXNIP induction but further induced TRX levels. These figures show that curcumin and SY52

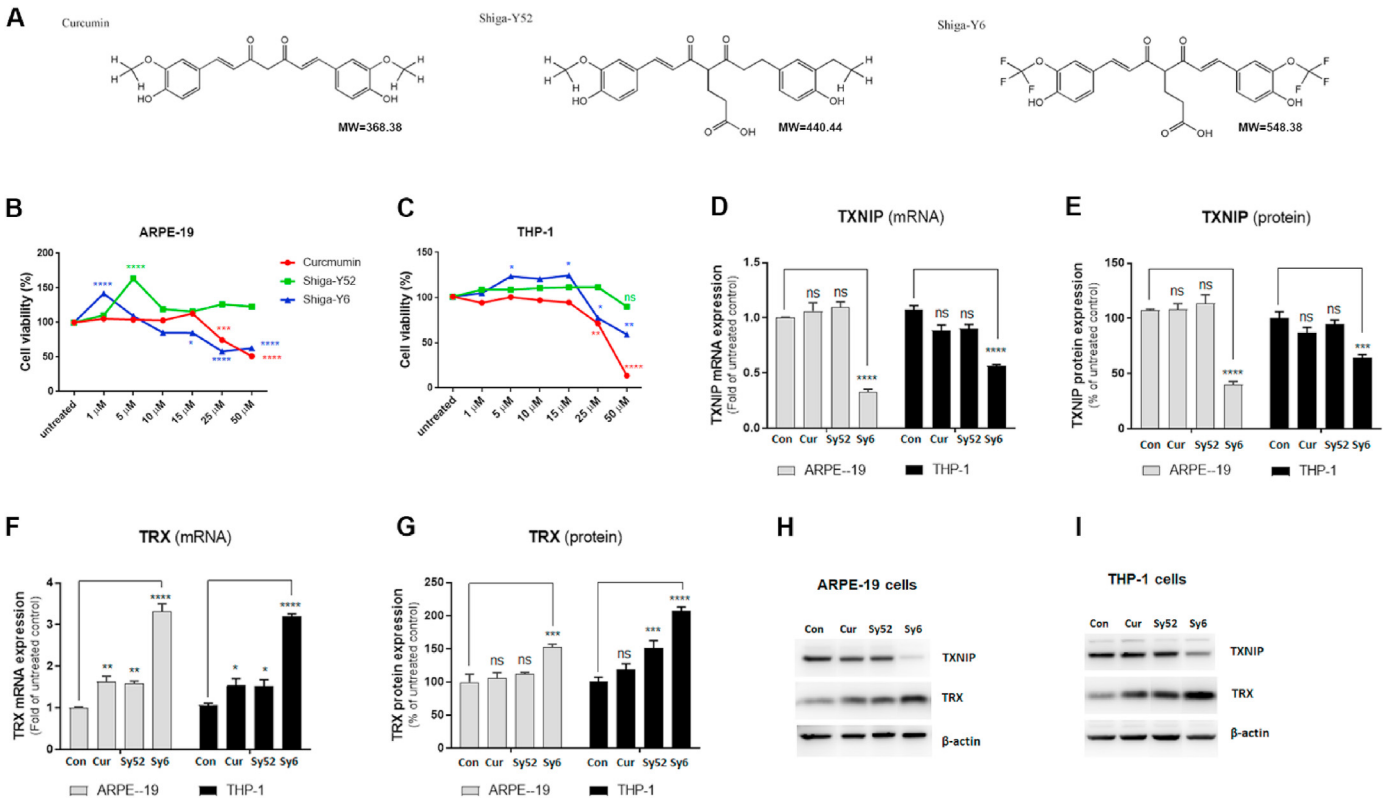

Fig. 1. Regulation of TXNIP and TRX expression by curcumin and derivatives in ARPE-19 cells and THP-1 macrophages.
(A) Chemical structures of curcumin and derivatives SY52 and SY6. **(B)** Dose-response effects of curcumin and derivatives on cell viability of ARPE-19 cells and **(C)** THP-1-derived macrophages. **(D–G)** Effects of curcumin and derivatives (5 μM dose) on TXNIP mRNA **(D)**; on TXNIP protein levels **(E)**; on TRX mRNA levels (**F**); and on TRX protein levels in ARPE-19 cells and THP-1 macrophages (**G**). **(H)** Representative immunoblots for TXNIP and TRX in control and treated ARPE-19 cells and **(I)** THP-1 macrophages. Data analysis and significance symbols as described in Methods.

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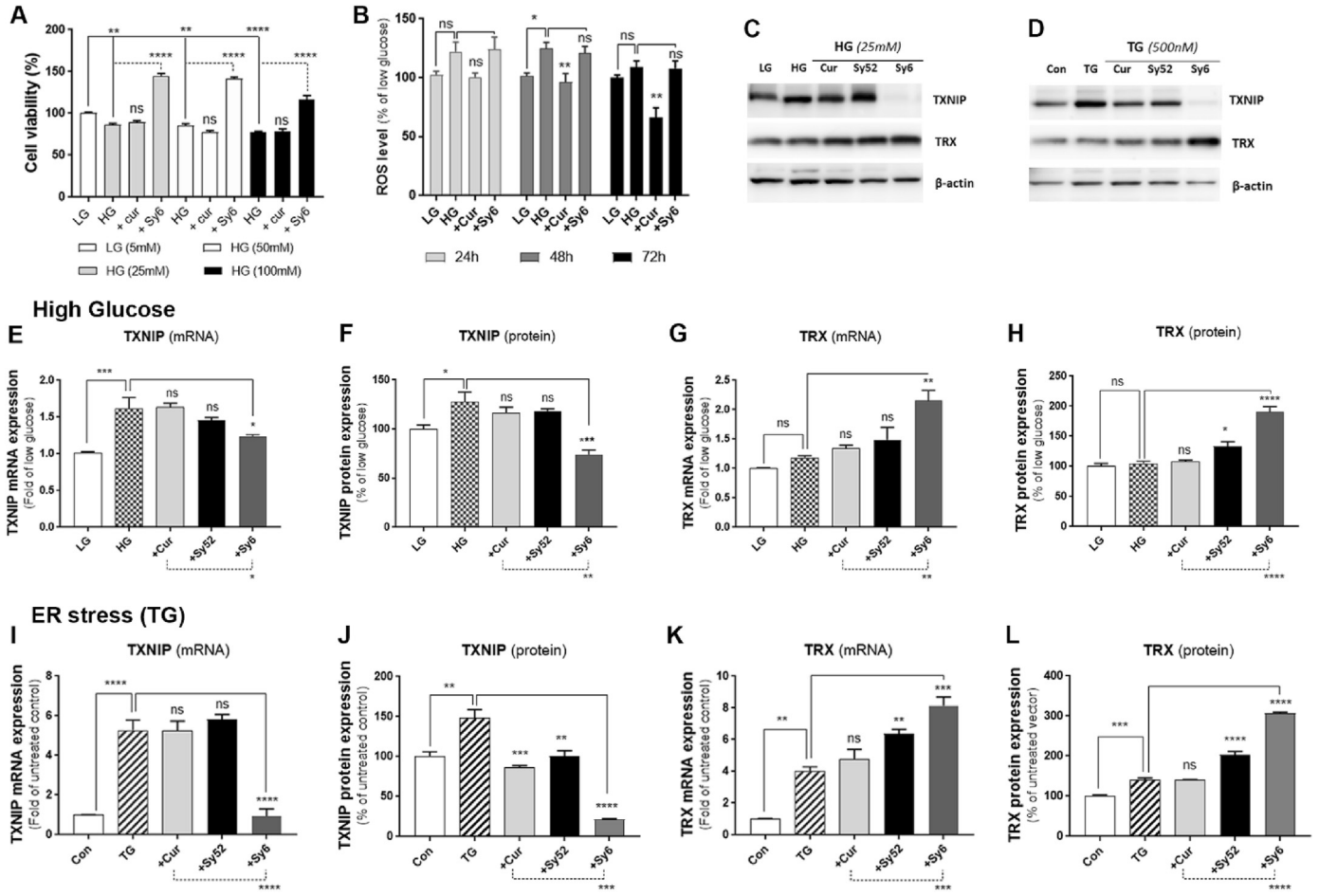

Fig. 2. High glucose and ER stress induced expression of TXNIP and TRX significantly modulated by Shiga-Y6 curcumin derivative in ARPE-19 cells.
High Glucose: (A) Glucose dose-response (25 mM, 50 mM and 100 mM) compared to 5 mM (LG) effects on ARPE-19 cells in the presence of curcumin, SY52 and SY6 (5 μM) on cell viability/mitochondrial activity assay. **(B)** Effect of curcumin and derivatives (5 μM) on high glucose (25 mM) induced reactive oxygen species (ROS) levels at different time-points. **(C)** Representative immunoblots of TXNIP and TRX protein normalized with β-actin in cells treated with high glucose and curcumin or derivatives. **(D)** Representative immunoblots of TXNIP and TRX protein normalized with β-actin in ARPE-19 cells treated with thapsigargin (TG), and curcumin or derivatives. SY6 significantly reduced high glucose induced TXNIP mRNA **(E)** and TXNIP protein **(F)**. SY6 significantly induced TRX mRNA **(G)** expression and protein **(H)** in the presence of high glucose.
ER stress: (I) SY6 significantly reduced TG (500 nM) induced TXNIP mRNA and protein **(J)** expression. SY6 significantly induced TRX mRNA **(K)** and protein **(L)** in the presence of TG (500 nM).

were ineffective or significantly less effective than SY6. By comparison, levels of secreted CCL2 and TNF-α from TG-treated cells were reduced by all curcumin compounds (Supplemental Fig. 1).

3.3. Curcumin and derivatives inhibited expression of LPS and TG-induced inflammatory genes

Comparison was made of the effects of curcumin and its derivatives on inhibiting expression of inflammatory genes in LPS- and TG-stimulated THP-1 macrophages. The stimulation of CCL2, TNF-α and IL-1β expression was considerably higher using the strong inflammogen LPS compared to the ER stress inducer TG. LPS stimulation of CCL2 (Fig. 3A), TNF-α (Fig. 3C) and NLRP3 mRNA (Fig. 3F) levels were highest at 6 h, while IL-1β mRNA (Fig. 3E) was highest at 24 h. It can be seen that mRNA expression of all markers and LPS stimulated secretion of CCL2 (Fig. 3B) and TNF-α (Fig. 3D) were significantly inhibited by curcumin, SY52 and SY6. Overall, these figures show that SY6 was generally more effective than the other compounds in inhibiting mRNA or secreted protein for these markers.

Comparing these results with TG-stimulated THP-1 macrophages, which has an inflammatory stimulating effect of a lower magnitude than LPS, showed curcumin and SY6 had similar effectiveness at reducing CCL2 mRNA (Fig. 3G) and secretion (Fig. 3H), and TNF-α RNA (Fig. 3I) and secretion (Fig. 3J), while SY52 was less effective. IL-1β mRNA expression was significantly inhibited by curcumin, SY52 and SY6, with SY6 being more effective (Fig. 3K). TG-stimulated expression of NLRP3 mRNA was only inhibited significantly by SY6 (Fig. 3L). Levels of TXNIP and TRX with representative immunoblot images for THP-1 macrophages treated with TG and cell viability assay under experimental conditions are shown in Supplemental Fig. 2.

3.4. TXNIP knockdown can result in enhanced expression of inflammatory genes in THP-1 derived macrophages

To further investigate how TXNIP expression affected inflammatory activation in THP-1 macrophages, siRNA-mediated knockdown of TXNIP expression was employed using cells treated with LPS or TG with and without co-treatment with SY6. There was a noticeable difference between responses by LPS- and TG-treated cells. LPS stimulation did not alter TXNIP mRNA expression and this was not significantly altered by cotreatment with SY6 (Fig. 4A). The effectiveness of TXNIP mRNA knockdown was approximately

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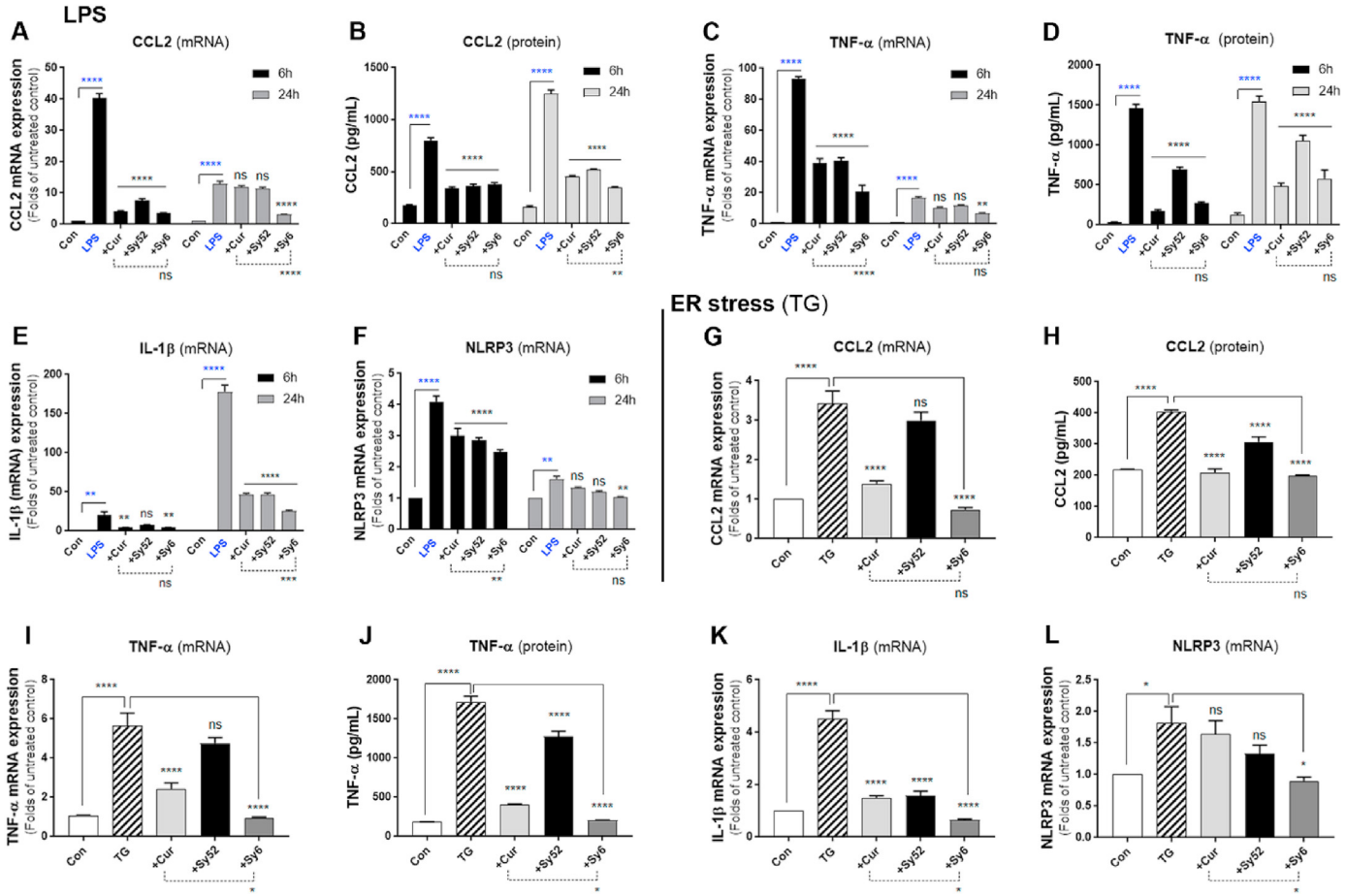

Fig. 3. Curcumin and derivatives reduced LPS and Thapsigargin induced inflammation in THP-1 derived macrophages.
LPS (A–F): Curcumin and derivatives reduced expression of CCL2 mRNA **(A),** CCL2 protein **(B);** TNF-α mRNA **(C),** TNF-α protein **(D);** IL-1β mRNA **(E);** NLRP3 mRNA **(F)** after 6 h and 24 h of LPS (100 ng/mL) stimulation. **ER stress (G–L):** Curcumin and derivatives reduced expression of CCL2 mRNA **(G),** CCL2 protein **(H);** TNF-α mRNA **(I),** TNF-α protein **(J);** IL-1β mRNA **(K);** NLRP3 mRNA **(L)** after 24 h of treatment with TG (300 nM).

90%. However, expression of CCL2 and TNF-α mRNA was significantly increased in TXNIP knockdown cells (Fig. 4B and C). This enhancement was greater in LPS stimulated cells. In these experiments, CCL2 mRNA was significantly inhibited in LPS stimulated cells by SY6 (Fig. 4B) while TNF-α RNA was not (Fig. 4C). However, this pattern was not observed for control or LPS stimulated expression of IL-1β (Fig. 4D) or NLRP3 (Fig. 4E). Silencing of TXNIP mRNA had no significant effect on stimulated or unstimulated cells.

In contrast, using TG treated THP-1 macrophages, a different pattern of gene expression was observed as a consequence of TXNIP knockdown (Fig. 4F–J). TG treated macrophages showed TXNIP stimulated expression (Fig. 4F), whereas LPS stimulation did not have this effect (Fig. 4A). TG stimulated expression of TXNIP was inhibited by SY6 in control transfected cells but not TXNIP siRNA transfected cells (Fig. 4F). CCL2 mRNA induction by TG was significantly reduced in TXNIP-silenced cells (Fig. 4G). This pattern was also observed for IL-1β (Fig. 4I) and NLRP3 (Fig. 4J), however TNF-α mRNA expression was not significantly different in TXNIP silenced cells stimulated with TG compared to controls. Representative western blot panels are shown for TXNIP protein in siRNA silenced cells treated in the same manner (Supplemental Fig. 3).

4. Discussion

The major findings of this study are that our novel fluorinated curcumin derivative SY6 significantly inhibited TXNIP expression in different cellular disease models; stimulated expression of the antioxidant TRX; showed similar or increased anti-inflammatory effects of curcumin and derivatives in cellular models of inflammation and ER stress; and TXNIP silencing could result in enhanced inflammation in LPS-stimulated macrophages but not TG treated cells. It had been initially hypothesized that lowering TXNIP levels would prevent the inhibitory binding to TRX, but the lowering of TXNIP levels also had a transcription stimulating effect on TRX expression. These studies point to the possible interactions of signaling pathways. TXNIP transcription is induced by glucose-stimulated translocation of the carbohydrate-response-element-binding protein (ChREBP) to the nucleus and binding to the carbohydrate response element [16], while TXNIP expression is negatively regulated by activation of AMP-activated protein kinase (AMPK) [17,18]. Curcumin was shown to inhibit gestational diabetes partially through activation of AMPK [19]. Activation of AMPK can result in increased expression of TRX as well as downregulated TXNIP [20]. A recent in vitro study using WI-38 human lung fibroblasts showed curcumin had significant protective effects from paraquat-induced toxicity by inhibiting TXNIP and NLRP3 expression and reducing inflammatory activity [21]. However, this study employed curcumin doses of 300 μM, which was much larger than those we demonstrated to have toxicity on ARPE-19 cells and THP-1 macrophages. The dose of 5 μM used in these experiments represents a reasonable physiological dose and showed significant effect for SY6. In experiments presented in Figs. 3 and 4 with LPS

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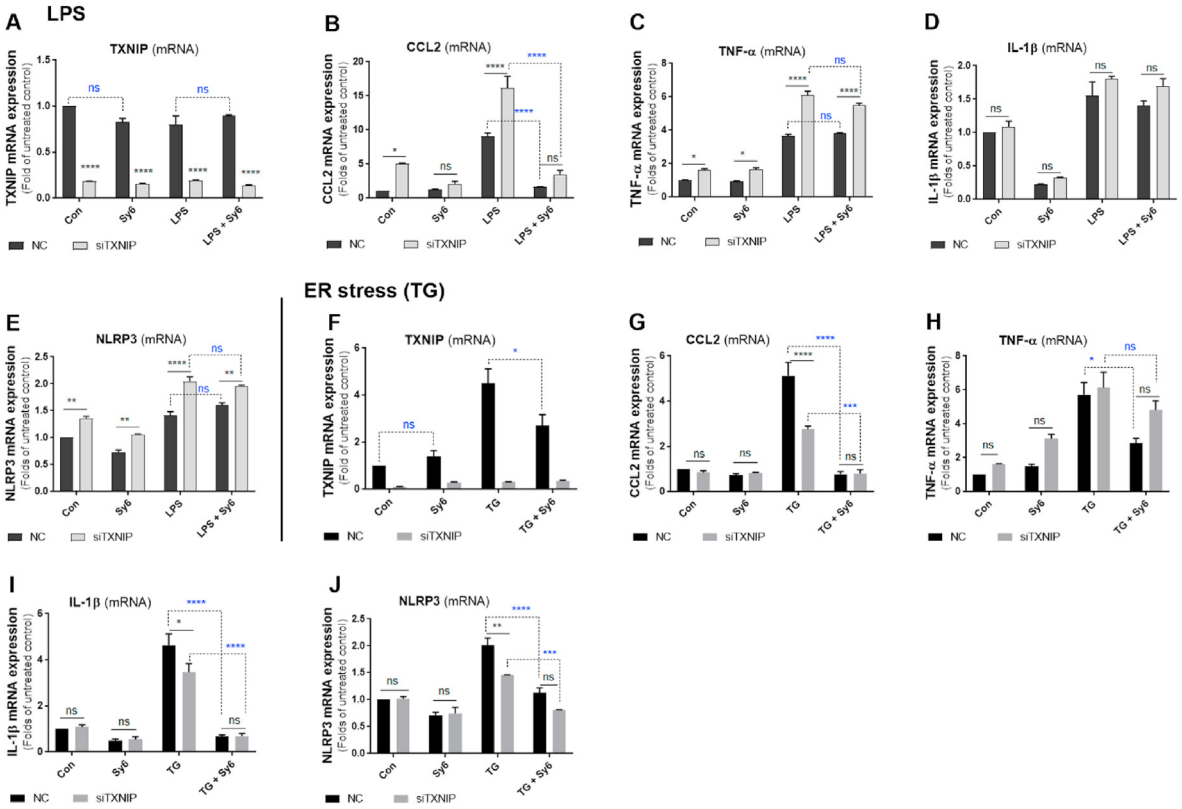

Fig. 4. TXNIP knockdown in THP-1-derived macrophage results in amplified inflammatory responses to LPS but not TG treatments.
TXNIP knockdown and LPS treatment. (A) siRNA knockdown of TXNIP mRNA was not affected by treatment with SY6, LPS or LPS and SY6. **(B)** TXNIP knockdown results in significantly enhanced expression of CCL2 mRNA in control and LPS treated cells. **(C)** TXNIP knockdown results in significantly enhanced expression of TNF-α mRNA in control, LPS and SY6-treated cells. **(D)** TXNIP knockdown did not result in enhanced expression of IL-1β mRNA. **(E)** TXNIP knockdown results in significantly enhanced expression of NLRP3 mRNA in control, LPS and SY6-treated cells.
TXNIP knockdown and TG treatment. **(F)** TXNIP mRNA expression in control and TXNIP knockdown cells. Comparison of effects of SY6, TG and SY6 and TG. **(G)** TXNIP knockdown resulted in significant inhibition of CCL2 mRNA in TG treated THP-1 macrophages. **(H)** TXNIP knockdown did not result in significant inhibition of CCL2 mRNA in TG treated THP-1 macrophages and enhanced expression in TG and SY6 THP-1 macrophages. **(I–J)** TXNIP knockdown resulted in significant inhibition of IL-1β **(I)** and NLRP3 mRNA **(J)** in TG treated THP-1 macrophages.

stimulation of macrophages, reduction in inflammatory gene expression did not correlate with levels of TXNIP. Each curcumin type was effective at reducing expression of CCL2, TNF-α, IL-1β and NLRP3. This was confirmed with TG treated cells, though SY52 was less effective than SY6. With LPS, the primary pathway for inflammatory activation is through NF-κB pathway, while TG induces ER stress through raising intracellular calcium that can activate different pathways. There was enhanced expression of CCL2 and IL-1β, but not TNF-α, by LPS stimulated cells with TXNIP knockdown, but these were reduced with TG treatment and TXNIP reduction.

Failures of glucose homeostasis leading to oxidative stress, inflammation and endoplasmic reticulum stress due to type-1 diabetes with loss of pancreatic β cells or due to type-2 diabetes with loss of insulin sensitivity are significant causes of morbidity. Complications from diabetes include significant vascular endothelial cell dysfunction linked to cardiovascular disease, cerebrovascular disease, renal disease and loss of eyesight amongst others. TXNIP has been linked to these conditions as a central regulator, and as such becomes a target for therapeutics [1,22,23]. TXNIP deficiency in experimental animal models was protective from the consequences of diabetes [24]. TXNIP affects different cellular functions not only facilitating oxidative stress through thioredoxin inhibition. Dissecting the multiple mechanisms of TXNIP under pathological conditions is inherently difficult, but identifying a novel class of agents that affects TXNIP expression could be useful. There has been much interest in curcumin and derivatives as they can alleviate the consequences of diabetes-induced endothelial dysfunction through reducing inflammation [25]. Curcumin compounds can have different mechanisms of action as many different signaling pathways can be downregulated, in particular NF-κB and JNK [26], JAK/STAT/NF-κB [27] and PI3/AKT/mTOR [28].

In summary, these data indicate directions for future studies for characterizing properties of curcumin derivatives. We have produced a number of different chemically modified curcumin derivatives that can be tested in these simple assays for modulation of TXNIP and TRX expression.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Japanese patent application has been submitted (#2020−141272) by the host institution (Shiga University of Medical Science) covering the usage of the novel curcumin derivatives Shiga-Y52 and Shiga-Y6. The intended patent application submission had no influence on the results presented in this publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2020.08.114.

References

[1] H. Parikh, E. Carlsson, W.A. Chutkow, L.E. Johansson, et al., TXNIP regulates peripheral glucose metabolism in humans, PLoS Med. 4 (2007) e158, https://doi.org/10.1371/journal.pmed.0040158.
[2] D.Z. Wondafrash, A.T. Nire'a, G.G. Tafere, D.M. Desta, et al., Thioredoxin-interacting protein as a novel potential therapeutic target in diabetes mellitus and its underlying complications, Diabetes. Metab. Syndr. Obes. 13 (2020) 43–51, https://doi.org/10.2147/DMSO.S232221.
[3] J. Hu, Y. Yu, The function of thioredoxin-binding protein-2 (TBP-2) in different diseases, Oxid. Med. Cell. Longev. (2018) 4582130, https://doi.org/10.1155/2018/4582130, 2018.
[4] P.C. Schulze, J. Yoshioka, T. Takahashi, Z. He, et al., Hyperglycemia promotes oxidative stress through inhibition of thioredoxin function by thioredoxin-interacting protein, J. Biol. Chem. 279 (2004) 30369–30374, https://doi.org/10.1074/jbc.M400549200.
[5] J. Lv, S. Bao, T. Liu, L. Wei, et al., Sulforaphane delays diabetes-induced retinal photoreceptor cell degeneration, Cell Tissue Res. (2020), https://doi.org/10.1007/s00441-020-03267-w.
[6] S. Matsuoka, H. Tsuchiya, T. Sakabe, Y. Watanabe, et al., Involvement of thioredoxin-binding protein 2 in the antitumor activity of CD437, Canc. Sci. 99 (2008) 2485–2490, https://doi.org/10.1111/j.1349-7006.2008.00979.x.
[7] H. Shimizu, T. Tsubota, K. Kanki, G. Shiota, All-trans retinoic acid ameliorates hepatic stellate cell activation via suppression of thioredoxin interacting protein expression, J. Cell. Physiol. 233 (2018) 607–616, https://doi.org/10.1002/jcp.25921.
[8] Y. Gao, J.-C. Qi, X. Li, J.-P. Sun, et al., Decreased expression of TXNIP predicts poor prognosis in patients with clear cell renal cell carcinoma, Oncol. Lett. 19 (2020) 763–770, https://doi.org/10.3892/ol.2019.11165.
[9] X. Zhang, J.-H. Zhang, X.-Y. Chen, Q.-H. Hu, et al., Reactive oxygen species-induced TXNIP drives fructose-mediated hepatic inflammation and lipid accumulation through NLRP3 inflammasome activation, Antioxidants Redox Signal. 22 (2015) 848–870, https://doi.org/10.1089/ars.2014.5868.
[10] S. Oliveira, T. Monteiro-Alfredo, S. Silva, P. Matafome, Curcumin derivatives for Type 2 Diabetes management and prevention of complications, Arch Pharm. Res. (Seoul) 43 (2020) 567–581, https://doi.org/10.1007/s12272-020-01240-3.
[11] E. Chainoglou, D. Hadjipavlou-Litina, Curcumin in health and diseases: Alzheimer's disease and curcumin analogues, derivatives, and hybrids, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21061975.
[12] D. Yanagisawa, H. Taguchi, S. Morikawa, T. Kato, et al., Novel curcumin derivatives as potent inhibitors of amyloid beta aggregation, Biochem. Biophys. Reports. 4 (2015) 357–368, https://doi.org/10.1016/j.bbrep.2015.10.009.
[13] I. Tooyama, D. Yanagisawa, H. Taguchi, T.K. Kato, et al., Amyloid imaging using fluorine-19 magnetic resonance imaging ((19)F-MRI), Ageing Res. Rev. 30 (2016) 85–94, https://doi.org/10.1016/j.arr.2015.12.008.
[14] H. Taguchi, D. Yanagisawa, S. Morikawa, K. Hirao, et al., Synthesis and tautomerism of curcumin derivatives and related compounds, Aust. J. Chem. 68 (2015) 224–229.
[15] U. Buyandelger, D.G. Walker, D. Yanagisawa, T. Morimura, I. Tooyama, Effects of FTMT expression by retinal pigment epithelial cells on features of angiogenesis, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21103635.
[16] H. Cha-Molstad, G. Saxena, J. Chen, A. Shalev, Glucose-stimulated expression of Txnip is mediated by carbohydrate response element-binding protein, p300, and histone H4 acetylation in pancreatic beta cells, J. Biol. Chem. 284 (2009) 16898–16905, https://doi.org/10.1074/jbc.M109.010504.
[17] M. Shaked, M. Ketzinel-Gilad, E. Cerasi, N. Kaiser, G. Leibowitz, AMP-activated protein kinase (AMPK) mediates nutrient regulation of thioredoxin-interacting protein (TXNIP) in pancreatic beta-cells, PloS One 6 (2011), e28804, https://doi.org/10.1371/journal.pone.0028804.
[18] J. Song, J. Li, F. Hou, X. Wang, B. Liu, Mangiferin inhibits endoplasmic reticulum stress-associated thioredoxin-interacting protein/NLRP3 inflammasome activation with regulation of AMPK in endothelial cells, Metabolism 64 (2015) 428–437, https://doi.org/10.1016/j.metabol.2014.11.008.
[19] X. Lu, F. Wu, M. Jiang, X. Sun, G. Tian, Curcumin ameliorates gestational diabetes in mice partly through activating AMPK, Pharm. Biol. 57 (2019) 250–254, https://doi.org/10.1080/13880209.2019.1594311.
[20] X. Hou, J. Song, X.-N. Li, L. Zhang, et al., Metformin reduces intracellular reactive oxygen species levels by upregulating expression of the antioxidant thioredoxin via the AMPK-FOXO3 pathway, Biochem. Biophys. Res. Commun. 396 (2010) 199–205, https://doi.org/10.1016/j.bbrc.2010.04.017.
[21] Y. Ren, Z. Yang, Z. Sun, W. Zhang, et al., Curcumin relieves paraquat-induced lung injury through inhibiting the thioredoxin interacting protein/NLR pyrin domain containing 3-mediated inflammatory pathway, Mol. Med. Rep. 20 (2019) 5032–5040, https://doi.org/10.3892/mmr.2019.10612.
[22] L. Perrone, T.S. Devi, K.-I. Hosoya, T. Terasaki, L.P. Singh, Inhibition of TXNIP expression in vivo blocks early pathologies of diabetic retinopathy, Cell Death Dis. 1 (2010) e65, https://doi.org/10.1038/cddis.2010.42.
[23] A. Shah, L. Xia, H. Goldberg, K. W, et al., Thioredoxin-interacting protein mediates high glucose-induced reactive oxygen species generation by mitochondria and the NADPH oxidase, Nox4, in mesangial cells, J. Biol. Chem. 288 (2013) 6835–6848, https://doi.org/10.1074/jbc.M112.419101.
[24] A. Shah, L. Xia, E.A.Y. Masson, C. Gui, et al., Thioredoxin-interacting protein deficiency protects against diabetic nephropathy, J. Am. Soc. Nephrol. 26 (2015) 2963–2977, https://doi.org/10.1681/ASN.2014050528.
[25] N. Wongeakin, P. Bhattarakosol, S. Patumraj, Molecular mechanisms of curcumin on diabetes-induced endothelial dysfunctions: txnip, ICAM-1, and NOX2 expressions, BioMed Res. Int. 2014 (2014) 161346, https://doi.org/10.1155/2014/161346.
[26] L. Qihui, D. Shuntian, Z. Xin, Y. Xiaoxia, C. Zhongpei, Protection of curcumin against streptozocin-induced pancreatic cell destruction in T2D rats, Planta Med. 86 (2020) 113–120, https://doi.org/10.1055/a-1046-1404.
[27] S. Geng, S. Wang, W. Zhu, C. Xie, et al., Curcumin suppresses JNK pathway to attenuate BPA-induced insulin resistance in LO2 cells, Biomed. Pharmacother. 97 (2018) 1538–1543, https://doi.org/10.1016/j.biopha.2017.11.069.
[28] G.A. Borges, S.T. Elias, B. Amorim, C.L. de Lima, et al., Curcumin downregulates the PI3K-AKT-mTOR pathway and inhibits growth and progression in head and neck cancer cells, Phytother Res. (2020), https://doi.org/10.1002/ptr.6780.

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